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Moisture-saturated zeolites – A new strategy for releasing nitric oxide†

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To cure ulcers inside the stomach and/or intestines caused by bacterial infection, a new strategy for releasing NO in the digestive system is proposed and verified. Nitric oxide, pre-adsorbed onto zeolite, when added to hydrochloric acid with a low pH (similar to that of gastric juice), releases NO into the solution; the amount of NO_x released can be tuned by altering the structure and modifier species of the zeolite. Various factors, including pH value, the temperature of acid solution, the volume ratio of liquid to solid, and the release time, are investigated to provide help understand the NO_x release mechanism from the moisture-saturated zeolites. We find that nitrite is produced in acid solution, which has advantages for controlled NO release because it provides an active storage mechanism for NO, and this is proved by *in situ* FT-IR experiments. Finally, we were able to simultaneously achieve the release of a large amount of NO and the capturing of nitrosamine by alumina-modified zeolite samples in the gastric juice mimic, affording a potential functional material to reduce the risk of ulcers and cancers in the digestive system.

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1. Introduction

Nitrogen oxides (NO_x) are valuable in biological and pharmacological applications. In particular, N_2O is an attractive agent for the alleviation of pain and anxiety during painful procedures,[1] and NO is essential for life at very low concentrations,[2] serving as a mediator for various vital functions such as vasodilatation,[3] blood pressure regulation and vascular smooth muscle cell relaxation,[4] neurotransmission,[5] immune stimulation,[6] and inhibition of platelet aggregation.[7] Some illnesses, such as hypertension, atherosclerosis, arterial thrombotic disorders and coronary heart disease, are a result of NO deficiency,[8] and consequently, the attractive strategy of delivering exogenous NO has been proposed to overcome this problem.[9]

Zeolites have been of increasing interest as gas storage and hosts for nanotechnology application because of their high adsorption capability.[10] Moreover, the low-toxicity and biocompatibility of zeolites are suitable for NO-delivery materials in biological systems,[11] and consequently, zeolites adopted as NO-delivery materials have attractive potential applications.

Recently, one important potential application of NO-loaded zeolites as an anti-bacterial material has been reported, and the bactericidal effect is definitely associated with the exposure to NO.[12] Bacteria damage the gastric mucosal integrity, inducing ulceration inside the gastric system,[13] and consequently, to suppress the stomach ulcers, NO-delivery materials could be used within the digestive system to release NO. This could provide a means of NO delivery into human body distinct from other methods and afford a way of healing ulcers other than the commonly used antibiotics.

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A problem with this idea is that traditional NO-delivery materials require activation to exclude water prior to adsorption of NO, and they need to be stored in rigorously anhydrous conditions, because they release NO upon contact with moisture – something that would no doubt hamper their application as NO-delivery materials. Thus, here we try to employ moisture-saturated zeolites (with their greater ease of storage) in the NO-delivery process and to release biologically relevant amounts of NO through a proton-induced mechanism. As a part of this, we have also investigated the adsorption and acid-induced desorption of NO in moisture-saturated zeolites.

In order to explore the possibility of releasing NO in the human stomach, a series of desorption experiments are performed in acid solution (pH = 1.2), and the influence of the temperature of solution, volume ratio of liquid to solid, pH value and release time are studied. Also, various types of zeolites are employed to store and release NO through temperature-programmed desorption (TPD) and acid treatment. Another purpose of this investigation is to explore the possibility of using the zeolite, once it releases the NO in the acidic solution, to adsorb carcinogenic compounds like nitrosamines, because these are widespread carcinogens that (even in trace amounts) can induce tumors and cancers in almost all organs of experimental animals.[14] Our alumina-modified zeolite samples thus have potential use in gastric juices to release NO and capture nitrosamines in a one-step process.

2. Experimental

2.1 Preparation of materials

Zeolites NaA, NaY and NaZSM-5 with a Si/Al ratio of 12.5 are commercially available powders,[15] while Hβ zeolite was provided by BASF, and Naβ zeolite was obtained by ion exchange from Hβ. HUSY (Si/Al = 2.87) zeolite, a FAU sample dealuminated by steam treatment, was supplied by Beijing Research Institute of Petroleum Processing (China). $Al(NO_3)_3{\cdot}9H_2O$ was purchased from Shanghai Zhenxin

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(China), and alumina was derived from the $Al(NO_3)_3{\cdot}9H_2O$ calcined at 773 K; other reagents with AR grade were used as received. *N*-Nitrosopyrrolidine (NPYR) was bought from Sigma. A mixture of NO/N_2 (v/v, 1/49) was prepared as the adsorbate, and nitrogen with 99.99% purity was used as the carrier gas in both NO adsorption and desorption experiments. Alumina-modified NaZSM-5 was prepared through an impregnation method. Typically, the calculated amount of $Al(NO_3)_3{\cdot}9H_2O$ was dissolved in 10 g distilled water, and 1.25 g of NaZSM-5 was then added into the solution and stirred at 303 K for 6 h, followed by evaporation at 353 K. Subsequently the sample was dried at 373 K overnight and calcined in an air flow at 773 K for 6 h, giving a sample denoted as *n*AZ where *n* represents the weight percent of alumina incorporated. All samples were conditioned at ambient temperature at 79% relative humidity prior to use.

500 ml of gastric juice mimic with a pH value of 1.2 was prepared by adding 0.35 g NaCl, 0.5 g glycine and 31.6 ml of 1 M HCl to distilled water. A quantitative amount of NPYR was then added.[14] The solution was kept at 277 K in a refrigerator to avoid volatilization of NPYR.

2.2 Characterization

Nitrogen adsorption and desorption isotherms of samples were measured at 77 K on a Micromeritics ASAP 2020 volumetric adsorption analyzer.

