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Published on Web 12/20/2005

NO-Releasing Zeolites and Their Antithrombotic Properties

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Abstract: Transition metal-exchanged zeolite-A adsorbs and stores nitric oxide in relatively high capacity (up to 1 mmol of NO/g of zeolite). The stored NO is released on contact with an aqueous environment under biologically relevant conditions of temperature and pH. The release of the NO can be tuned by altering the chemical composition of the zeolite, by controlling the amount of water contacting the zeolite, and by blending the zeolite with different polymers. The high capacity of zeolite for NO makes it extremely attractive for use in biological and medical applications, and our experiments indicate that the NO released from Co-exchanged zeolite-A inhibits platelet aggregation and adhesion of human platelets in vitro.

Introduction

Nitric oxide (NO) is a crucial biological agent in the cardiovascular, nervous, and immune systems.[1] NO synthesized by endothelial cells that line blood vessels mediates a number of vital functions, including vasodilatation[2,3] and inhibition of platelet[4] and inflammatory cell[5,6] activation and adhesion. In addition, NO is an important neurotransmitter and neuromodulator in the peripheral and central nervous systems,[7] and its synthesis in high concentrations contributes to the cytotoxic effects of inflammatory cells on invading pathogens.[8]

The delivery of exogenous NO is an attractive therapy for a number of ailments, but the range and diversity of its effects make target specificity a major concern, which has held back the development of some NO applications. To overcome this problem requires the development of materials that can store significant quantities of NO and then deliver it to specific sites in the body. There is currently particular interest in using NO delivery materials to prevent life-threatening complications associated with thrombosis formation at the surface of medical devices such as stents and catheters.[9]

A number of materials have been proposed as delivery agents for exogenous NO. Perhaps the chemically most advanced are those based on polymers or silica functionalized with secondary amines, which, on reaction with NO, form ionic diazenium diolates that can be used to increase the thromboresistivity of polymers.[9,10,11] Two molecules of NO react with each amine (giving rise to the trivial name NONOate) and are released on contact with moisture at an appropriate pH. Other methods of release have also been explored, including the light-activated release of NO from metal-containing polymers.[12]

A specific potential use for NO-releasing materials in the cardiovascular arena centers on the prevention of thrombosis on artificial surfaces that come into contact with blood, particularly in relation to procedures that require extracorporeal circuits, like bypass and renal replacement therapy, catheter implantations, or stents that are used in interventional cardiology to improve the patency of partially blocked arteries.[9] Current best practice to prevent thrombosis in these situations involves the administration of heparin, other anticoagulants, or antiplatelet agents to the patient, with the inherent risk of hemorrhagic[13,14]

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 10.1021/ja0503579 CCC: $33.50

or paradoxical thrombotic[15] complications. Stent coatings that release antithrombotic,[16] antiinflammatory,[17] and antimitogenic[18] agents to reduce in-stent thrombosis or restenosis have had some success, but NO might be expected to have all of these effects if delivered at the appropriate rate for a sufficient duration. Other potential uses of these types of solids, as shown by a number of different biological effects, include antibacterial coatings[19] and wound-healing promotors.[20]

Zeolites have well-known applications in ion exchange, gas adsorption, and catalysis (including DeNO$_x$, the removal of nitrogen oxides from exhaust gases[21,22]), and they are of increasing interest as hosts for nanotechnology applications.[23] New zeolites[24] and new methods for zeolite preparation[25,26] continue to attract attention as catalysts, but their use in medicine is limited to nontoxic medical diagnosis tools and clotting enhancers.[23] Zeolites have also been studied as microporous storage media for gases such as hydrogen.[27]

A great deal is known about the interaction of NO with zeolites, particularly from catalytic and separation studies. Pressure swing adsorption experiments indicate that there are two types of adsorbed NO in zeolites, commonly called reversibly and irreversibly adsorbed NO.[28−30] As its name suggests, reversibly adsorbed NO is a weakly held, predominantly physisorbed species. In contrast, irreversibly adsorbed NO is relatively strongly held and is not spontaneously released from the zeolite even at very low pressures. Irreversible NO is primarily chemisorbed, as has been shown by single-crystal X-ray diffraction[31] and IR spectroscopy[32] and supported by recent modeling experiments,[33] where the NO interacts with the extraframework cations in zeolites through the nitrogen atom to form either mononitrosyl or dinitrosyl complexes. Irreversible adsorption of this kind is a good method of storing a gas in a material as it is not lost too easily. However, suitable methods of initiating delivery of the gas must be found before the materials can be utilized in applications. Here we show how a suitable nucleophile (water) can be used to displace the "irreversibly" adsorbed NO from ion-exchanged zeolite-A at biologically relevant temperatures. The release of NO can be customized by altering the chemical composition of the zeolite. Experiments with human platelets confirm that NO released from a zeolite is sufficient to abolish platelet activation and adhesion in vitro, pointing to potential applications in coating surgically implanted catheters that are ordinarily regarded to be prothrombogenic. The tuneable nature of NO release from cation-exchanged zeolites extends the potential applications to tubing and instruments used in procedures such as bypass surgery and renal replacement therapy.

