Fabrication of a hydrophobic smart nanocontainer with pH-sensitive surface activation for controlled release

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Abstract. A hydrophobic pH sensitive nanocontainer was fabricated using smart surfaces covalently attached to a porous alumina support. The smart surface was synthesized using a mixture of aliphatic and aminated silanes and optimized to be hydrophobic at pH>7 and hydrophilic at pH<5. The hydrophobic nanocontainer thus synthesized was able to retain a cargo of the model molecule safranine at neutral pH. When pH decreased, safranine was liberated at a high rate due to the large pores of the alumina. It is expected that the nanocontainer here presented could constitute the basis of a cancer treatment as an effective drug delivery system in chemotherapy.

1. Introduction
Nanocontainers are porous nanostructures that can be used to store a molecular load and release it in a controlled fashion [1]. Due to this property they have attracted interest in a number of fields such as: agriculture [2,3], catalysis [4], corrosion [5], molecular detection [6], drug delivery [7], etc. First generation nanocontainers were usually hollow materials that made a slow liberation of molecules with no control other than simple diffusion. Some of them were organic in nature such as polymers [8], lipids [9] and proteic nanocapsules [10]. Inorganic nanocontainers appeared a while later and they were based on silica [11], ceramics [12], carbon [13], gold nanoparticles [14] and lately, alumina [15].

Nanomaterials based on silica or alumina have unique properties such as high-volume aspect ratio, biocompatibility, good thermal stability, homogeneous porosity, low toxicity [16] and pore diameter with acceptable size [17]. But most important, it is the presence of hydroxyl groups on their surfaces. These hydroxyl groups make them susceptible of surface modification by silanes, which allows introduction of virtually any chemical group. Some of these functionalities can confer to these supports the ability to hold their cargo and release only upon activation by a change in the physical or chemical properties of their environment. Numerous examples of these so called “smart nanocontainers” are reported in the literature where controlled release is triggered by light, temperature, pH, etc [18,19].

One property that can be manipulated to create a smart nanocontainer is hydrophobicity. It has been reported that a hydrophobic pore can remain dry even under conditions of high pressure [20]. To open a hydrophobic pore, it is necessary then to generate a change on the surface energy of the interfaces at the contact line between air, liquid and solid. Rios et al. demonstrated the fabrication of a hydrophobic nanovalve that remained closed at neutral pH but opened up at pH values below 5 [21,22]. This principle can be applied in the fabrication of a smart nanocontainer. Up to our knowledge, there is in the literature only one report that used a pH sensitive smart hydrophobic surface to generate a control release from a hydrophobic nanocontainer. Wang et al. demonstrated such mechanism based on the modification of a
silica porous nanoparticle with an aminated silane [23]. However, this nanocontainer presented a very slow release, probably due to the small size of the pores.

Herein we are presenting a new smart hydrophobic nanocontainer that can hold their cargo at neutral pH but release it at values of pH below 5. It is based on nanoporous alumina as a solid support which show the advantage of a much larger pore diameter. This allows a much shorter liberation time without sacrificing the retention of the cargo during the off-state of the valve. It is expected that the resulting material may be applied as an effective drug delivery system based on its properties.

2. Experimental
Aluminium sheets with purity of 99.9% and area 4.2 cm² were used to synthesize anodized anodic aluminium oxide membranes (AAO). Before the first anodization step, the substrate was degreased in ethanol for 5 min and washed in distilled water. Then, the aluminium was put in a solution of perchloric acid and ethanol with ratio 1:4 v/v at 10 °C and subjected to a current density 100 mA/cm² for 4 min with stirring to obtain a mirror type bright surface. The synthesis of ordered porous anodic aluminium oxide was carried out by two-step anodization process [17,24]. Anodization took place in an aqueous solution of oxalic acid 0.3 M under a constant potential difference of 60 V between the anode and cathode for 3 hours at room temperature. After that, the oxide layer had to be removed using a mixture of chromic (2.0%) and phosphoric acid (6.0%) until disappearance of the white colouration on the substrate. The second-step anodization was performed under the same conditions of potential and temperature increasing the time to 6 hours to obtain an ordered array AAO.

3-aminopropyltrimethoxysilane (C₃H₇NOSi) and dodecyltrimethoxysilane (C₁₃H₂₇NOSi) were obtained from Gelent Inc. Smart surfaces were synthetized on glass slides and AAO. Glass slides were pre-treated for cleaning with piranha solution (40% H₂O₂ – 60% H₂SO₄) for 30 min at 80 °C, washed with distilled water five times and dried at 115 °C. The substrates were modified by immersing them for 24 hours in solutions that contained 2.0%v/v DTMS and 0.0 – 2.0%v/v APTS using toluene as solvent, under constant agitation to guarantee homogeneous modification and found the optimal mixture. After that, substrates are washed with toluene and methanol and cured at 120 °C for 1 hour.