Instantaneous adsorption of NO in a gas stream was performed at 310 K. 60 mg of zeolite (20–40 mesh) were added to a Pryrex glass-reactor as an adsorption bed, and the $NO–N_2$ mixture with 0.8 μmol of NO was injected every 1 min into the nitrogen flow,[16] with the injection number of NO being fixed to 30. Subsequently, the adsorbent was purged by the N_2 flow with a rate of 30 ml min^{-1} for 1 h. To detect the desorbed amount of NO in the TPD experiment, about 25 mg of zeolite pre-adsorbed with NO was heated from 310 K to 773 K at 10 K min^{-1} and maintained at this temperature for 1 h, with the desorbed NO being collected and detected every 10 min. A CrO_3 tube was used to oxidize any reduced NO_2 prior to trapping, and the total desorbed amount of NO_2 was obtained by adding all data collected during the experiment. The other portion of zeolite pre-adsorbed with NO (30 mg) was immediately put in 15 ml hydrochloric acid solution with pH = 1.2. The experiment was carried out at 310 K for 2 h, and the desorbed amount of NO was detected every 30 min. The nitrogen oxides were purified by successive NaOH traps and then oxidized to NO_2, which was determined by a photometric method.[17] Although NO_2 released from the zeolite was removed by NaOH and thus NO became predominant in the desorbed gases, the finally detected nitrogen oxides are still denoted as NO_x. The acid solution was centrifuged at 3000 rpm for 15 min and then 10 ml of solution was added to 40 ml sulfanilamide and *N*-1-naphthylethylenediamine dihydrochloride solution to determine the concentration of nitrite.[15,18] To explore the ability of the sample in trapping nitrosamine and releasing NO in a combined process, the sample of zeolite pre-adsorbed with NO (30 mg) was added to 15 ml of the gastric juice mimic, and the whole experiment was carried out at 310 K for 2 h, with the amount of desorbed NO being detected every 30 min. Finally, the residual mixture was centrifuged at 3000 rpm for 15 min to separate the solution and the solid. 10 ml of the clean solution was extracted by 60 ml dichloromethane and concentrated to a final volume of 25 ml. The residual concentration of NPYR in the gastric juice mimic was determined by an improved spectrophotometric method.[14]

To investigate the storage of NO in zeolites, NaA and NaY were used to continually adsorb NO for 1 h and then purged for 1 h by a nitrogen flow to remove physically adsorbed NO. These zeolites pre-adsorbed with NO were stored in open vials so that both the temperature and the atmospheric humidity of ambient environment would directly affect the NO-storage process. Subsequently, the release of NO was tested every week by a TPD experiment, and in addition the zeolite pre-adsorbed with NO that had been stored for 5 weeks, named zeolite-5, was added to the acid solution to detect the release of NO.

In situ FT-IR spectra of NO adsorbed on zeolites was recorded on a Nicolet 5700 FT-IR spectrometer. A sample disc with an area density of 20 mg cm^{-2} was purged with N_2 for 5 min at 310 K prior to taking a background spectrum, and then exposed to a stream of $NO–N_2$ (1.98% of NO by volume) at a rate of 5.0 ml min^{-1} for 30 min, followed by a purge with N_2 for 60 min. All spectra were taken at 310 K and the corresponding background subtracted from them.

3. Results and discussion

3.1 Heat- and acid-induced release of NO_x from zeolites

Fig. 1A and B show NO_x TPD curves of NaA and NaY pre-adsorbed with NO at 310 K or 298 K. These zeolites were conditioned at 79% relative humidity prior to adsorption of NO, therefore, both the temperature of NO adsorption and the flow rate of carrier gas influence the content and distribution of moisture in the zeolite, affecting the subsequent adsorption of NO, because water in the zeolite will hinder adsorption of NO.[7] More water molecules were removed from the zeolite when NO was adsorbed at 310 K, and consequently a larger amount of NO_x was detected in the corresponding NO_x TPD experiments (Table 1). Zeolite NaA had a large desorption of NO_x in the range 473–773 K, whilst several peaks appeared for NaY (Fig. 1B). Moreover, the NaY pre-adsorbed with NO at 298 K could desorb more NO_x (5.8 μmol g^{-1}) than that treated at 310 K (4.4 μmol g^{-1}) in the TPD test below 593 K (Fig. 1B).

Table 1 lists the release of NO_x from NaA and NaY in acid solution for 2 h. About 13.5 μmol g^{-1} of NO_x were detected on NaA pre-adsorbed with NO at 310 K, more than that treated at 298 K (8.3 μmol g^{-1}), in agreement with the TPD results (Fig. 1A). Surprisingly, less NO_x was released for the NaY pre-adsorbed with NO at 310 K in comparison with that treated at 298 K. There are two possible reasons for the larger amount of NO_x released from NaA than NaY in acidic solution – one is the high cation content of NaA, and the other is its more easily destroyed structure. The NO_x released in acid solution may be the nitrous surface species adsorbed on the weak sites of zeolite, *i.e.* that desorbed at low temperature