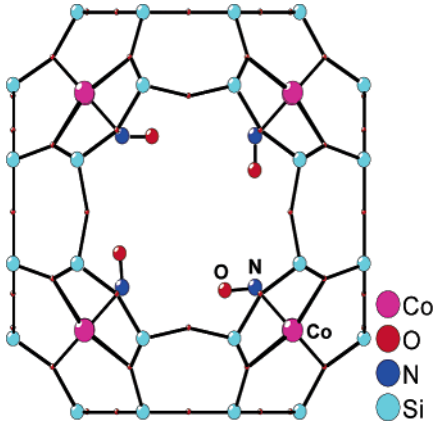

Figure 1. Structure of the cobalt−NO complex in zeolite-A as revealed by single-crystal X-ray diffraction (data taken from ref 31). The cobalt is bound to three oxygen atoms of a six-ring unit in the zeolite framework and bonds to the nitrogen of a bent mononitrosyl ligand. Oxygen atoms of the zeolite framework are shown smaller than the NO oxygen for clarity.

Zeolite-A (given the three letter framework code LTA[34]) is manufactured in >1 M tonne amounts annually for use as a detergent builder and water softener. Zeolite-A[35,36] consists of alternating SiO_4 and AlO_4 tetrahedra that share corners to produce the open framework depicted in Figure 1, with exchangeable extraframework cations residing in the channels. Zeolite-A is a poor DeNO$_x$ catalyst but is used in a number of adsorption applications. It is therefore an ideal choice of zeolite for studies of NO storage. Also, it has a well-known affinity for water, often being used by organic chemists to dry solvents.

Figure 2 shows the adsorption and desorption isotherms for NO on Co-exchanged zeolite-A and illustrates the natural affinity of the zeolite for NO, as even at low pressures the majority of the gas remains bound inside the zeolite (i.e. is irreversibly adsorbed). The hysteretic nature of the adsoption/desorption isotherm means that the zeolite can be used to hold NO at pressures below those needed to load NO onto the material. This is ideal for the storage of significant amounts of NO. Similar hysteretic adsorption has recently been seen for hydrogen storage in other types of nanoporous material.[37] The maximum amount of NO adsorbed by cobalt-exchanged zeolite-A is approximately 1.7 mmol/g of zeolite, although reduction of the NO pressure leads to loss of the reversibly adsorbed

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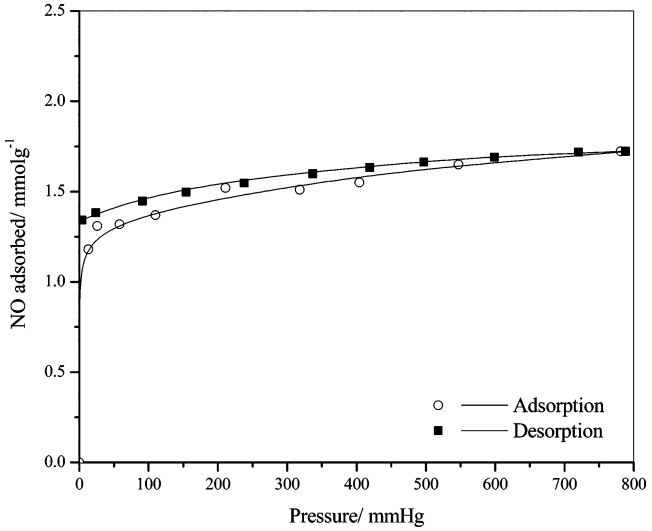

Figure 2. Adsorption/desorption isotherm of nitric oxide on Co-exchanged zeolite-A. The irreversible nature of the adsorption of the majority of NO on the zeolite is indicated by the hysteresis.

(weakly physisorbed NO) leading to a maximum storage capacity of ~1.2−1.3 mmol/g. The only remaining problem is then how to release (deliver) the NO from the zeolite when required. Fortunately, zeolites also interact strongly with water, and our hypothesis was that exposure to moisture would result in replacement of the NO by water in the zeolite, leaving the gaseous NO to diffuse out of the zeolite. Our aim in this study was to synthesize metal-containing zeolites that store NO and release it in biologically relevant amounts on contact with water. We envisaged that the rate of NO release could be modified by altering the chemical composition of the material for a range of different medical and biological applications in which exogenous NO might be beneficial.

Results and Discussion

Samples of zeolite-A were synthesized according to the procedure given in ref 38. The transition exposed to dry NO and stored in sealed vials under argon at room temperature ready for use.

