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Nanosponge formulations as oxygen delivery systems

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ABSTRACT

Three types of cyclodextrin nanosponges were synthetized cross-linking α, β or γ cyclodextrin with carbonyldiimidazole as cross-linker. Nanosponges are solid nanoparticles previously used as drug carriers. In this studies cyclodextrin nanosponges were developed as oxygen delivery system. For this purpose the three types of nanosponges suspended in water were saturated with oxygen and *in vitro* characterized. The nanosponge safety was tested on Vero cells. Their ability to release oxygen in the presence and in the absence of ultrasound (US) was determined over time. Oxygen permeation through a silicone membrane was obtained using a β-cyclodextrin nanosponge/hydrogel combination system. Nanosponge formulations might be potential gas delivery systems showing the ability to store and to release oxygen slowly over time.

Gases play important role in medicine, either for diagnostic or treatment purposes. It is sometime difficult to deliver oxygen in appropriate form and dosage in clinical practice. The deficiency of adequate oxygen supply, named hypoxia, is related to various pathologies, from inflammation to cancer (Möller et al., 2007; Cavalli et al., 2009). Therefore the design of delivery systems providing oxygen would be necessary (Evan et al., 2004; Wagner, 2008).

Cyclodextrin nanosponges (NS) are biocompatible nanoporous nanoparticles, obtained by the cross-linking of cyclodextrins (Trotta and Tumiatti, 2003), with the capacity of encapsulating active molecules (Cavalli et al., 2006; Trotta and Cavalli, 2009; Swaminathan et al., 2007, 2010) due to the cooperation of cyclodextrin (CD) cavities and cross-linker network. NS high capacity for CO_2 and methylcyclopropene storage was previously observed.

In this study NS formulations were developed as oxygen delivery systems for topical applications. For this purpose three types of NS (α, β, γ NS) using α, β, or γ-CD cross-linked with carbonyldiimidazole (1:4 molar ratio) were synthetized. Briefly, to obtain NS, a solution of anhydrous cyclodextrin in DMF was allowed to react with carbonyldiimidazole under stirring at 80–100 °C for 4 h (Scheme 1).

Once the reaction was over the obtained solid was crashed, washed with water and recovered by filtration. Possible by products were removed by Soxhlet extraction with ethanol. The resulted material was insoluble and not swellable in all tested solvents (i.e. water, DMF, DMSO), thus proving the presence of the cross-linked network. The NS formation was also evaluated by SEM analyses. The surface areas of NS were determined using a Surface Area Analyser ASAP 2020 (Micromeritics).

For the formulation of oxygen carriers the three types of NS were pre-treated to obtain nanoparticle populations with average diameter below one micrometer. For this purpose NS were suspended in water and milled with a PM 100 ball mill (Retsch, Germany) for 30 min.

Sizes and surface charge of NS in aqueous suspensions were then determined at 25 °C using a 90 Plus instrument (Brookhaven, NY, USA) and the morphology were evaluated by transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

To prepare oxygen-encapsulating nanosponges, different quantities of αNS, βNS or γNS were weighted and introduced in a vial with filtered water. After homogenization with an high-shear mixer (Ultraturrax) at 24,000 rpm for 2 min NS dispersions were sealed and saturated with oxygen using an oxygen purge and monitoring the gas concentration up to 35 mg/l in the external aqueous phase.

The stability of the oxygen-encapsulating NS stored at 25 °C was evaluated over time by measuring the oxygen concentration into the sealed vial and the NS sizes.

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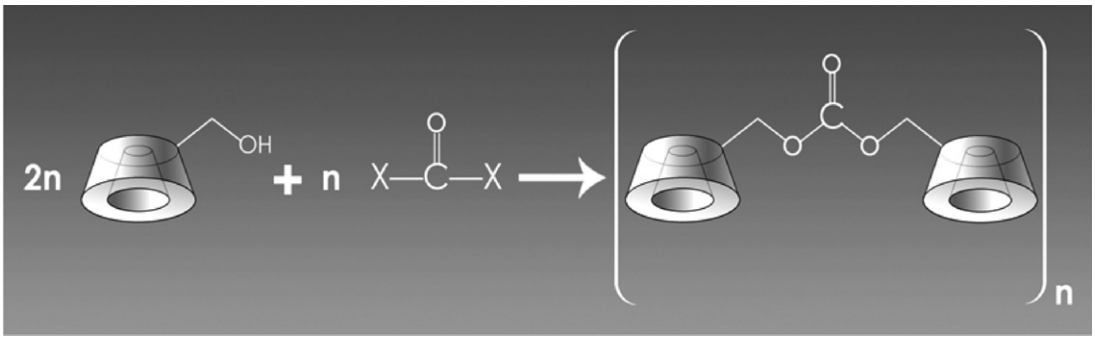

Scheme 1. Scheme of nanosponge synthesis where X is the imidazoyl group.