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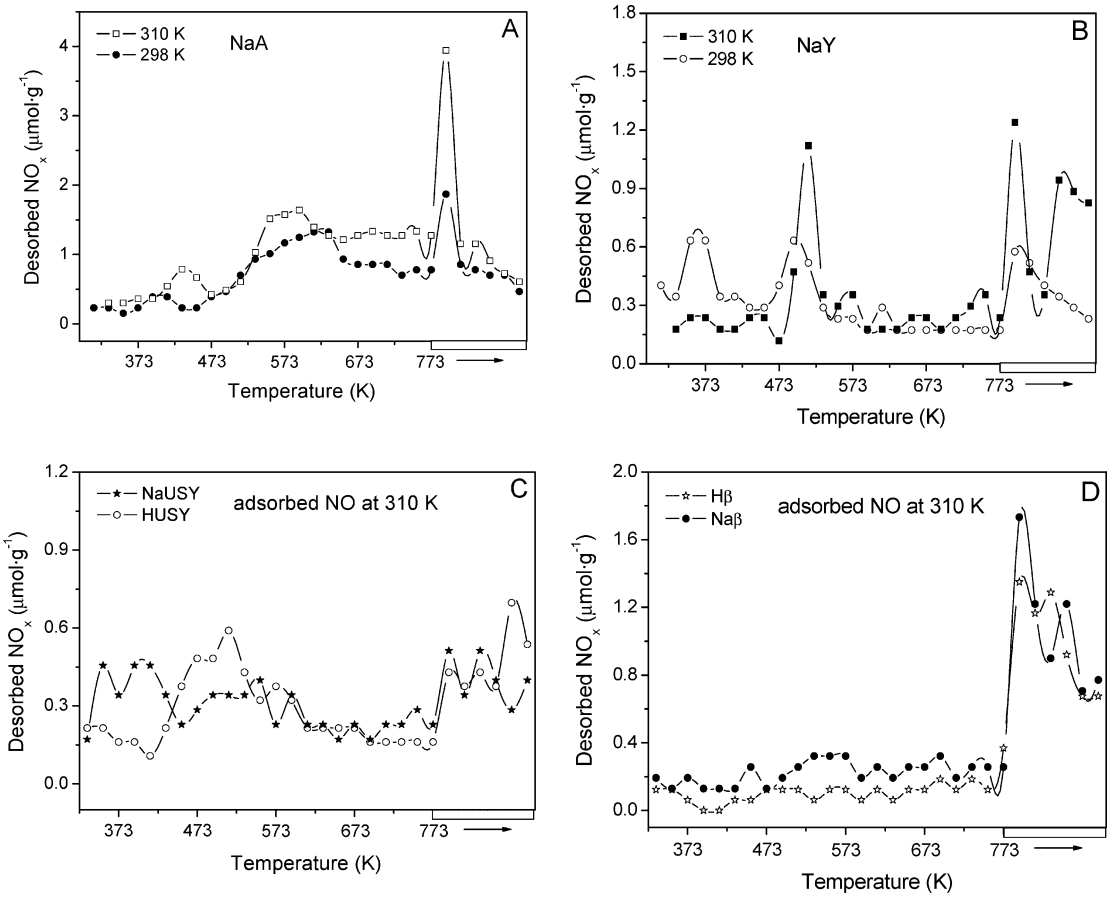

Fig. 1 NO_x TPD curves on the zeolites pre-adsorbed with NO at different temperatures.

Table 1 The results of heat- and acid-induced release of NO_x on NaA and NaY[a]

Sample		NaA		NaY			
Adsorption temperature (K)		310		298	310	298	
NO_x desorbed in NO TPD (μmol g^{-1}) (*A*)		30.75		21.83	11.20	9.78	
pH value		1.2	7.0	1.2	1.2	1.2	1.0
NO_x released in acid solution (μmol g^{-1})	30 min	4.35	0.20	3.18	0.28	0.33	0.49
	60 min	4.47	0.20	2.57	0.11	0.33	0.33
	90 min	2.90	0.20	1.56	0.11	0.22	0.16
	120 min	1.81	0.13	1.00	0.11	0.27	0.16
	Total (*B*)	13.53	0.73	8.31	0.62	1.15	1.15
Desorption proportion (%) (*B*/*A*)		44.00	2.37	38.07	5.54	11.76	11.76
[NO_2^-] (μmol g^{-1})[b]		7.30	12.96	10.46	0.50	3.19	0.54

[a] Acid-induced release was carried out at 310 K, and the *V*/*m* ratio was 500 cm^3 g^{-1}. [b] This represents the amount of NO_2^- formed in acid solution when zeolite pre-adsorbed with NO was placed in the solution for 2 h.

in the TPD process. Therefore, the NaA pre-adsorbed with NO at 298 K was added to the acid solution with pH = 1.2 for 2 h, and then it was separated from solution and examined by TPD to explore whether any NO_x remained. 1.63 μmol g^{-1} of NO_x was detected on the sample, indicating the existence of some nitrous surface species on the zeolite leached by acid solution. Consequently, we speculate that these NO_x species adsorbed on weak sites are the first to be released in acid solution. Following this, a portion of NO_x is released from the destroyed structure of the zeolite. These residual NO_x species on NaA were adsorbed on strong sites, so that they are not released by exposure to acid solution for 2 h.

Fig. 2A illustrates the release of NO_x from NaA pre-adsorbed with NO at 310 K in acid solution for 4.5 h. An initial release of NO_x was observed, followed by a gradual drop in the rate of release. This initial abrupt release originated from the desorption from weak sites and the destroyed structure of NaA. Finally, 15.9 μmol g^{-1} of NO_x was released in acid solution, equaling about half of that desorbed in the NO_x TPD process. Almost all of the NaA dissolved in acid solution because of its high aluminium content, and only about 5.1 wt% of the zeolite remained. In addition to the nitrite and nitrate in acid solution, some other nitrous species were held on the strong adsorption sites of the zeolite, as

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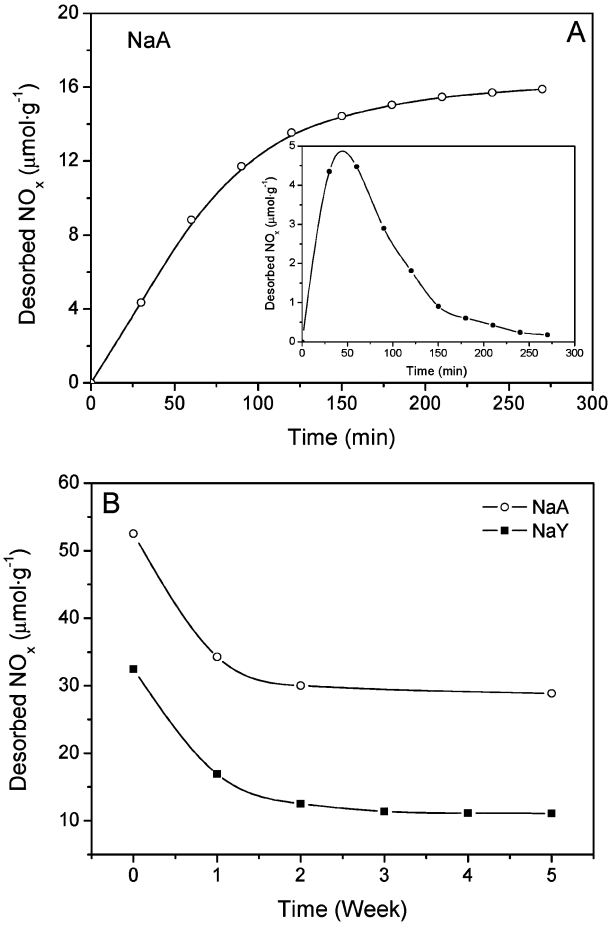