Zeolites are well-known as extremely good drying agents and interact strongly with water, and this has led to their use in numerous applications, including as a method of rapidly absorbing water from blood to aid in clotting after serious wounding.[39] It is not surprising then that the NO is released very quickly when contacted by water in a physiological solution. Unfortunately, it is difficult to quantify the release rate of NO in this fashion accurately because the powder zeolite tends not to disperse well in the liquid, with much remaining floating on the surface. An alternative, and much more repeatable, method is to flow a wet gas (of controlled relative humidity) through the sample at a steady rate and measure the NO concentration of the gas using chemiluminescence measurements. The NO release profiles of NO-loaded cobalt exchanged zeolite-A expressed as a total concentration of NO in the gas stream versus time (a) and a cumulative total amount of NO released/g of zeolite (b) are shown in Figure 3. In this case the flow rate of gas used was 200 mL/min and the relative humidity of the gas was 11%. The total amount of NO released is approximately 1 mmol of NO/g of zeolite. The duration of NO release approaches 2500 s with a half-life ($t_{1/2}$, the time it takes for half the NO to be released) of ~340 s. The total amount of NO released is also very consistent between different samples as long as care is taken to ensure that the chemical composition of the zeolite and its dehydration properties are the same. The average amount of NO released from 130 separate measurements on different samples was 1.02 mmol of NO/g with a standard deviation of 0.05. Storage of the NO-loaded zeolites under a dry atmosphere also has no effect on the total NO released by the materials. Samples of NO-loaded cobalt-exchanged zeolite-A were stored under argon in sealed ampules for up to 16 weeks. The quantification of the NO released by these samples is shown in Figure 4, with each measurement repeated at least 16 times on different samples. There is no drop off in total NO released indicating that under dry conditions cobalt-exchanged zeolite-A is a stable NO storage material with a gas storage capacity similar to that of the best NO-storing polymers reported and significantly higher than most.[10,11]

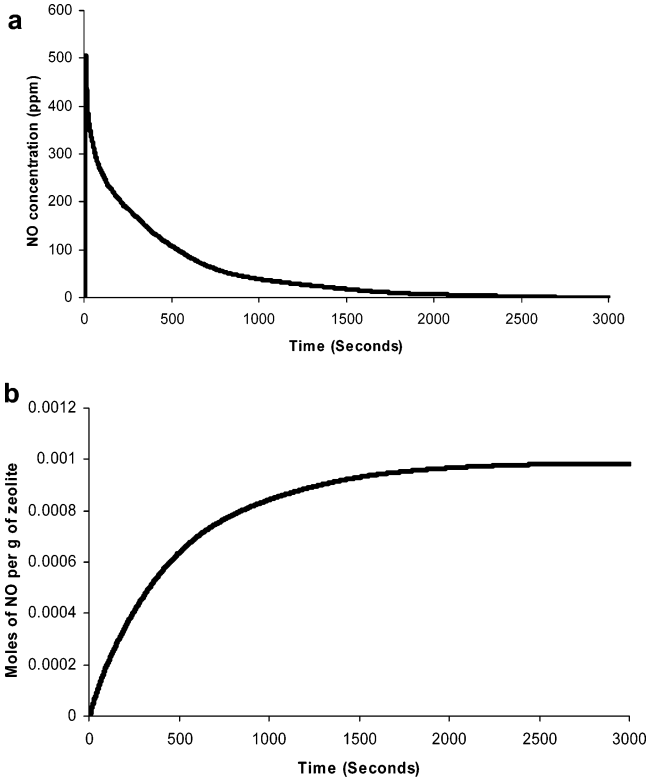

Figure 3. Two NO-release profiles for NO-loaded Co-exchanged zeolite-A: (a) NO concentration (ppm) in the gas stream passing through the chemiluminescence detector versus time; (b) total mount of NO released by the material with time (per g of zeolite). The conditions for this experiment were 31 mg of zeolite and a flow rate of 200 mL/min of wet argon at a relative humidity of 11%.

The amount of moisture present in the gas flow is also important in controlling the release of the NO. Increasing the relative humidity (while keeping the flow rate constant) to 22% increases the rate of NO release ($t_{1/2}$ = 208 s), while an almost dry gas (relative humidity 1.5%) increases the $t_{1/2}$ to more than 3000 s. It should be remembered though that there are two

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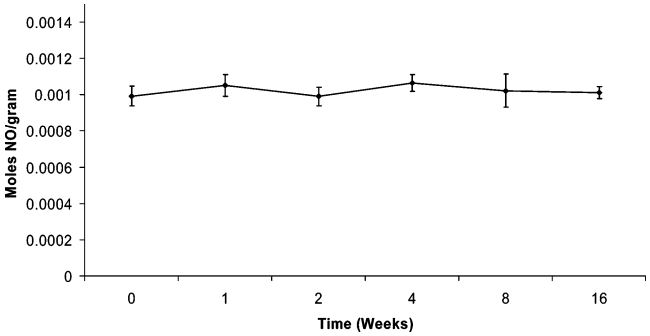

Figure 4. Storage stability experiments showing the total amount of NO released by a cobalt-exchanged zeolite-A after storage under dry conditions for different amounts of time. The results are the average of at least 16 different measurements for each storage period, and the error bars indicate the standard deviations of the measurements.

