Glucose-Triggered Release Using Enzyme-Gated Mesoporous Silica Nanoparticles

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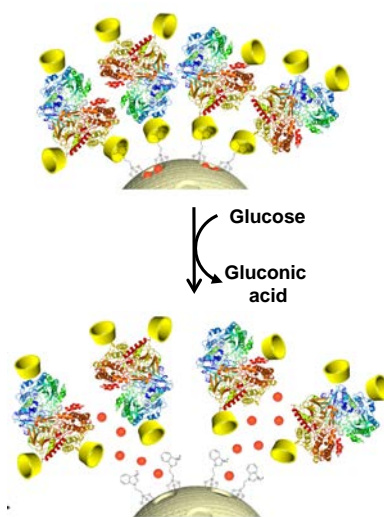
A new gated nanodevice design able to control cargo delivery using glucose as trigger and cyclodextrin-modified glucose oxidase as capping agent is reported.

Mass transport control at the nanometric level is a captivating area of research that has attracted the attention of scientists in the latest years. Especially, the development of gated systems able to retain a cargo and release it after the application of a stimulus has demonstrated to be an excellent approach for the development of smart nanodevices for advanced delivery applications.[1] In this context, silica mesoporous supports are widely used as inorganic scaffolds thanks to their unique characteristics such as high homogeneous porosity, inertness, robustness, thermal stability, the presence of tunable pore sizes, homogeneous pore distribution and high loading capacity.[2] Moreover, via decoration of the mesoporous material with a wide collection of organic moieties, linkers and capping agents, researchers have prepared nanovalves that can be triggered with target chemical[3] (such as redox molecules, selected anions, pH changes and biomolecules) physical [4] (such as light, temperature or magnetic fields) and bio-chemical (such as enzymes, antibodies, or DNA) stimuli.[5]

In particular the development of enzyme-responsive bio-gated mesoporous silica nanoparticles is appealing. In previously reported examples tailor-made sequences anchored on the mesoporous support are hydrolized by target enzymes allowing the selective release of the entrapped cargo. However in these cases enzymes act as triggers but as far as we know there are not examples where enzymes were used as functional gating elements.[6] In this context, and as a new approach, we envisioned the potential design of gated materials in which the enzymes could act as caps and the uncapping process would be triggered by the product obtained by the enzyme's activity on a target substrate. The combination of the promising features of silica mesoporous supports as containers, enzymes as caps and substrates as trigger would result in a sophisticated but simple way to prepare selective substrate-responsive gated mesoporous materials for different applications. As a proof-of-concept we have selected herein glucose oxidase as capping enzyme and glucose as the substrate to trigger cargo release.

The designed capped support is depicted in Scheme 1. It is based on the use of mesoporous silica nanoparticles loaded with a suitable reporter (i.e. ruthenium bipyridine complex) and containing anchored methylbenzymidazole moieties on the pore outlets. The mesopores are then capped with an active CD-modified-glucose oxidase (CD-GOx) through the formation of an inclusion complex between the cyclodextrins and the propylbenzymidazole group anchored to the solid support. The presence of the substrate glucose combined with the catalytic action of CD-GOx to produce gluconic acid was expected to induce protonation of the benzymidazole group that might result in the inclusion complex dethreating and the subsequent cargo release.

Scheme 1. Solid **S3** expected performance.

In this context, to the best of our knowledge, the use of glucose as trigger for a gated system has only been described by Lin et. al..

In their work, they reported a saccharide-responsive controlled release of insulin and cAMP using phenylboronic acid-functionalized silica mesoporous nanoparticles.[6] However, this later system was based in a simple displacement reaction yet no enzymes were used in its design.

In order to prepare the gated nanodevice, silica mesoporous (MCM-41-type) nanoparticles (ca. 100-150 nm) were selected as inorganic scaffold. The MCM-41 support was loaded with a suitable dye ($[Ru(bpy)_3]Cl_2$) and the external surface was functionalized with 3-iodopropyltrimethoxysilane (solid **S1**). Then, a nucleophilic substitution reaction of the anchored 3-iodopropyl moiety with benzimidazole resulted in a solid functionalised with 1-propyl-1-H-benzimidazole groups (solid **S2**).