Fig. 2 (A) Release of NO_x from NaA in acidic solution with pH = 1.2 at 310 K (inset: desorbed amount of NO_x at regular intervals). (B) Thermal release of NO_x in TPD test from the zeolite pre-adsorbed with NO and stored for different periods under ambient conditions.

previously mentioned. NO_2^- is an active storage form of NO and also has physiologically important functions in the human body,[19] so that nitrite dissolved in acid solution might have an effect on the storage of NO.

Table S1† shows the influence of pH value and volume of acid solution on the release of NO_x from NaY pre-adsorbed with NO at 310 K. The volume ratio of solution to solid did not seem to be vital in NO_x release because the solution itself was sufficient to destroy the zeolite structure and to release NO_x once the V/m ratio reached 333 cm^3 g^{-1}. However, pH value was quite important for NO_x release in acid solution. Larger amounts of NO_x were detected on NaY in the acid solution with pH = 1.0 than that with pH = 1.2, because the high concentration of protons in the solution led to NO_x desorption from weak sites and easily destroyed the zeolite framework to release NO_x. Less NO_x was detected after the sample was placed in the acid solution for 1 h. However, for the sample adsorbed NO at 298 K, the amount of NO_x detected in the acid solution with pH = 1.0 and 1.2 were equal (Table 1), implying that a pH value of 1.2 was low enough for the release of nitrogen oxides.

Table 2 displays the temperature of the acid solution on the release of NO_x from zeolite. Normal human body temperature (310 K) and room temperature (298 K) were adopted in tests for the potential application of the zeolites. All samples released a larger amount of NO_x in the acid solution at 310 K than at 298 K, whether the adsorption of NO was carried out at 310 K or 298 K. A higher solution temperature accelerated the corrosion of zeolite framework, damaging the structure, and less of the sample survived in the solution at 310 K for 2 h, leading to the release of more NO_x in solution. In addition, a corresponding relationship between the amount of NO_x released and nitrite concentration emerged in the acid solution for all zeolites, and higher concentration of nitrites were present at 298 K than at 310 K, implying that a complex reaction occurred in the desorption process, and that nitrite might be the predominant nitrous surface species for NO release. Furthermore, lower amounts of NO_x and higher concentrations of nitrite were detected on NaA that adsorbed NO at 310 K in the neutral solution than in the solution with pH = 1.2 (Table 1). This is attributed to the fact that sodium nitrite is a very weak nitric oxide releaser at physiological pH, and nitric oxide formation resulting from the decomposition of free nitrous acid is strongly pH dependent.[20]

3.2 *In situ* FT-IR investigation into the release of NO_x from zeolites

Fig. 3 illustrates the *in situ* FT-IR spectrum of NO adsorbed on NaA, NaY and NaZSM-5 to investigate the nitrous surface species formed on zeolites. The bands at 1905 and 1849 cm^{-1} formed quickly upon contact of NaA with NO (Fig. 3A), and they were assigned to NO and $(NO)_2$, respectively.[21] These bands were unchanged for a short period, indicating the immediate saturation of the sample with gaseous NO.

Table 2 The release of NO_x from zeolites in acid solution[a]

Sample[b]		NaA		NaY		NaZSM-5	
NO_x desorbed in NO TPD (μmol g^{-1}) (*A*)		21.83		11.20		9.87	
Desorption temperature (K)		310	298	310	298	310	298
NO_x desorbed in acid solution (μmol g^{-1})	30 min	3.18	0.75	0.28	0.34	2.24	0.78
	60 min	2.57	0.75	0.11	0.07	0.93	0.48
	90 min	1.56	0.80	0.11	0.07	0.44	0.42
	120 min	1.00	0.86	0.11	0.07	0.55	0.36
	Total (*B*)	8.31	3.15	0.62	0.55	4.16	2.04
Desorption proportion (%) (*B*/*A*)		38.07	14.44	5.54	4.90	42.12	20.67
$[NO_2^-]$ (μmol g^{-1})[c]		2.53	10.46	0.50	3.19	2.42	5.70

[a] The pH value of acid solution was 1.2, and the V/m ratio was 500 cm^3 g^{-1}. [b] NaA adsorbed NO at 298 K, but NaY and NaZSM-5 adsorbed at 310 K. [c] This represents the amount of NO_2^- formed in acid solution when the zeolite pre-adsorbed with NO was placed in the solution for 2 h.