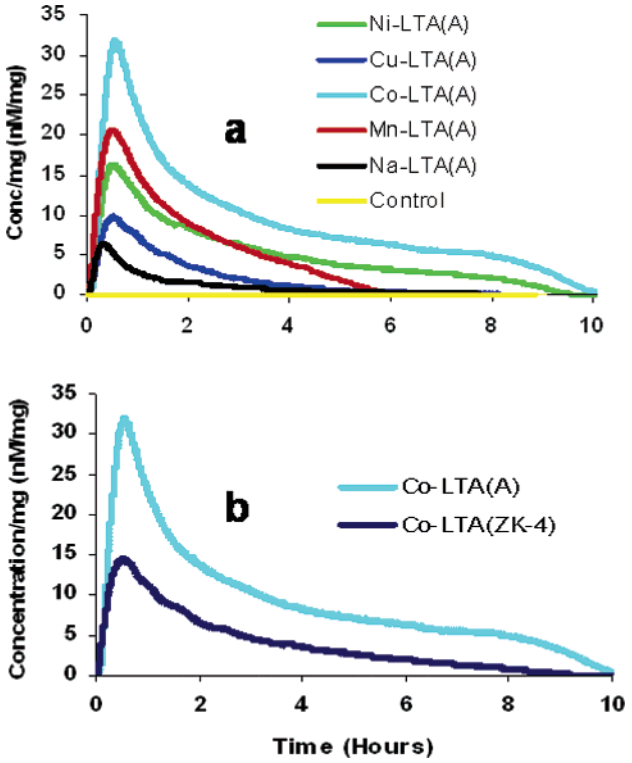

Figure 5. NO-release profiles of NO-loaded metal-exchanged zeolite-A. Top: Effect of metal ion on NO release. The control is a Co^{2+}-exchanged zeolite that has not been exposed to NO. The electrode response results have been normalized to give the concentration of NO in solution/mg of zeolite material. Bottom: Dependence of NO release on Co^{2+} exchange level. Co−zeolite-A (Co−LTA(A)) contains 19.8 wt % cobalt, while Co−zeolite-ZK4 (Co−LTA(ZK4)) contains 9.0 wt % cobalt. All zeolite samples were from the same synthesis batch to minimize particle size differences in the materials.

components to the storage of NO and that any physisorbed NO will be released even in completely dry conditions.

An alternative way to measure the release of NO from the zeolites involves contacting the zeolite with a moist gas that is subsequently bubbled through a liquid phase (phosphate buffered saline, pH 7.4 at 25 °C). The amount of NO dissolved in the solution is then measured electrochemically. This allows the flow rate to be reduced down to 5 mL/min showing that even in moisture-saturated argon the release of the NO now takes place much more slowly and lasts up to 10 h (Figure 5). Again this is consistent with the reduced flux of water on the sample from the much reduced flow rate. Figure 5 also shows the NO release profiles measured electrochemically for a number of other transition metal-exchanged zeolite-A samples in contact with an argon flow that has been saturated with water vapor. The order of how much NO is released for each metal agrees well with the NO adsorption properties of transition metal zeolites in pressure swing adsorption studies.[28,29] Co-exchanged zeolites released the most NO while the original sodium form of the zeolite released least NO. At first glance the Cu-exchanged zeolite-A results seem anomalously low, especially since Cu−zeolites are well-known deNO_x catalysts and that Cu-exchanged zeolites do adsorb significant quantities of NO.[28,29] This is because the zeolite is overexchanged, with more Cu^{2+} ions in the channels than is strictly necessary for charge balance reasons. Many of the "extra" Cu^{2+} ions are probably present as hydroxide species and so reduce the availability of the metal ions for NO coordination.

The amount of NO released by the zeolite depends not only on which transition metal is present but also on how much of the metal is there. Zeolite-ZK4 is a variant of zeolite-A that has the same framework structure and so has the same framework code (LTA). However, there are fewer exchangeable cations in zeolite-ZK4 as there is less aluminum in the framework than in zeolite-A. This means that there are fewer metal cation sites in the channels of the structure to bind NO. It is clear from Figure 5b that Co-exchanged zeolite-A releases more NO than Co-exchanged zeolite ZK4, consistent with the reduced level of cobalt in the ZK4 structure. Interestingly, Co-exchanged zeolite-A samples are self-indicating; when initially dried they are a bright blue, and when loaded with NO they are a gray/blue but change to pink as the NO is displaced by water. These color changes emphasize the importance of the metal−NO interactions in the storage of the gas and the replacement of these by metal−water interactions on delivery of the NO.

Bioactivity. The need for improvements in the biocompatibility of materials is a very important target. This is particularly true for blood-contacting solids that are used in vascular grafts and in extracorporeal tubing used in coronary bypass surgery and kidney dialysis. Life-threatening complications can occur if thrombosis formation (platelet aggregation and adhesion together with coagulation) is induced by artificial materials that are in contact with blood. Thrombus formation in healthy circulatory systems is inhibited in a number of ways, including the production of small quantities (approximately 1 pmol min^{-1} mm^{-2}; see ref 9) of NO by the endothelial cells that line the blood vessels and also by platelets. A potentially important strategy for reducing postoperative complications is to make medical devices out of an NO-releasing material, thereby mimicking the antithrombotic action of the endothelial cells.