These intermediate supports (**S1** and **S2**) were characterized using standard procedures (see Supporting Information). X-ray diffraction pattern of **S2** (Figure 1) shows the mesoporous characteristic (100) diffraction peak indicating that the loading process with the dye and the further functionalization to obtain the benzimidazole domains have not modified the structure of the mesoporous scaffolding. Moreover, contents of 0.48 mmol g^{-1} SiO_2 of dye and 0.54 mmol g^{-1} SiO_2 of benzimidazole on solid **S2** were determined by elemental analysis and thermogravimetric studies.
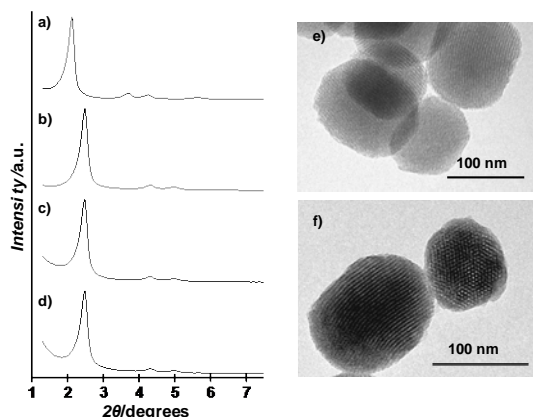

Figure 1. Powder X-ray diffraction pattern of a) MCM-41 as made, b) calcined MCM-41, c) solid **S1** and d) solid **S2** showing the typical reflections of the MCM-41 hexagonal array. TEM representative images of e) calcined MCM-41 and f) solid **S2** showing the typical porosity of the MCM-41 mesoporous matrix.

For the preparation of the final gated material, CD-GOx was synthesized as previously reported[7] and a mixture of **S2** and CD-GOx in water at pH 7.5 was stirred for 24h. After that time, the capped solid was centrifuged and washed with water at pH 7.5 three times to obtain the orange final solid **S3**. (see Supporting Information for further details)

In order to test the gating properties of the solid, portions of 150 μL of a suspension of **S3** (6.7 mg mL^{-1}) were diluted with 3 mL of water at pH 7.5 in the presence of glucose (0.1 $molL^{-1}$) and stirred for a certain time. As a control experiment another portion of 150 μL of the suspension of **S3** was diluted in the same proportion and stirred. During the experiments, at a given time, 0.3 mL of each suspension was filtered, and the absorbance at 453 nm of the released $[Ru(bpy)_3]Cl_2$ was measured.

The delivery kinetics profile of the ruthenium dye in the presence and absence of glucose is shown in Figure 2. In absence of glucose (curve a) solid **S3** shows a very poor dye release, indicating tight pore closure. On the other hand, the delivery of the dye is induced when glucose is present in the solution (curve b) due to displacement of the CD-GOx as consequence of CD-GOx-induced oxidation of glucose to gluconic acid and protonation of the benzimidazole group. Thus, the presence of glucose allows the release of 51% of the entrapped dye. Additionally, the figure also shows how release from the capped system can be triggered on command by adding glucose at a certain time (curve c).

The obtained results confirmed the triggering event hypothesis; i.e. whereas solid **S3** displayed no release, the presence of the substrate of the enzyme acting as cap induced the delivery of the entrapped guest. Moreover, to confirm that the opening protocol was due to protonation of the benzimidazole which induced the dethreating of the inclusion complex, pH changes in the final aliquots were measured. Thus, only in the experiments in which glucose was present a change from pH 7.5 to pH < 5.2 was observed confirming the enzyme-mediated conversion of glucose in gluconic acid.
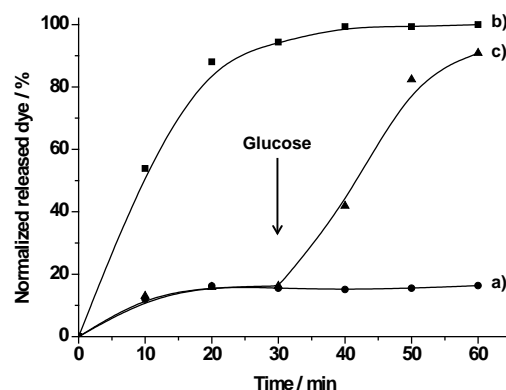

Figure 2. Release profile of $[Ru(bpy)_3]Cl_2$ from solid **S3** at pH 7.5 a) in the absence of glucose, b) in the presence of glucose c) with glucose addition at 30 min.