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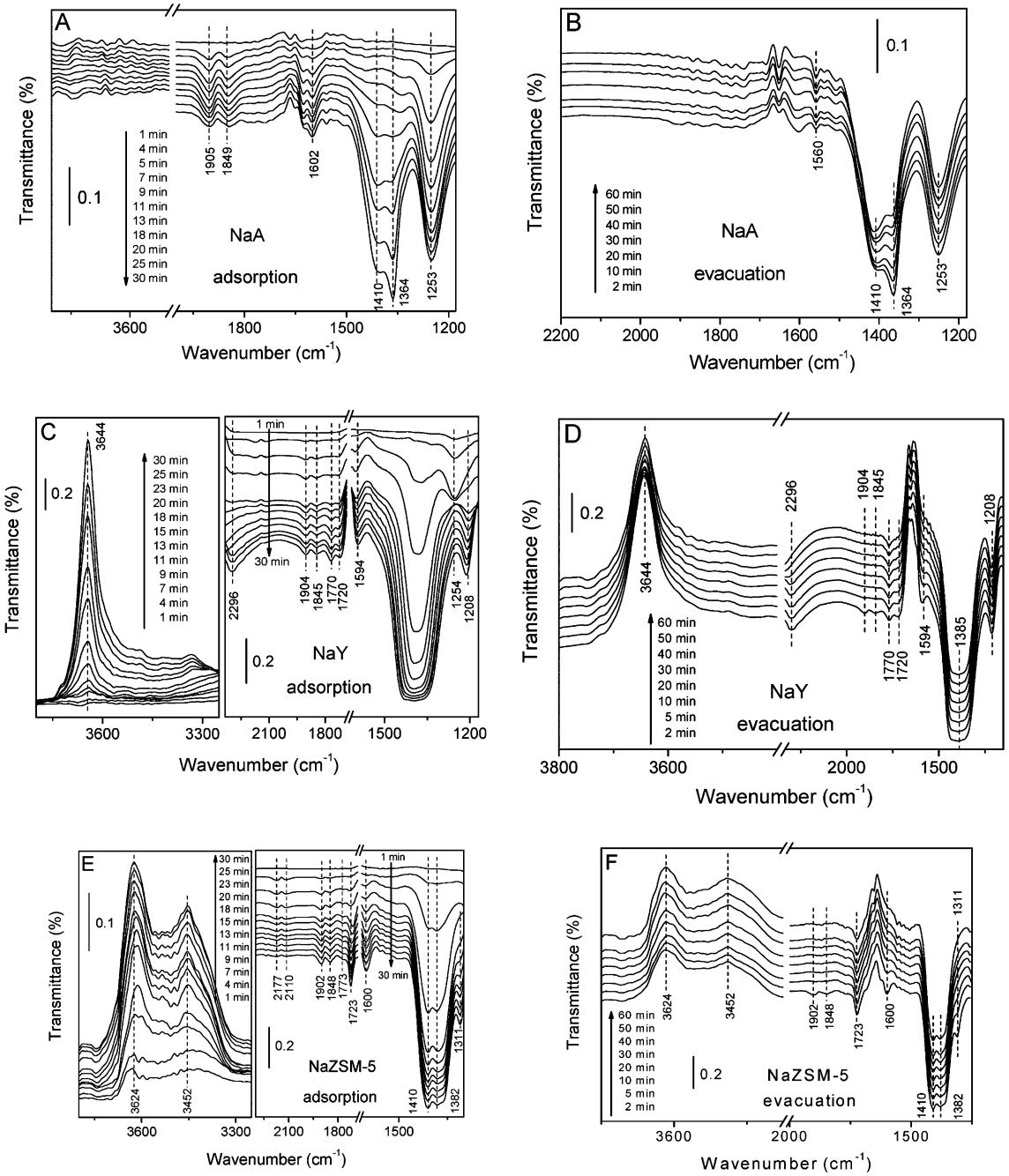

Fig. 3 *In situ* FT-IR spectra of NO (1.98% by volume) adsorbed on (A, C, E) and released from (B, D, F) NaA, NaY and NaZSM-5 at 310 K.

The bands at 1626, 1602 and 1560 cm^{-1} were assigned to bridging, bidendate and monodentate nitrates, respectively.[22] Concomitantly, a doublet with transmittance maxima at 1410 and 1364 cm^{-1}, which can be attributed to the υ_3 mode splitting of surface nitrate adsorbed on Na^+ sites,[23] appeared on the sample. The band at 1253 cm^{-1} was associated with adsorbed bidentate nitrite,[23,24] and further exposure of NaA to NO strengthened the bands of nitrates and nitrites. After the sample was purged with N_2, however, the bands of NO and $(NO)_2$ (1905 and 1849 cm^{-1}) disappeared quickly (Fig. 3A), because NO was weakly adsorbed, whilst the bands of nitrates and nitrites remained, reflecting their stability on zeolite.

Unlike NaA, NaY had a negative band at 3644 cm^{-1} in its spectrum, and its intensity grew as the adsorption of NO proceeded (Fig. 3C), which was attributed to the desorption of water from the sample.[23] In addition to the bands of NO and $(NO)_2$ that emerged at 1904, 1845 and 1720 cm^{-1} on NaY,[21]a band at 2296 cm^{-1} due to N_2O was also observed.[25] Some nitrate bands emerged around 1770, 1594, 1385 and 1208 cm^{-1} in the spectrum of NaY;[23] in particular, the 1208 cm^{-1} band might result from a nitrite species (Fig. 3C). When the sample was exposed to NO for 4 min, a band at 1254 cm^{-1} appeared for NaY ascribed to bidentate nitrites formed with the participation of Na^+ ions.[25,26] Its intensity increased at first, but soon declined and finally disappeared; meanwhile a nitrate

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band at 1208 cm^{-1} formed and became larger (Fig. 3C). The pre-adsorbed water and residual oxygen molecules in the zeolite would interact with NO to form nitrites and nitrates, promoting the transformation of nitrite to nitrate species.[25] Purging the sample at 310 K for 30 min removed the bands of NO, $(NO)_2$ and N_2O because of their instability, but the nitrate bands remained even when the purge continued for 1 h, implying the stability of nitrates on the sample (Fig. 3D).

The stability of nitrous species formed on the surface of the zeolite appears to be as follows: Na^+-nitrate > Al-nitrates > nitrites, consistent with the literature.[23] Two differences were observed, the first was that larger amounts of NO_x were detected in the TPD process than in the acid solution (Tables 1 and 2), because nitrates formed on zeolite could be reduced to NO_x at elevated temperature,[23] but not decomposed in acid solution. Secondly, nitrite species were absent on NaY but observed on NaA, resulting in more NO_x being desorbed from NaA than NaY (Table 2).