To overcome the problem of the nondispersion of zeolite powders in the liquid phase, samples of zeolite were prepared as pressed disks with small amounts of polymers as binders. The Co-exchanged zeolite samples, in 75:25 or 50:50 wt % mixtures with powdered poly(tetrafluoroethylene) (PTFE) or poly(dimethylsiloxane) (PDMS), were prepared as pressed disks and subsequently dehydrated and loaded with NO in the same way as the powdered samples. Pressed disks made from only zeolites are slightly brittle; some material breaks off easily under stirring, and so the polymer is there primarily to add mechanical stability to the disks. However, this process clearly affects the NO release properties of the materials. Figure 6 shows that on

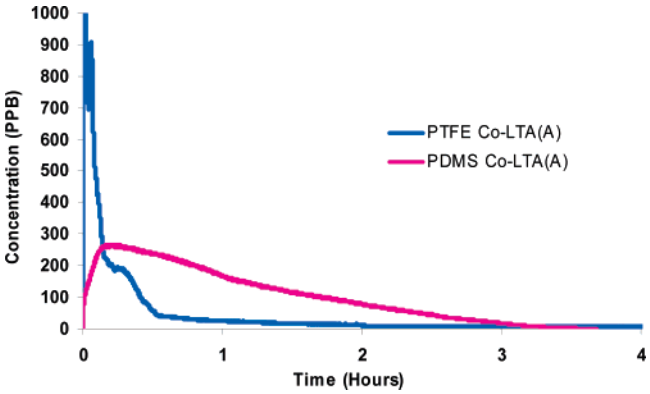

Figure 6. Chemiluminescence response to NO (in ppb) from two disks of cobalt-exchanged zeolite-A (8.5 mg) mixed with PTFE and PDMS (8.5 mg, respectively) immersed in water.

exposure to liquid water (25 °C) the release of NO is slowed considerably as compared to the powdered zeolite, with $t_{1/2}$ of 509 s for the PTFE disk and a $t_{1/2}$ of 3076 s for the PDMS disk. In addition, the total amount of NO released is very much reduced compared to the free zeolite powders (1.4×10^{-5} and 2.4×10^{-5} mol of NO/g of zeolite for PTFE and PDMS, respectively). This is not at all surprising given that the preparation of the disk will affect the accessibility of the zeolite to the NO considerably. However, given the small quantities of NO required for biological action this is not a major drawback in terms of testing the materials for their antithrombotic behavior. Clearly the nature of polymer used in any formulation can have a significant affect on the rates of release and is another method of tuning the release rate to match any potential application. Figure 7c shows that the electrochemically measured released profile of the Co−zeolite-A/PTFE disk is very similar to that seen in plasma. The NO-loaded disks were stored under an inert atmosphere in a manner similar to that described above for the powdered zeolite samples. The disks were stored for up to several weeks before being used in further experiments.

Zeolite/PTFE disks were suspended by a stainless steel wire holder below the surface of platelet-rich plasma (PRP) in the cuvette of a four-channel platelet aggregometer (Chronolog, Labmedics, Stockport, U.K.) at 37 °C. After a short induction period (1 min), platelet aggregation was initiated using the thromboxane A_2 analogue, U46619 (8 μM) and then measured as a change in turbidity (light transmission) of PRP against a platelet poor plasma (PPP) reference. The results (Figure 7) show that a NO-loaded Co-exchanged zeolite-A/PTFE sample completely inhibits platelet aggregation. Addition of the NO scavenger, oxyhemoglobin (40 μM), prevents the inhibitory effect, confirming the central role for NO in the inhibitory process and excluding the possibility that the effects of the NO−zeolite were merely cytotoxic. Furthermore, a Co-exchanged zeolite/PTFE sample that has not been loaded with NO failed to inhibit U46619-mediated aggregation. Parallel experiments