Oxygen-encapsulating NS cytotoxicity was determined by measuring the inhibition of cell growth using MTT assay. Vero cells were seeded in a 96-well plate using Modified Eagle Medium. After 24 h, Vero cells were treated with increasing volumes (5–10–15 μl) of oxygen-encapsulated NS, diluted in a final volume of 100 μl of medium. The cell viability was determined at 48 h measuring the absorbance at 490 nm using a 96-well plate reader.

In vitro oxygen release from the NS formulations was evaluated by injecting 10 ml of each oxygen encapsulating NS aqueous dispersion (5 mg/ml) into a small Teflon vial containing 40 ml of NaCl solution (0.9%, w/v) in hypoxic conditions (O_2 = 0.4 mg/l) and dipped into a thermostatic water bath to enable US propagation. An oxymeter was present in the vial to determine the oxygen concentration up to 60 min in the absence and in the presence of US having frequency of 2.5 MHz ± 0.4%, acoustic pressure of 2.6 MPa, D = 50% of duty cycle and applied for 15 min (n = 3).

Considering topical applications, oxygen permeation studies were then carried out using a designed apparatus consisting of two compartments separated by a silicon membrane (Fig. 1). NS were tested as aqueous nanosuspension or as gel.

Thirty ml of NaCl solution and 40 ml of hypoxic NaCl solution (0.4 mg/l) were placed in the donor and receiving compartments respectively. Then 10 ml of an aqueous dispersion (5 mg/ml) of oxygen-filled nanosponges were injected in the donor compartment and the oxygen concentration in the receiving compartment was monitored for 40 min in the absence and in the presence of 15 min US (n = 3). Oxygen permeation was then evaluated using a Pluronic F127 hydrogel (10%) containing the oxygen-filled βCD-nanosponges as donor phase (n = 3).

The NS synthetic procedure formed porous solid particles with the three types of CD ranging from colloidal to micrometer sizes as shown by SEM analysis (Fig. 2a) and having surface area values between 40 and 50 m^2/g. Studies are in progress to determine the pore sizes of the different types of NS.

After milling the average diameter of the three oxygen-encapsulating NS ranged between 400 and 550 nm with narrow size distribution and high zeta potential values (−30 mV), which can prevent NS aggregation. TEM (Fig. 2b) showed regular spherical shape of all NS confirming their sizes.

The oxygen-encapsulating NS showed no toxicity toward Vero cells after 48 h of incubation at each concentration tested. Fig. 3 reports the results obtained for βNS formulation.

No aggregation over time and no changes of oxygen concentration in the aqueous phase were evidenced storing NS suspensions at 25 °C for 15 days in sealed vials.

The oxygen concentration released from the three NS, diluted in hypoxic solutions is reported in Fig. 4. The three release profiles showed an initial burst effect with a rapid oxygen delivery up to about 6 mg/l followed by a slower and prolonged release over time (Fig. 4).