Fig. 3E shows the results of an *in situ* FT-IR experiment performed on NaZSM-5 to investigate nitrous surface species. Removal of pre-adsorbed water from NaZSM-5 caused the negative bands at 3624, 3452 and 1642 cm^{-1}. A series of bands appeared in the spectrum at 1723, 1600, 1410, 1382 and 1311 cm^{-1} when the adsorption was prolonged, indicating the capacity of NaZSM-5 to produce nitrate species similar to NaA and NaY.[27] In addition, bands of NO and $(NO)_2$ were observed for NaZSM-5 at 1902 and 1848 cm^{-1}, and the bands at 2177 and 2110 cm^{-1} were assigned to N_2O and NO^+, respectively.[22,28] Purging the sample at 310 K removed the bands of N_2O (2177 cm^{-1}), NO^+ (2110 cm^{-1}), NO (1902 cm^{-1}) and (symmetric) $(NO)_2$ (1848 cm^{-1}). Concomitantly, two bands due to N_2O_3[26] were observed at 1868 and 1558 cm^{-1}, while a band due to N_2O_4 appeared at 1773 cm^{-1} even the sample was purged for 60 min.[29] Bidentate and unidentate nitrates (1600 and 1311 cm^{-1}) on NaZSM-5 disappeared upon purging for 5 min, indicating their low stability. The main nitrous surface species on NaZSM-5 were nitrates and nitrogen oxides, and these remained with increasing time (Fig. 3F). Consequently, water and residual oxygen molecules in the zeolite led the NO adsorbed to be converted to nitrites, nitrates and/or NO_x species, resulting in different release property of NO-delivery materials in acid solution (Table 2). Clearly, these samples possess NO_x and/or nitrite species that can be reduced to NO, and release a large amount of NO_x in acid solution, as demonstrated in Table 2. In addition, nitrite is inferred as an active storage mechanism for NO, because a large amount of NO_x was detected on NaA in acid solution but no obvious NO_x peaks were found in the FT-IR spectra (Fig. 3B). To confirm this, 100 μl of 5.03 mM $NaNO_2$ solution was added to 15 ml acid solution to detect the relationship between HNO_2 decomposition and release of NO_x at different temperatures and pH values. It was apparent that a larger amount of NO was released at relatively low pH or high temperatures resulting from decomposition of HNO_2 (Table S2†). Thus, the amount of NO released is related to the desorbed nitrite in acid solution. Actually, zeolite itself also has the ability to transform nitrite back into NO in the physiological solution (pH = 7.4),[30] which provides a possibility for using the samples with pre-adsorbed NO in other digestive systems with low proton concentrations.

3.3 The effect of storage, cation and modification of zeolite on the release of NO_x and adsorption of NPYR

In practice, pre-adsorbed NO materials need to release NO after storage for various periods, and therefore Fig. S1 examines the release of NO_x in the TPD test from the NaY pre-adsorbed with NO and stored under ambient conditions for up to 5 weeks. Large amounts of NO_x were detected at temperatures below 623 K when pre-adsorbed NaY was tested immediately. As the storage time was prolonged, such desorption became weak, indicating that the weakly adsorbed NO_x was replaced by moisture and/or other species present in the atmosphere. The released amount of NO_x from NaY became constant after two weeks storage. Finally, about 11 μmol g^{-1} of NO_x was desorbed from the zeolite stored for 5 weeks (Fig. 2B).

Storage of zeolite pre-adsorbed with NO also had an influence on the acid-induced release of NO_x. For NaA, thermal release of NO_x decreased from 52.52 to 28.28 μmol g^{-1}, while the acid-induced release amount varied from 19.40 to 4.28 μmol g^{-1} (Table 3). Nitrite was the primary species on NaA, and it was easily reduced to NO in acidic microenvironment. Nonetheless, acidic impurities such as atmospheric CO_2 adsorbed on the moisture-saturated zeolite would form an acidic microenvironment in the sample, which would easily induce a portion of the nitrite to reduce to NO (Table S2†), resulting in the lower release ability. In contrast to the thermal release of NO_x from NaY, which fell from 32.47 to 11.09 μmol g^{-1} (Fig. 2B), the acid-induced release of NO_x

Table 3 The results of the NO_x TPD and NO_x desorption in acid solution experiments[a] on various samples

Sample	NO_x desorbed in TPD (μmol g^{-1}) (*A*)	NO_x released in acid solution (μmol g^{-1})					Desorption proportion (%) (*B*/*A*)	$[NO_2^-]$ (μmol g^{-1})[b]
		30 min	60 min	90 min	120 min	Total (*B*)		
NaA-fresh	52.52	9.48	4.65	3.18	2.10	19.40	36.94	8.76
NaA-5	28.88	1.25	1.11	0.96	0.96	4.28	14.82	7.97
NaY-fresh	32.47	1.42	0.77	0.66	0.27	3.12	9.61	0.71
NaY-5	11.09	1.19	1.95	0.43	1.84	5.41	48.84	1.64
HUSY	8.96	0.44	0.33	0.28	0.22	1.27	14.17	0.54
NaUSY	9.17	0.26	0.26	0.19	0.13	0.84	9.17	0.55
Hβ	8.65	0.12	0.06	0.18	0.06	0.42	4.82	0.84
Naβ	11.68	0.06	0.06	0.13	0.06	0.32	2.75	0.35

[a] The acid-induced release of NO_x was carried out at 310 K and the pH value of solution was 1.2. [b] This represents the amount of NO_2^- formed in acid solution when the pre-adsorbed sample was placed in the solution for 2 h.

