Microporous and Mesoporous Materials 229 (2016) 83–89

Contents lists available at ScienceDirect

Microporous and Mesoporous Materials

journal homepage: www.elsevier.com/locate/micromeso

Storage and delivery of nitric oxide by microporous titanosilicate ETS-10 and Al and Ga substituted analogues

Moisés L. Pinto [a, *], Ana Cristina Fernandes [b], Fernando Antunes [b], João Pires [b], João Rocha [c]

[a] CERENA, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal
[b] Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal
[c] Department of Chemistry, CICECO-Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form
15 April 2016
Accepted 15 April 2016
Available online 19 April 2016

Keywords:
Nitric oxide
Titanosilicate
NO release
Toxicity
Adsorption

ABSTRACT

Exogenous administration of nitric oxide may be a therapy for several pathologies because this molecule regulates many biological systems. Here, the storage and release of NO by microporous titanosilicate ETS-10 and samples where the silicon was substituted by aluminium (ETAS-10) or gallium (ETGS-10) are studied. The Al- and Ga-doped materials exhibit an increase in the storage capacity of 95% and 55%, respectively, the highest values observed, so far, for microporous titanosilicates. ETAS-10 releases more NO and ETGS-10 almost the same amount as ETS-10. In ETAS-10 and ETGS-10, the irreversibly adsorbed NO amount increases relatively to ETS-10. Tests of NO release in haemoglobin solutions indicate that biologically relevant amounts are release and that ETS-10 and ETGS-10 display a release slower than ETAS-10, more adequate for a sustained delivery. Cytotoxicity studies show that the samples have very low toxicity (cell viability above 87%, after 72 h) at high concentration (0.45 mg cm^{-3}). Tests at variable ETS-10 concentration further confirm the low cytotoxicity of this material, even at high concentrations (up to 1.8 mg cm^{-3}).

1. Introduction

The discovery of nitric oxide, NO, as a signalling molecule in the regulation of the cardiovascular systems, prompted many studies on the NO regulating effect in the human body. It is now known that NO is involved in important physiological processes, like vasodilatation, prevention of platelet aggregation and thrombus formation, neurotransmission, and wound repair [1]. NO delivery to the human body is becoming an attractive therapeutic alternative for a large number of pathologies. Certain molecular donors, usually based on diazeniumdiolates and S-nitrosothiols, that deliver NO directly from solutions are well established, while new compounds have been recently developed [2]. The use of these compounds in therapy is, however, inadequate due to their wide non-selective distribution in the body and ensuing unwanted side effects. Moreover, many by-products of these molecular donors may form carcinogenic compounds, following the NO release. NO itself is very toxic at high concentration and, thus, targeted delivery and concentration control are crucial.

New and improved materials and technologies to store and target-deliver NO in biological amounts may provide an alternative to overcoming the problems associated with systemic and homogeneous delivery. For NO-delivery, appropriate releasing kinetics is as important as good storage capacity. Indeed, the ideal material should release NO for several hours at a known constant rate: release of a considerable amount of NO in a burst is often deleterious and may induce permanent tissue damage. Usually, a slow releasing kinetics is preferred because it allows for a safer control of the NO concentration for longer periods.

Nanoporous materials have potential for releasing NO at a localized site in the human body, as shown by studies on zeolitic materials, which adsorb and release NO upon contact with moisture [3–5]. The capability of gas storage and production of NO over zeolites was also demonstrated [6,7]. In this context, metal organic frameworks were studied taking advantage of the presence of open metal sites in their porous structure for NO coordination [8–11]. Moreover, porous materials based on natural clays pillared with transition metal oxides exhibit interesting NO-delivery properties

* Corresponding author.
E-mail address: moises.pinto@tecnico.ulisboa.pt (M.L. Pinto).

http://dx.doi.org/10.1016/j.micromeso.2016.04.021
1387-1811/

and low toxicity [12,13]. Our group has previously shown that microporous titanosilicate ETS-4 bearing unsaturated Ti^{4+} framework centres is an exceptional material for the storage and slow release of NO [14]. In a subsequent work, Cu^{2+} and Co^{2+} ion-exchanged ETS-4 was studied in order to adjust the NO storage-release cycle [15].

Here, we wish to report on the excellent NO storage and release properties of another structure type of porous titanosilicate, ETS-10 [16], and materials obtained from it by isomorphous substitution of framework silicon by Al^{3+} or Ga^{3+} (respectively, ETAS-10 and ETGS-10) [17–20] (Figure S1 in Supplementary Material). These metal centres cannot easily leach from the material, avoiding toxicity problems. In contrast with ETS-4, the Ti^{4+} centres in ETS-10 are hexacoordinated and not available for coordination with molecules inside the channels. Thus, in ETS-10 NO is expected to coordinate the cations hosted inside the channels, in a way similar to that of classic zeolites. Considering that ETAS-10 and ETGS-10 have the structure of ETS-10 but different chemical compositions they allow studying the influence of the chemical composition on the NO storage and release, specially due to the increase in the number of surface charges arising from substitution of the framework silicon by Al^{3+} or Ga^{3+}. Because biocompatibility is an essential feature of any drug-delivery material, the cytotoxicity of ETS-10, ETAS-10 and ETGS-10 is also evaluated here, for the first time. We envisage the application of these materials in topical applications for wound healing and skin regeneration therapeutics.