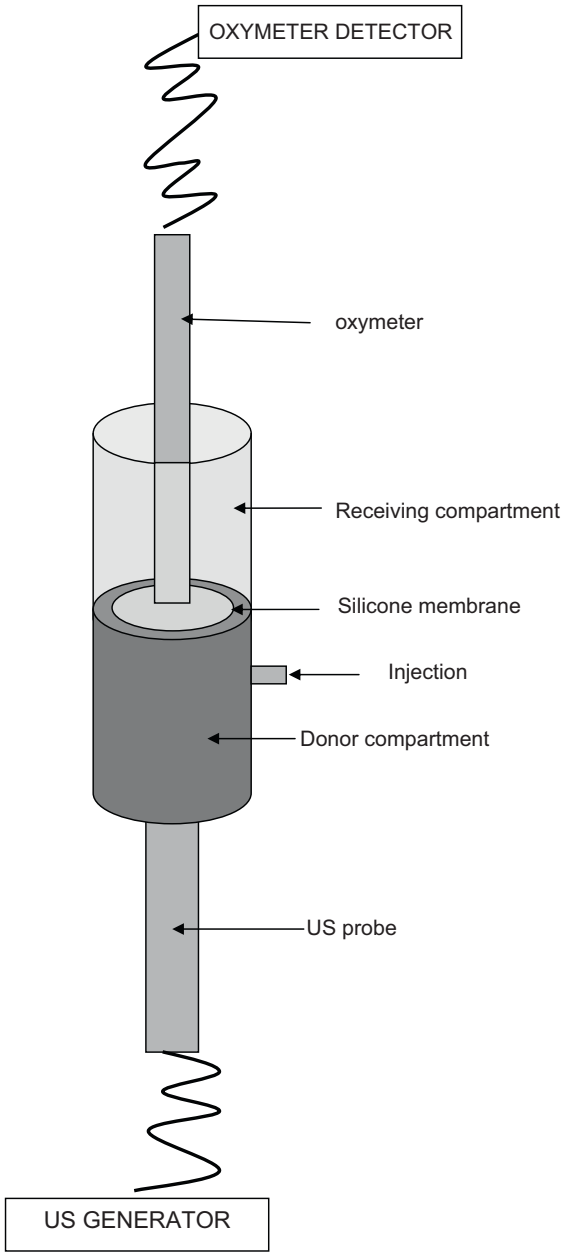

Fig. 1. Scheme of the apparatus for oxygen permeation studies.

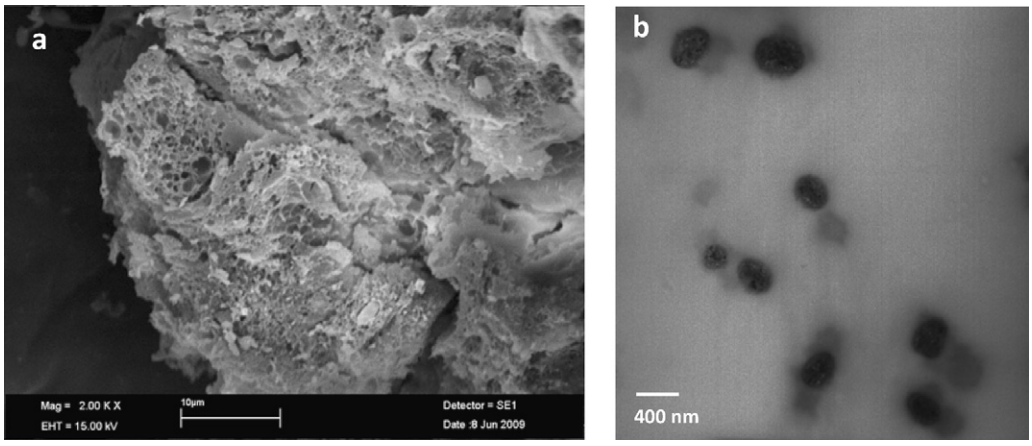

Fig. 2. (a) SEM image of βNS and (b) TEM image of oxygen encapsulating βNS (magnification 35,000×).

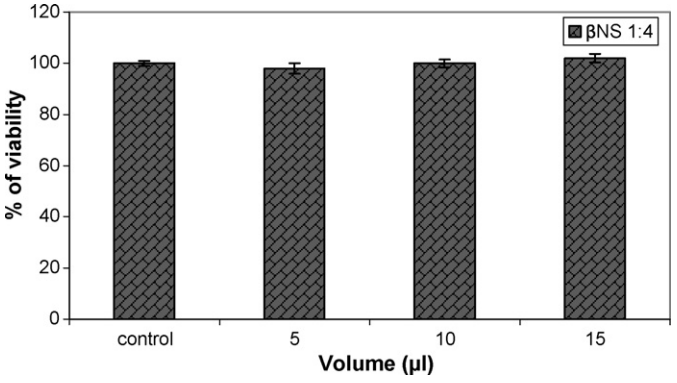

Fig. 3. Effect of βNS formulations at different concentrations on Vero cells viability after 48 h. Each point represents the mean ± S.D. ($n = 3$).

US increased of about 58% ± 2% (after 4 min of sonication) the gas release from the NS. Fig. 5 reports the oxygen release profile from βNS in the presence and in the absence of US. Statistically comparable results were obtained with α and γ nanosponges. For comparison the experiment was also carried out using oxygen not encapsulated in nanosponges. The release profile of the oxygen solution showed a different kinetic reaching about 3 mg/ml. Moreover US had no effect on oxygen solution injected in the absence of NS proving the formulation ability as gas carrier.

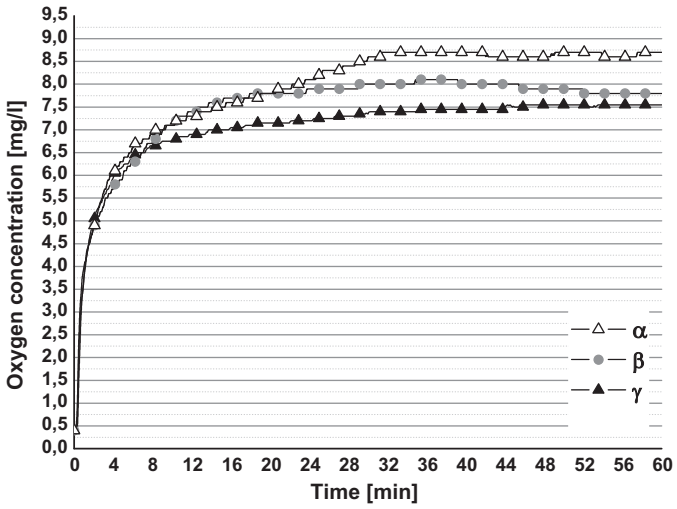

Fig. 4. Oxygen release from nanosponges in hypoxic condition (standard error = 0.1 mg/l).