On the other hand, we believe that solid **S3** or similar systems could be of application in the development of glucose-induced delivery systems or in the design of colorimetric probes for glucose determination. In this context, and as a further step in the characterization of the delivery system, the response of the hybrid material **S3** as a function of the glucose concentration was studied and the results are illustrated in Figure 3. From these data a linear release of the dye from **S3** in the $1x10^{-2}$-$1x10^{-4}$ $molL^{-1}$ glucose concentration range was found. Moreover a detection limit of glucose of $1.5x10^{-4}$ $molL^{-1}$ was calculated which was in the range of other glucose-responsive reported detection systems.[8] Taking into account that the general range of blood glucose concentration in healthy and diabetic humans is about 3–8 mM and 9–40 mM, respectively,[9] we can envision the potential of this enzyme-gated systems for the design of chromo-fluorogenic probes for glucose detection.

Figure 3. Released dye from solid **S3** in the presence of different glucose concentrations.
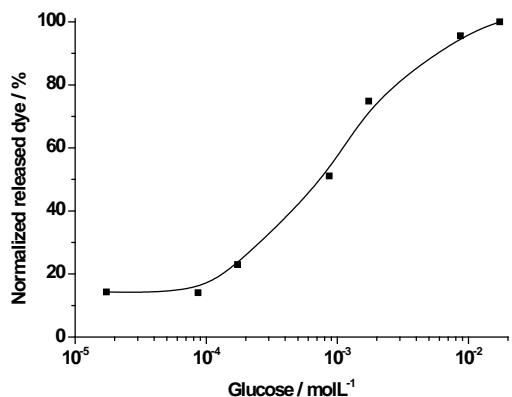
Moreover, as it is well-known enzymes are selective catalytic centres. In our case, although numerous sugars and derivatives of glucose have been reported to be substrates of GOx, the enzyme shows far greater activity with glucose. In this scenario, the selectivity in the opening protocol in **S3** was tested in the presence of different saccharides such as mannose, fructose, galactose, maltose and saccharose at a concentration of 1×10^{-3} molL^{-1} and following the same described procedure. As it can be seen in Figure 4 a selective uncapping was observed in the
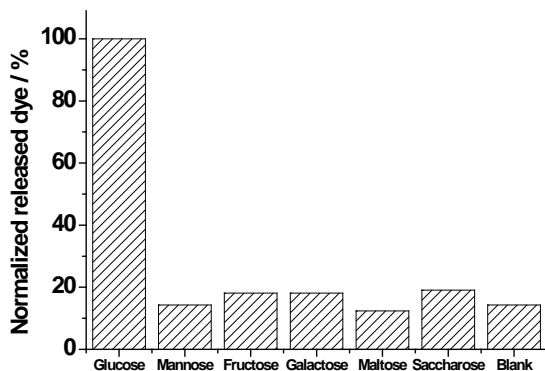
presence of glucose confirming the selective triggering.

Figure 4. Released dye from 1 mg of solid **S3** in the presence of different analytes (1×10^{-3} molL^{-1}).

In summary, we have reported a new approach for the development of gated materials using enzymes. In this proof-of-principle, the mesoporous material is capped with an active CD-modified-glucose oxidase (CD-GOx) through the formation of inclusion complexes between the cyclodextrins and the propylbenzymidazole group anchored to the solid support. In the presence of glucose as target substrate a cascade response results in a delivery of the entrapped cargo. In particular, CD-GOx converts glucose to gluconic acid which induces the protonation of benzimidazole which results in a dethreating of the inclusion complex and delivery of the dye. The approach is simple and versatile and may allow to inspire the design of high selective on-command delivery systems based on enzyme's activity on target substrates for different applications.

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