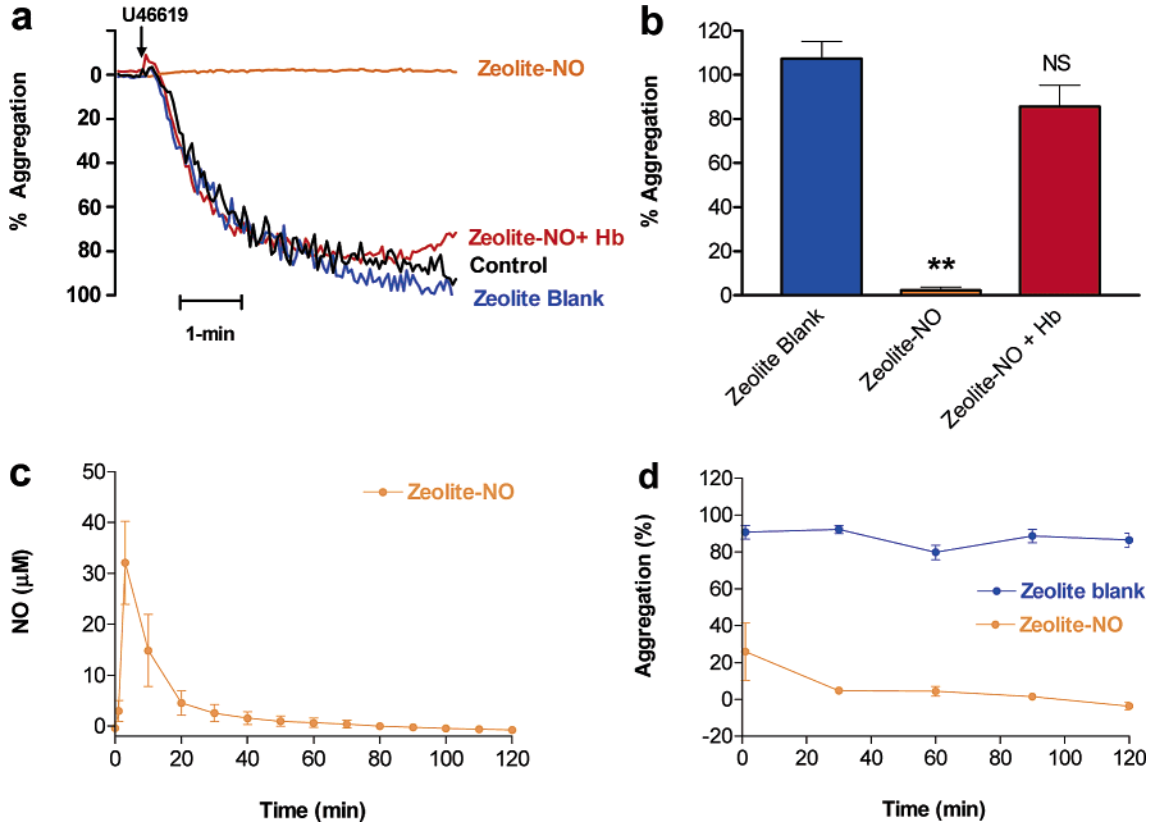

Figure 7. Effect of NO-loaded Co−zeolite-A and the NO-free Co−zeolite A (both 75% in PTFE) on platelet aggregation in response to U46619 (8 μM): (a) aggregation recordings from a single experiment in the presence and absence of the NO scavenger, oxyhemoglobin (Hb; 40 μM); (b) mean ± standard error of the mean (SEM) data for aggregation under different conditions, expressed as % of platelet aggregation in the absence of zeolite [$^{**}P < 0.01$, Dunn's multiple comparison test after nonparametric Kruskal Wallis comparison for samples with different variances; NS = not significant ($n = 6$)]; (c) electrochemically detected NO generation profile from NO-loaded Co−zeolite (75% in PTFE) suspended in PRP ($n = 5$); (d) time−course of the inhibitory effect of NO-loaded Co−zeolite A and the NO-free counterpart (both 75% in PTFE) on agonist-induced platelet aggregation at timed intervals after immersion of the zeolite in PRP ($n = 6$; $P < 0.001$, 2-factor ANOVA).

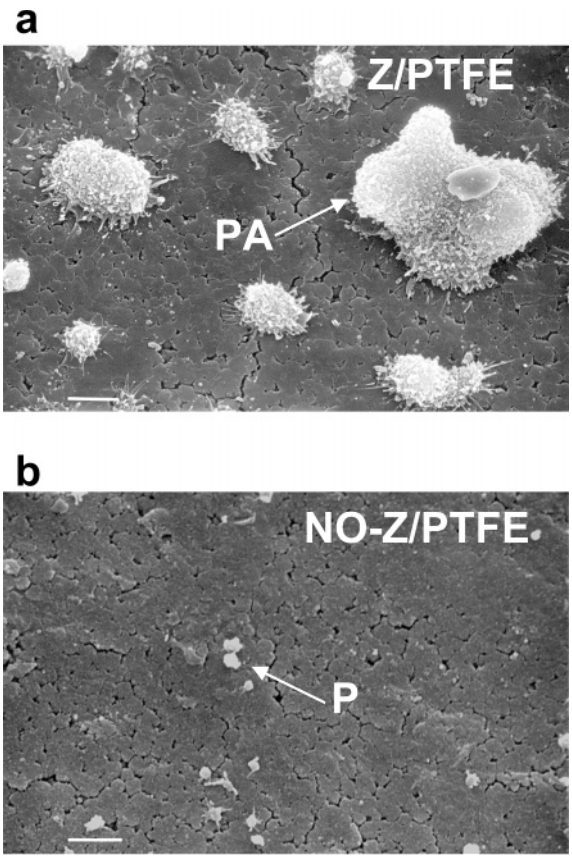

Figure 8. Scanning electron micrographs of the surface of (a) untreated Co−zeolite-A/PTFE disks (Z/PTFE) and of (b) NO-loaded Co−zeolite-A/PTFE disks. (a) shows large platelet aggregants (PA) on the surface on the surface of the untreated zeolite/PTFE disk, while (b) shows only a few, isolated platelets (P) on the surface of the NO-treated zeolite−PTFE disk. The scale bar is 10 μm.

were conducted to determine the concentration of NO generated by 75% NO-loaded zeolite disks in PTFE suspended in PRP (Figure 7c). The profile of release is similar to that shown in Figure 6, suggesting that plasma components have little impact on the water-mediated release of NO from this compound. In addition, a time−course study for the effect of equivalent disks on platelet aggregation (Figure 7d) indicate a prolonged inhibitory effect throughout the 2 h period of study, which is not shared by the NO-free counterpart. However, it is worth noting that an element of the persistent inhibition is likely to be mediated via formation of a durable NO store in plasma[40] rather than necessarily through the persistent delivery of NO directly from the zeolite. Indeed, it is clear that the zeolite/PTFE NO release profile is not ideal for maintaining a protracted antithrombotic effect, as the NO release is exhausted after ~30 min (Figures 6 and 7c). The release profile for the zeolite/PDMS disks (Figure 6) shows a considerably lower peak NO concentration and a considerable prolonged release profile. This indicates that it may be possible to tune the release profile for a protracted thrombotic effect.