After modification, AAO were loaded in a 0.1 M solution of safranin O (SO) in ethanol. Membranes were immersed in SO solution during 2 hours with lineal stirring, after that SO solution was extracted and AAOs were washed with distilled water and dried at 120 °C for 30 minutes. The releasing process was monitored by measuring the absorbance of the SO in solution as a function of time.

AAOs were characterized by infrared spectroscopy (FTIR Bruker Tensor II - Software OPUS Spectroscopy) to identify functional groups and surface APTS concentration. Scanning electron microscope SEM (FEI Quanta 650 FEG – Software EDX Genesis) was used to evaluate the morphology and structure of AAOs. Contact angle were performed (Dataphysics SCA20 – Software AvaSoft 7.8) with buffer solutions as liquid droplet (0.1 M phosphate buffer pH 7 and 0.1 M acetate buffer pH 5) to analyse the hydrophobic and hydrophilic character of the surfaces. Release of SO was carried out in a UV-Vis spectrometer (AVANTES-AvaSpec).

3. Results and discussion
Figure 1 shows SEM images of the synthesized membranes. Figure 1(a) agrees with the expected honeycomb porous structure and pore diameters between 80 nm–110 nm approximately is observed. 6 h anodization produces typically membranes of ~75 µm thickness as can be seen in Figure 1(b).

Hydrophobicity switching of the surface (i.e. change from hydrophobic to hydrophilic) was accomplished through the use of a mixture of silanes, aliphatic and aminated. In this work we have used DTMS as aliphatic silane and APTS as aminated silane. Glass slides served as substrates to test the behaviour of the smart surface. Figure 2 shows the contact angle of buffer drops with different pH values as a function of APTS composition (DTMS concentration in the modifying solution was kept constant). A solution of 1.8% APTS generated the desired effect, this is, presenting hydrophobic behaviour at neutral pH and becoming hydrophilic at pH 5.
Figure 1. SEM characterization of AAO membranes. (a) shows the honeycomb porous structure. (b) shows the thickness of the membrane.

Once the right proportion of molecules in the surface was found, AAOs were modified and characterized. Contact angles on the membrane were 95.2 ± 1.30 at pH 7 and 61.3 ± 4.97 at pH 5. This confirms that the behaviour of the smart surface on the membrane is similar to the one on glass although differences on alumina and silica surface chemistry [25].

Figure 2. Smart surface hydrophobicity behaviour.

Functional groups in the modified membranes were characterized by ATR-FTIR. Typical absorption bands associated with the organosilane groups present in APTS and DTMS on AAO are observed in Figure 3. The characteristic peaks between 2820 cm\(^{-1}\) and 3000 cm\(^{-1}\) can be assigned to the presence of methyl and methylene as observed in Figure 3(a). Amine stretching appears as a broad absorption between 2300 cm\(^{-1}\) and 3400 cm\(^{-1}\), different intensities correspond different APTS concentration. The increasing absorption near 1560 cm\(^{-1}\) with raising the amount of APTS agrees with the corresponding peak of NH bending [21]. The characteristic absorption of the silane films appears between 1150 cm\(^{-1}\) and 1000 cm\(^{-1}\) as observed in Figure 3(b), which is attributed to the -Si-O asymmetric stretching in -Si-O-Si because of the condensation reaction that occurred among silanols themselves in the film [26,27]. In 1029 cm\(^{-1}\) spectra shows a peak that correspond to the Si-O-C stretching due -Si-O-CH\(_3\).
The release behaviour of modified AAO was measured in buffer solutions of pH 5 and 7 with respect to time as observed in Figure 4. There was no obvious burst release of SO from modified AAO in phosphate buffer suggesting a good stability of the system if were to be used in medical applications. Nevertheless, the cumulative release of SO from modified AAO was accelerated dramatically when the pH values decreased from 7 to 5.

It is clear then that the nanocontainer is able to hold a molecular load and release it upon changes of pH as expected. The capacity of the surface to exercise a change in its hydrophobicity is associated to the surface charge of the groups that are present. Since the terminal amine is the only group that can change its character depending of pH, is this group we associate to the “smartness” of the nanocontainer. Further studies are necessary to understand the chemistry of the surface and quantify the concentration of the chemical groups as well as its physical-chemical properties.

4. Conclusions
A new hydrophobic smart nanocontainer was presented which was able to hold a molecular load at pH 7 and promptly release it when pH decreased to 5. The nanocontainer was synthesized using porous anodic alumina as support and a surface modification based on a mixture of silane molecules, aliphatic and aminated. Model molecule Safranine O was encapsulated using an ethanol solution. The molecular cargo was released at pH 5, with a relatively high rate due to the size of the pores. It is expected that this type of smart nanocontainers can take part on effective therapies for cancer treatment.
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