Biophysical Chemistry 182 (2013) 11–15

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Anticancer drug delivery system based on calcium carbonate particles loaded with a photosensitizer

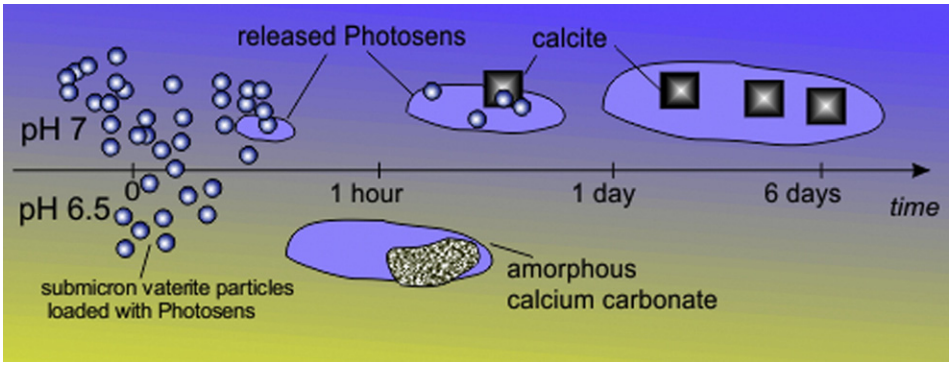
Yulia Svenskaya [a,b], Bogdan Parakhonskiy [a,c,*], Albrecht Haase [d], Vsevolod Atkin [b], Evgeny Lukyanets [e], Dmitry Gorin [b], Renzo Antolini [d]

[a] BIOtech Center Dept. of Industrial Engineering, University of Trento, via delle Regole 101, 38123 Mattarello, Italy
[b] Saratov State University, Astrakhanskaya Street 83, 410012 Saratov, Russia
[c] A.V. Shubnikov Institute of Crystallography, Russian Academy of Science, Leninskiy Prospect 59, 119333 Moscow, Russia
[d] Department of Physics, University of Trento, via Sommarive 14, 38123 Povo, Italy
[e] Organic Intermediates and Dyes Institute, B. Sadovaya 1/4, 123995 Moscow, Russia

HIGHLIGHTS

- Vaterite particles were loaded with the photosensitizer Photosens used in PDT.
- The drug release process was triggered by a $CaCO_3$ crystal phase transitions.
- Release dynamics were found to be sensitive to the environmental pH.
- The phase transitions speed up with increasing particle size.
- This allows creation of a controllable photosensitizer delivery system, releasing its payload under acidy conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 April 2013
Received in revised form 9 July 2013
Accepted 15 July 2013
Available online 24 July 2013

Keywords:
Calcium carbonate
Vaterite
Photosens
Drug delivery
Cancer
Phthalocyanine

ABSTRACT

In photodynamic therapy (PDT), photosensitizers are required to arrive in high concentrations at selective targets like cancer cells avoiding toxicity in healthy tissue. In this work, we propose the application of porous calcium carbonate carriers in the form of polycrystalline vaterite for this task. We investigated the loading efficiency for the photosensitizer Photosens in vaterite micro- and nanocarriers. A possible release mechanism depending on the surrounding pH was studied, showing a fast degradation of the carriers in buffers below pH 7. These results hold out the prospect of a novel PDT drug delivery system. Variation of particle size or additional coatings allow custom-design of workload release curves. An intrinsic cancer-sensitivity can be expected from the pH-dependent release in the acidic microenvironment of cancer tissue.

1. Introduction

One of the major challenge in nanomedicine is the development of systems for targeted substance delivery, which requires understanding of fundamental biochemical processes such as cellular uptake

* Corresponding author at: BIOtech Center Dept. of Industrial Engineering, University of Trento, via delle Regole 101, 38123 Mattarello, Italy.
E-mail address: bogdan.parakhonskiy@unitn.it (B. Parakhonskiy).

0301-4622/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bpc.2013.07.006

mechanisms [1,2] or intracellular transport [3], and also the intelligent design of adequate carriers [4–6]. An effective matrix for such a delivery system is calcium carbonate ($CaCO_3$) [7-10]. $CaCO_3$ exists in three different anhydrous crystalline polymorphs: calcite, aragonite, and vaterite. Under standard conditions for temperature and pressure, calcite is the stable phase, while aragonite and vaterite are the metastable forms that readily transform into the stable phase. Vaterite is an ideal candidate for a drug delivery system because it has large porosity, large surface area, and can decompose rapidly under relatively mild conditions [10,11]. Vaterite is the least stable phase of $CaCO_3$ since in contact with water it slowly dissolves and recrystallizes to form calcite. Previous studies described the possibility of synthesizing spherical mono-dispersed vaterite particles in the size range from 2 to 10 μm [11] and from 400 nm to 2.4 μm [12]. Vaterite containers allow for different substance loading methods such as adsorption [13–15] and co-precipitation [8,14]. A release mechanism based on a crystal phase transition has recently been demonstrated [12,15,16]. Cytotoxicity and influence on cell viability have been excluded in cell culture studies with 400 nm vaterite containers. Apart from this efficient cellular uptake of substance-loaded containers was observed [15].

To exploit vaterite containers as a drug delivery system in photodynamic therapy (PDT), photosensitizers have to be incorporated, delivered to the target, and released within the cells. Exposure to light at the photosensitizers absorbance wavelength then induces singlet oxygen generation, a photochemical reaction of type II [17,18]. The singlet oxygen can oxidize cellular macromolecules like lipids, nucleic acids, and amino acids leading to cancer cell apoptosis [19].