2. Materials and methods

2.1. Materials synthesis and characterization

The microporous silicates ETS-10, ETAS-10 and ETGS-10 were synthesized according to procedures previously described [17,20,21], using titanium choroid as Ti source. The synthesis and purity was confirmed by powder X-ray diffraction (Bruker D8 Discover and Bruker D8 Advance diffractometers) and scanning electron microscopy (SEM). SEM images were obtained in microscope (Hitachi S4100) using a 15 kV electron beam. Energy dispersive X-ray spectroscopy (EDS) analysis of the powders yielded their chemical composition.

Nitrogen (Air Liquid, 99.999%) adsorption-desorption isotherms were measured at −196 °C using a liquid nitrogen cryogenic bath, in a volumetric automatic apparatus (Micromeritics, ASAP 2010). Prior to the measurement the samples were outgassed at 300 °C for 2.5 h at a pressure lower than 0.133 Pa.

Zeta potential (ζ-potential) of the water (Millipore Q) suspensions (5 mg cm^{-3}) was measured (Microtrac; Stabino) with time and the value at equilibrium recorded. Particle size was determined in the same water suspensions by dynamic light scattering (Microtrac; NANO-flex). Particle size measurement was preformed immediately after the measurement of the zeta potential in the same cell to avoid steeling and aggregation of particles.

The aluminium and gallium content of ETAS-10 and ETGS-10 was determined by ICP-AES at the laboratory of analysis of Instituto Superior Técnico. Prior to analysis, the materials were digested in a concentrated mixture of hydrochloric and nitric acids in Teflon lined autoclaves until full dissolution of the material. The resulting solution was diluted and analysed by ICP.

2.2. Nitric oxide adsorption and release

The gravimetric method was used to record the NO kinetic adsorption/desorption profiles of the adsorbent materials using a microbalance (C.I. Electronics, Disbal) connected to a vacuum line. In the adsorption-release studies in the gas phase samples were outgassed at 100 °C for 2 h under a vacuum better than 10^{-2} Pa. Release studies were carried out in the gas and liquid phase. NO was introduced in the system until an 80 kPa pressure. The microbalance was connected to a computer and the weight was recorded during 72 h at 2 points per minute, after which the NO release started under vacuum and the weight decrease was then recorded for 48 h. In all the gas experiments, the temperature was maintained at 25 °C using a water bath (Grant, GD120), with 0.05 °C precision. The reproducibility of the obtained kinetic curves is ±2%, estimated from replicate measurements.

The oxyhaemoglobin assay was used to study the NO release in the liquid phase [22]. The samples were ground with poly(tetrafluoroethylene) (PTFE, 25 mm particle size powder, from Aldrich) (75 sample: 25 PTFE %) to avoid their dispersion on the liquid phase poly(tetrafluoroethylene) (PTFE, 25 mm particle size powder, from Aldrich) (75 sample: 25 PTFE %) [3]. The mixture was pressed into 5 mm diameter disks under 8 tons for 30 s. Then, 6 mg of the disks were introduced into small glass container with a PTFE vacuum valve connected to a vacuum line and the samples were outgassed for 2.5 h at 100 °C. When the material returned to room temperature, NO was introduced, and the valve closed and left until the equilibrium was reached, up to for 3 days. After evacuation for 1 min to remove NO in the gas phase, the container was filled up with helium to atmospheric pressure. For the NO release studies, the samples were added to 3 mL of oxyhaemoglobin solution (1 μM) in a quartz container, which was vigorously shaken. Briefly, an oxyhaemoglobin solution was prepared by dissolving 20 mg of lyophilized human haemoglobin in 1 mL of 0.1 M phosphate buffer (pH = 7.4). Purification and desalting were performed by passing the resulting oxyhaemoglobin solution over a column of Sephadex G-25 [22]. The kinetics experiments were performed in a 0.1 M phosphate buffer (pH = 7.4) at ambient temperature and spectra were taken at 10-min intervals during 2 h, using an UV/Vis spectrophotometer (Genesys 10S UV-Vis Spectrophotometer from Thermo Scientific). Blank experiments were also conducted on the samples without NO, following the same procedure.

2.3. Cytotoxicity essays

HeLa cells (American Type Culture Collection, Manassas, VA, USA) were cultured at 37 °C in a humidified atmosphere with 5% CO_2. The cell culture medium consisted of RPMI-1640 supplemented with 10% of fetal calf serum (FBS), 2 mM Glutamine, and 100 U/mL penicillin and 100 μg/mL streptomycin, all from HyClone.

HeLa cells were seeded in 96-well plates (Orange Scientific) with 7500 and 2500 cells/well (for 24 h and 72 h sample assay, respectively) in a final volume of 100 μL cell culture media. After 24 h incubation 10 μL sample was added. Before the experiment, the medium was removed and replaced by 100 μL/well of fresh new medium. Then 10 μL alamarBlue® was added directly to each well, the plates were incubated at 37 °C for 4 h and the fluorescence signal was measured on a Spectra Max Gemini EM from Molecular Devices, with a SoftMax Pro software. Cell viability was calculated with the following equation:

$$Cell\ viability = F_{sample} \Big/ F_{control}$$

where F_{sample} is the fluorescence of the cells incubated with samples and where $F_{control}$ is the fluorescence of the cells incubated without the sample. The number or replicates for each concentration and material was 4. All the results were statistically treated by ANOVA tests. For some selected materials (ETS-10) a concentration study was made. In this case, the concentration of the materials varied between 0.113 and 1.800 mg cm^{-3}. Survival was evaluated,