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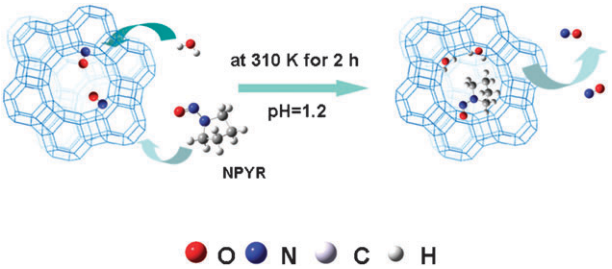
changed from 3.12 to 5.41 μmol g^{-1} in solution (Table 3). The nitrous species in NaY no doubt interacted with moisture and other impurities during the storage, which might promote the acid-induced release of NO_x. Based on the results mentioned above, it seems beneficial to condition NaY at 79% relative humidity for storage of NO under ambient conditions, which provides a new strategy for gas storage.[31]

The cations of zeolite also affect the adsorption and release of NO. Different property and distribution of cation certainly influence release of NO_x because of the various cation radii and electronegativities. Fig. 1C and D show the promoting effect of Na^+ on the desorption of NO_x in the TPD process, since Na^+ ions in the zeolites is favorable for NO_x storage under experimental conditions.[27] Although larger amounts of NO_x are desorbed from NaUSY and Naβ samples than from their acidic analogues in the TPD test, less NO_x was released from them in acid solution (Table 3), which resulted from the affinity of the cation towards nitrogen oxide.[27] No zeolite cation could suppress desorption of nitrogen oxide at high temperature, but some cations in the zeolite were able to hold NO_x in acidic solution provided the structure of zeolite was retained. In addition, ion exchange of zeolite certainly occurred in acid solution because of the high proton concentration, which would change the affinity of NO_x for the cation and then raise the pH of acid solution, affecting the release of NO_x (Table 3). Thermal desorption and acid-induced release of NO_x from zeolite characterize the different features of the zeolite: the former mirrors the absolute capability of zeolite in adsorbing NO, while the latter reflects the practical performance of NO-delivery in potential biological applications.

To further examine how the metal cation of the zeolite affects the adsorption and release of NO, two samples of USY, HUSY and NaUSY, were employed, and their performances are illustrated in Table 3 and Fig. 1C. Different from the unique aluminium state of NaY, USY sample had three types of Al species, tetrahedrally coordinated Al in the framework, and octahedrally coordinated and other coordinated extra-framework Al species with greater accessibility to NO. Although USY had the similar BET surface area and Al content to NaY, less NO was desorbed in the TPD process, but more was released in acid solution in comparison with NaY (Table 3), implying the special function of extra-framework Al species on the acid-induced release of NO_x. In addition, compared to NaY, a larger part of the USY sample remained in acid solution for 2 h because of the lower Al content in the framework of USY, which is beneficial for corrosion resistance. Subsequently, both NaY and NaUSY pre-adsorbed with NO were placed in gastric juice mimic to investigate their capability for releasing NO and capturing nitrosamines (Scheme 1). The complex composition of the gastric juice mimic affects the release property of zeolites, and therefore the amount of NO_x released differed from that in hydrochloric acid solution (Table 4). Nonetheless, more NO_x was released from NaUSY than from NaY, confirming the contribution of extra-framework alumina in releasing NO_x. Similarly, alumina pre-adsorbed with NO released about 4.78 μmol g^{-1} of NO_x in the gastric juice mimic, more than NaUSY and NaY. If we calculate the release ability of samples (Q_r) as the amount of NO_x released divided by their surface area, alumina was the



Scheme 1 Releasing NO and capturing nitrosamines by zeolite in a gastric juice mimic.

winner, as shown in Table 4. However, smaller amounts of NPYR were removed by alumina because of its small surface area.[32] NaUSY has a lower Al-content than NaY, and its partially collapsed channels formed during the dealumination process were unfavorable for adsorption of NPYR,[14] and consequently smaller amounts of NPYR was captured in comparison with NaY.

On the basis of results mentioned above, both the extra-framework alumina and unobstructed pore-structure of zeolite seem to be helpful for its release of NO_x and adsorption of nitrosamine. Therefore, we tried to modify NaZSM-5 with alumina because it is relatively stable in acid solution and alumina has low toxicity in organisms and is highly efficient at releasing NO_x. Fig. 4A and B show the NO_x TPD curves on *n*AZ samples, and it is clear that more NO_x desorbed at higher temperature as more alumina modifiers were loaded on the zeolite, except for 10AZ, which released a smaller amount of NO_x (23.53 μmol g^{-1}) than alumina (30.64 μmol g^{-1}) in the TPD process. Unlike alumina, for which the desorption of NO_x occurred around 453 K, the *n*AZ samples hold the NO_x more tightly so that the peak of desorption shifts to higher temperature (Fig. 4A and B), indicating that the composite strengthened the interaction with NO and redistributed the nitrous surface species. A different trend was observed in the acid-induced release of NO_x in the gastric juice mimic, in which the largest amount of NO_x was released from the 2.5AZ sample. Apart from the advantage in expanding the absolute storage ability of NO in the vessel (Fig. 4C), modification of ZSM-5 with alumina has another benefit, namely the majority of NaZSM-5 remains in acid solution because of the lower aluminium content in its framework in comparison with NaY or NaA. This is helpful for the subsequent adsorption of carcinogens such as nitrosamines in human gastric juice. In fact, three samples – 1AZ, 2.5AZ and 5AZ – adsorbed more NPYR than the parent NaZSM-5 (Table 4). Modification of zeolite with a small amount of alumina through impregnation did not damage the structure of the host, and the hydrophobic feature of alumina was helpful for adsorbing NPYR in aqueous solution.[14] However, introducing more alumina in NaZSM-5 reduced the surface area and pore volume in the 10AZ sample, so that its performance became poorer (Fig. 4D). To exclude the influence of surface area, we also calculated the Q_e values of adsorbents (Table 4), from which it is clear that *n*AZ composites had better performances than NaZSM-5, or indeed NaY. Together with the results of NO_x release, it is clear that alumina-coated NaZSM-5 is the most promising candidate for release of NO_x and capture of NPYR in gastric juice mimics.