Platelet aggregants (PA; Figure 8a) on the surface of the zeolite/PTFE (Z/PTFE) were visualized using scanning electron microscopy, which conclusively demonstrates the extent to which platelet aggregation and adhesion is inhibited at the surface of NO-treated zeolite/PTFE (NO−Z/PTFE; Figure 8b), on which only a small number of isolated platelets (P) can be seen.

The toxicology of zeolites has been extensively studied, and the frameworks themselves are regarded as benign materials. Zeolite-A, because of its wide range of uses in detergent powders, has been particularly well studied in terms of its potential toxicity. There are no reported adverse effects on the body from zeolite frameworks, except possible pulmonary problems caused by breathing small particles of zeolite dust.[41] Unlike zeolites for detergent applications, the zeolites reported in this paper contain transition metal ions, and the toxicity of these ions must also be taken into account. While zeolites have been proposed as materials for the inhibition of transition metal ion permeation in the skin[42] and to inhibit the toxicity of gadolinium in orally ingested MRI contrast agents,[43] long-term contact with blood will undoubtedly lead to some leaching of metal ions. As long as this leaching does not lead to excessive increases in local concentrations above normal in vivo values, then there will be no adverse effects, but this must be studied in greater detail.

Concluding Remarks

Gas storage by porous materials is currently an important topic but tends to concentrate on the storage of gases for energy applications.[44] We have demonstrated that zeolites have great potential as NO storage and release materials for biological and medical applications. Their preparation and loading with NO is relatively facile, the release of NO occurs by simple reaction with water, and the amount of NO released can be tailored by altering both the type and number of metal cations in the structures. Taking into consideration the other advantages of zeolites, such as their low cost and nontoxicity, these materials clearly have great potential as NO storage materials. We have also demonstrated that NO-releasing zeolites inhibit platelet aggregation and adhesion in plasma, a potentially important application of such solids in the prevention of thrombus formation. This work overturns the conventional idea that zeolites can be used only to destroy NO and adds a new delivery system to the armament of researchers developing NO-based therapies. The multiple roles for NO at different concentrations means that the potential for NO-release materials extends far beyond the confines of reduced thrombogenicity of prosthetic grafts, catheters, and extracorporeal blood conduits; it could also encompass other medical uses including antimicrobial adjuncts to prevent infection, for example, in chronically implanted catheters and in wound dressings for enhanced angiogenesis.

Experimental Section

Synthesis of Zeolites. Zeolites Linde type A and ZK-4 possessing the LTA framework topology were synthesized as previously published.[38]

Linde Type A. Sodium hydroxide (2.892 g) was dissolved in distilled water (320 mL) and divided equally into two nalgene bottles. To half

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Table 1. Elemental Analysis (%) of Metal-Exchanged Zeolites with Metals Reported Relative to Al Content

metal	sample a	sample b
Zeolite-A, Formula $Na_aM_bSi_{96}Al_{96}O_{384}$		
Co	0	48
Ni	10	43
Cu	0	96[a]
Mn	8	44
Zeolite-ZK4, Formula $Na_aM_bSi_{114}Al_{78}O_{384}$		
Co	36	21

[a] This sample is ~100% overexchanged.

was added sodium aluminate (33.032 g), into the other half was added sodium metasilicate (61.92 g), and both portions were stirred until dissolved. Then the silicate solution was transferred quickly into the aluminate solution and mixed until homogeneous. This was sealed in a nalgene bottle (500 mL) and heated at 99 °C for 4 h. This was allowed to cool to room temperature, filtered, washed with distilled water until the filtrate is below pH 9, and dried at 100 °C overnight.

ZK-4. Sodium hydroxide solution (3 g, 50 wt %) and sodium aluminate (10.75 g) were added to distilled water (145 mL) and stirred until dissolved. Tetramethylammonium hydroxide solution (25 wt %, 146 g) and silica sol (38 g, AS-30) were stirred for approximately 30 min. Solutions were combined, mixed thoroughly, sealed in a nalgene bottle, and incubated at 25 °C for 24 h. This was then heated at 100 °C for 30 h, cooled to room temperature, filtered, washed with distilled water (1 L), and dried at 100 °C overnight. The zeolites were calcined in flowing oxygen at 600 °C (maintained for 600 min) with a heating rate of 10 °C min^{-1} to remove organic content.

Powder X-ray diffraction data were collected on a STOE STADIP diffractometer operating on monochromated Cu $K\alpha_1$ radiation. Powder data were collected in Debye−Scherrer geometry using 0.5 mm quartz capillaries over a period of 12 h to determine purity of the synthesized phases.

Ion Exchange of Zeolites. Metal ion-exchanged zeolites were prepared as follows. Typically, the zeolite (5 g) was placed in a 0.05 M solution of the metal acetate (400 mL, distilled water) and stirred for 24 h. The products were recovered by filtration, washed with distilled water (400 mL), and dried at 100 °C overnight. Elemental analysis was carried out to determine the amount of metal ions exchanged by an Agilent 7500 Series ICP-MS spectrometer (Table 1).