So far, the main negative side effect of PDT is caused by its insufficient selectivity of action: a high concentration of photosensitizer is required for cancer treatment at the tumor site, but causes incidental toxicity in healthy tissue. This side effect could be strongly reduced by targeted delivery to the region of interest. The proposed delivery system will achieve this exploiting a pH-dependency of the carrier degradation dynamics.

2. Materials and methods

2.1. Materials

Calcium chloride, sodium carbonateacetic acid, and sodium hydroxide were purchased from Sigma-Aldrich and used without further purification. The photosensitizer Photosens, a mixture of sulfonated aluminum phthalocyanines $AlPcS_n$, with n = 2, 3 or 4 (the mean n = 3.1), was obtained from the Organic Intermediates and Dyes Institute (Moscow, Russia). It has strong absorption bands with a maximum at 675 nm wavelength [20], and can be activated at 100 J/cm^2 light power [21]. It is applied in clinical practice since 2001 (Registration Certificate Ministry of Health of Russian Federation No. 000199.01-2001) in both diagnostics [22,23] and treatment, from lip, pharynx, larynx, and tongue lesions to lung and esophageal tumor therapy [24–26].

2.2. Particle preparation and characterization

Spheroid-like calcium carbonate microparticles of 3.6 ± 0.5 μm size were fabricated using a previously reported protocol [10]: 1 mL of Na_2CO_3 (0.33 M) was loaded into a glass vessel, then an equal volume of $CaCl_2$ (0.33 M) was added and stirred at 500 rpm for 1 min. For the preparation of sub-micron vaterite spheres with a size 650 ± 30 nm, the protocol described in [12] was applied: the chosen concentrations of $CaCl_2$ and Na_2CO_3 were 0.33 M. Ethylene glycol (EG) was added to this reaction solution (Na_2CO_3 and $CaCl_2$ were dissolved each in 2 ml water and 10 ml EG). The solution was stirred with 500 rpm at room temperature for 3 h. The mixed solution turned opaque almost instantly. The synthesized $CaCO_3$ particles were carefully washed with ethanol and dried for 30 min at 60 °C.

The drug Photosens was loaded into the obtained vaterite containers by the adsorption method: 5 mg of dried $CaCO_3$ particles were taken for each sample, 1.5 ml of 0.5 mg/ml aqueous solution of the drug were added. The adsorption took place during 2.5 h of shaking. Micron-sized particles were centrifuged at 3200 ×g for 1 min, sub-micron particles for 3 min. Afterwards the supernatants were removed and collected.

To study the morphology and microstructure, dried particles were sputtered with gold and imaged with scanning electron microscopes (SEM), a MIRA II LMU (Tescan) at an operating voltage of 20 kV and a Phillips XL 30 at 5–30 keV.

2.3. Loading and release process

Optical studies of the loading process were performed using a two-photon laser scanning microscope Ultima IV (Prairie Technologies) with a 100 × objective (NA 1.0, water immersion, Olympus) and an ultra-short pulsed laser (Mai Tai Deep See HP, Spectra-Physics) at an excitation wavelength of 800 nm.

A spectrofluorometer (Cary Eclipse, Varian) was used to measure the Photosens uptake efficiency and release profile. The weight of the particle samples was kept constant (5 mg) in all experiments. Some loss of containers during the washing may have occurred, but did not exceed a few percent. As a measure of the amount of Photosens in solution, its fluorescence intensity was recorded at 684 nm. A calibration curve was obtained from fluorescence measurements of known concentrations of Photosens. Then samples were diluted in water or buffer solution to ensure that measurements were within the linear range of the calibration curve. The total amount of adsorbed molecules was deduced by subtracting the measured amount of unloaded and washed-off molecules of the supernatant from the initial amount of 0.5 mg/ml of Photosens, which had been added to the system.

To study the release of the drug under varying pH, a line of acetate buffers from pH 4.5 to pH 7 was created. Vaterite containers loaded with Photosens were suspended in these buffers (5 mg particles in 30 ml) and incubated at room temperature in carefully sealed centrifuge tubes. After different incubation times (5 min to 6 days), the samples were centrifuged at 2600 ×g for 3 min and the concentration of the released Photosens in the supernatant was measured by spectrofluorimetry. To study the calcium carbonate phase change during the release process, samples were monitored by SEM. Samples were dried from 10 μl of particle suspension based on SEM image analysis, the calcium carbonate phases were determinate via their specific properties: vaterite being spherical and polycrystalline (Fig. 1), calcite being a rhombohedral monocrystal (Fig. 2A), and amorphous calcium carbonate showing up as non-regular structures with grain sizes less than 20 nm (Fig. 2C, D).

3. Results and discussions

3.1. Particle loading

To study the influence of the carrier size on the loading efficiency, two lines of the vaterite particles were synthesized: vaterite containers with an average size of 650 ± 30 nm (hereafter referred to as “small”) and of size 3.6 ± 0.5 μm (hereafter referred to as “big”). In 5 mg of small particles 0.067 ± 0.007 mg of Photosens could be incorporated, which amounts to 1.4 ± 0.4% (w/w). For big particles the uptake was 0.047 ± 0.003 mg, corresponding to 0.9 ± 0.2% (w/w). The loading efficiencies of big and small particles are comparable which proves a deep internalization of the drug into the calcium carbonate matrix, a factor of 6 between small and big particle loading would reflect an adsorption only to the external surface. These efficiencies are in the same order as those of other substances loaded via adsorption into porous carriers [8,27,28]. Scanning electron microscopy images and two-photon fluorescent images of the loaded particles are presented in Fig. 1. The SEM images in Fig. 1A and B show the spherical shape of the vaterite