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Table 4 Surface properties of porous silica samples and their capability for releasing NO_x and adsorbing *N*-nitrosopyrrolidine (NPYR)[a]

Sample	NaY	NaUSY	NaZSM-5	1AZ	2.5AZ	5AZ	10AZ	Al_2O_3[b]
S_{BET} ($m^2\ g^{-1}$)	729	730	267	237	233	228	224	15
S_{micro} ($m^2\ g^{-1}$)	684	649	243	206	204	197	193	6.6
V_p ($cm^3\ g^{-1}$)	0.36	0.41	0.13	0.12	0.12	0.12	0.11	0.028
V_{micro} ($cm^3\ g^{-1}$)	0.32	0.30	0.11	0.10	0.10	0.09	0.09	0.003
Q_r ($\mu mol\ g^{-1}$)[c]	0.55	1.92	5.73	4.95	6.24	5.85	5.06	4.78
Q_r ($nmol\ m^{-2}$)[c]	0.75	2.63	21.46	20.89	26.78	25.66	22.59	307.20
Q_e ($mg\ g^{-1}$)[c]	0.48	0.43	0.30	0.42	0.50	0.37	0.26	0.01
Q_e ($\mu g\ m^{-2}$)[c]	0.66	0.59	1.12	1.77	2.15	1.62	1.16	0.64

[a] The acid-induced release of NO_x was carried out at 310 K. The concentration of NPYR in gastric juice mimic was 1.72 mg L^{-1}, and the pH value was 1.2. [b] This sample was obtained by calcining $Al(NO_3)_3{\cdot}9H_2O$ at 773 K. [c] Q_r and Q_e represent the NO_x released and NPYR adsorbed in solution within 2 h, respectively.

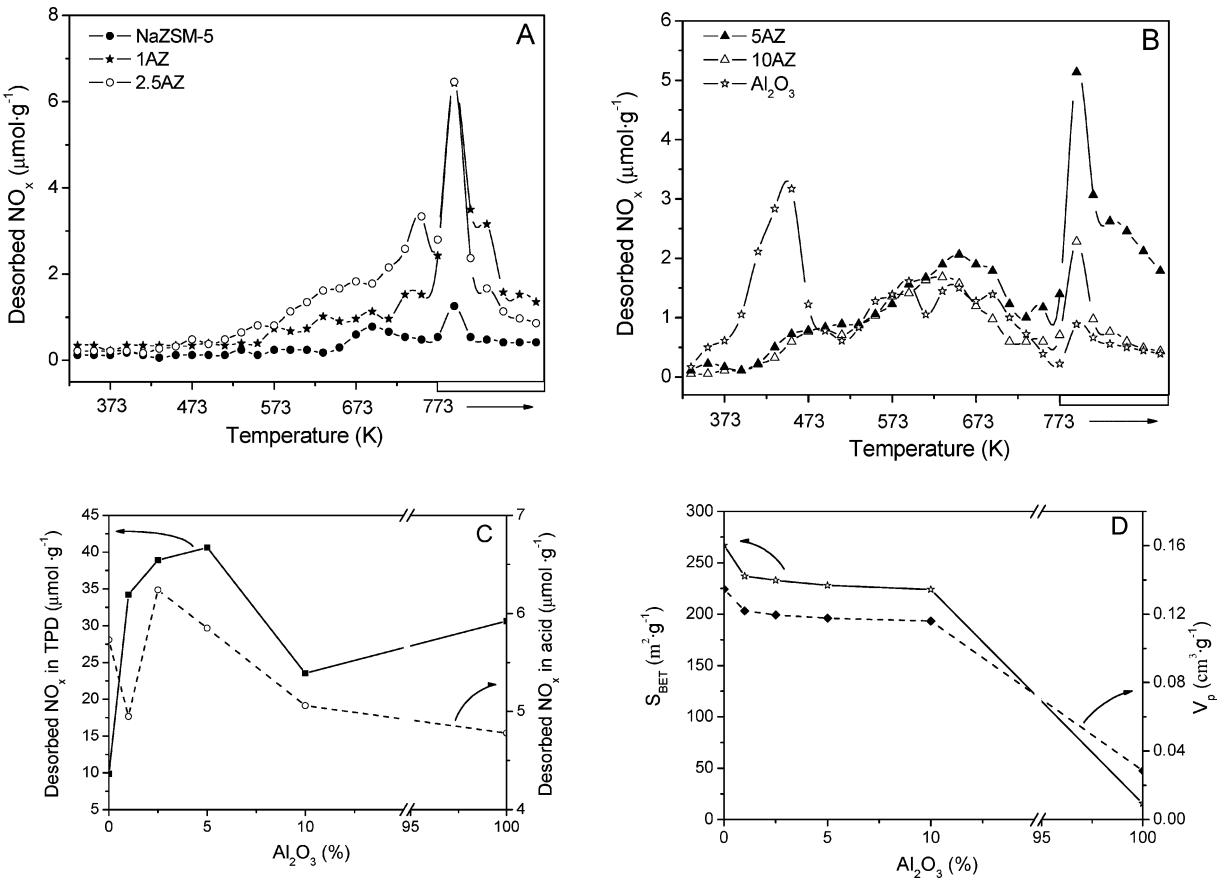

Fig. 4 (A, B) NO_x TPD curves of NaZSM-5, alumina and *n*AZ samples. The relationships between the alumina content of the samples and (C) the amount of desorbed NO_x and (D) structural parameters.

4. Conclusion

From our studies, we conclude the following:

(1) It is feasible to use moisture-saturated zeolites to adsorb NO and release NO_x in acidic solution. Large amounts of NO_x are still detected on zeolites stored for 5 weeks.

(2) Low pH value and high desorption temperature of solution are beneficial for the acid-induced release of NO_x from the zeolites. The content, type and distribution of cations in the zeolite play an important role in NO adsorption and release.

(3) It is also feasible to release NO_x and to adsorb nitrosamines in acid solution such as a gastric juice mimic, properties that can be tuned by choosing the structure, counterion and modifier species of the zeolite.

(4) The main forms of nitrous surface species in zeolite are nitrites and nitrates as well as nitrogen oxides, among which nitrite acts as an active storage species for NO in acid solution, providing the possibility of NO-delivery in acidic gastric juice mimics. This new strategy provides prospects for NO-delivery in practical medical applications, avoiding thrombus formation and bacterial infections in the digestive system.

This study is a preliminary approach to develop the functional zeolites for release of nitrogen oxide in the digestive

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system. Further investigations are required to clearly identify detailed characteristics of the processes involved.

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