NO Adsorption/Desorption Isotherms. The adsorption/desorption of nitric oxide gas in microporous materials was measured using a gravimetric adsorption system. A CI instruments microbalance was thermally stabilized to eliminate the effect from external environment. The microbalance has a sensitivity of 0.1 μg and reproducibility of 0.01% of the load. The pressure of adsorption system was monitored by two BOC Edwards Active gauges in the ranges of 1×10^{-8}−1×10^{-2} and 1×10^{-4}−1×10^{3} mbar, respectively. The sample (~130 mg) was initially outgassed at 573 K under 1×10^{-4} mbar, until no further weight loss was observed. The sample temperature was then decreased to 298 K and kept constant by a circulation water bath with temperature accuracy ±0.02 K. The counterbalance temperature was kept the same as that of the sample to minimize the influence of temperature difference on weight readings, and the sample temperature was monitored using a K type of thermocouple, located close to sample bucket (<5 mm). The variation in sample temperature was minimal (<0.1 K) throughout the experiment. NO gas was introduced into the system until the desired pressure was achieved, and the mass uptake of the sample was measured as a function of time until the adsorption equilibrium was achieved. In this manner an adsorption isotherm was collected by incrementally increasing the pressure and noting the mass gain of the sample at equilibrium. Desorption of nitric oxide gas adsorbed in the samples was performed by gradually decreasing the system pressure to a desired value (until 2×10^{-2} mbar).

NO Loading and Release Experiments. The ion-exchanged zeolite (~0.3 g) was dehydrated for 2 h at 300 °C in vacuo (0.5 mmHg). This was cooled to room temperature and exposed to approximately 2 atm of dry NO (99.5%, Air Liquid) for 30 min, evacuated, and exposed to dry argon. Evacuation and exposure to argon occurred three times.

Quantification of NO Release by Chemiluminescence. NO measurements were performed using a Sievers NOA 280i chemiluminescence NO analyzer. The instrument was calibrated by passing air through a zero filter (Sievers, <1 ppb NO) and 89.48 ppm NO gas (Air Products, balance nitrogen). The flow rate was set to 200 mL/min with a cell pressure of 8.5 Torr and an oxygen pressure of 6.1 psig. To measure NO release from zeolite powders, nitrogen gas of known humidity was passed over the powders, the resultant gas was directed into the analyzer, and the concentration of NO in ppm or ppb was recorded.

To measure the release of NO from pressed disks, the samples were sealed in a glass vial with a septum through which the gas was sampled. Nitrogen was supplied to the vial at a rate to maintain atmospheric pressure inside the vial. Distilled water (0.5 mL) was injected to initiate NO release.

Quantification of NO Release Using Electrochemical Methods. A flow of argon (saturated with water vapor, 5 mL min^{-1}) was passed over a known amount of the NO-loaded zeolite. The gas was then bubbled through phosphate-buffered saline solution (pH 7.4, 10 mL) in which a previously calibrated NO electrode (World Precision Instruments, ISO-NO Mark II) was immersed. The concentration of NO was measured over the course of several hours. All experiments were repeated three times and gave reproducible results. A similar technique was used for measurement of NO in PRP samples (1 mL), except the cuvette was incubated in the platelet aggregometer (37 °C) and was constantly stirred (100 rpm). These experiments were repeated 6 times, and results are illustrated as mean ± se of mean.

Storage Experiments. Vials of NO-loaded Co−LTA were evacuated immediately after loading with NO, and the atmosphere was replaced by dry argon. The flasks were then stored for up to 16 weeks, and the measurements of NO release were repeated at regular intervals.

Platelet Aggregation. The zeolite was ground with PTFE in the desired ratio (75% zeolite/25% PTFE). The mixture was then pressed into disks (5 mm diameter, ~20 mg) under 2 tons for 30 s. The disks were then dehydrated and loaded with NO as described for the powder samples.

Venous blood was drawn from the antecubital fossa of six healthy volunteers (aged 20−40 years) into citrated tubes (0.38% final concentration). Volunteers had not taken any medication known to affect platelet aggregation within the last 10 days. Platelet-rich plasma (PRP) was obtained from whole blood by centrifugation (350*g*; 20 min; room temperature). Platelet-poor plasma (PPP) was obtained by further centrifugation of PRP (1200*g*; 5 min; room temperature).

Zeolite/PTFE disks were suspended in stainless steel wire holders below the surface of the PRP in the aggregometer cuvette, ensuring that they did not interfere with the light beam or the mechanical stirring (1000 rpm). After variation of incubation periods (1−120 min), platelet aggregation was initiated by addition of U46619 (8 μM). Aggregation was measured as a change in turbidity (light transmission) of PRP against a PPP blank.

Electron Microscopy. Parallel aggregation experiments were conducted on separate samples prior to gentle washing of the zeolite/PTFE disks in phosphate buffered saline, fixing in 3% glutaraldehyde and osmium tetroxide, dehydration in graded acetone, and critical point drying with carbon dioxide. The surface of the disks was examined using a Phillips 505 scanning electron microscope following gold−palladium alloy sputter coating (SC500 sputter coater, Emscope Laboratories).

Acknowledgment. P.S.W. and R.E.M. thank the Leverhulme Trust for support, and R.E.M. thanks the Royal Society for the Provision of a University Research Fellowship. M.S.C. is supported by a British Heart Foundation Fellowship (FS/2001060), and S.F. and B.X. are supported by an EPSRC project grant (GR/T09712/01). We thank Mr. S. Mitchell for his help with the electron microscopy.

JA0503579