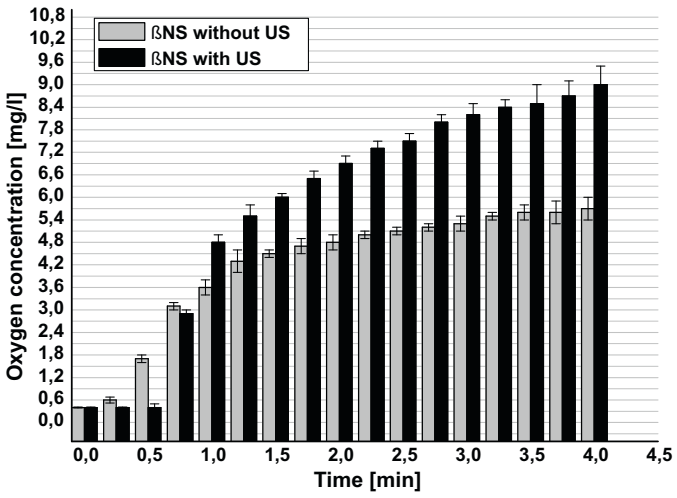

Fig. 5. Oxygen release from βNS formulations over time in the presence and in the absence of US (standard error = 0.1 mg/l).

The oxygen permeation profiles from the NS aqueous suspensions are reported in Fig. 6 showing that βNS favoured a higher oxygen permeation than αNS and γNS after 40 min.

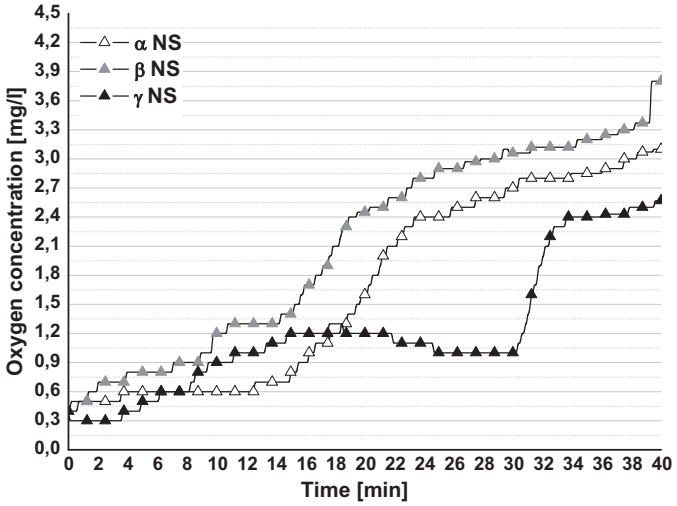

Fig. 6. Oxygen permeation through a silicone membrane in the absence of US (standard error = 0.1 mg/l).

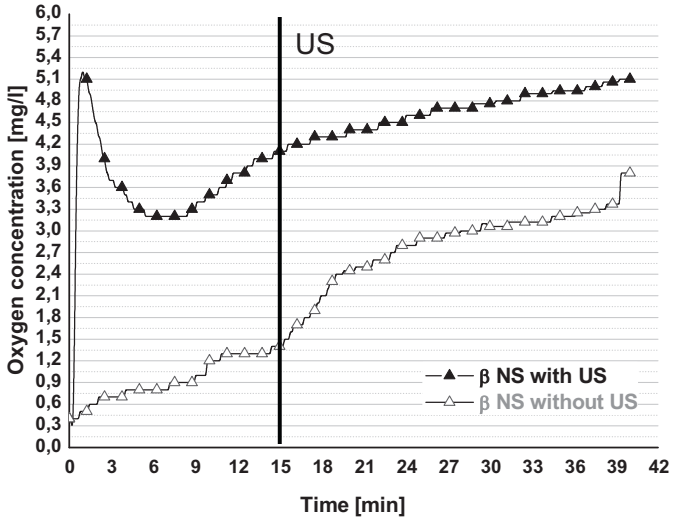

Fig. 7. Oxygen permeation through a silicon membrane in the absence (standard error = 0.1 mg/l) and in the presence of US (standard error = 0.2 mg/l) (βNS formulation).

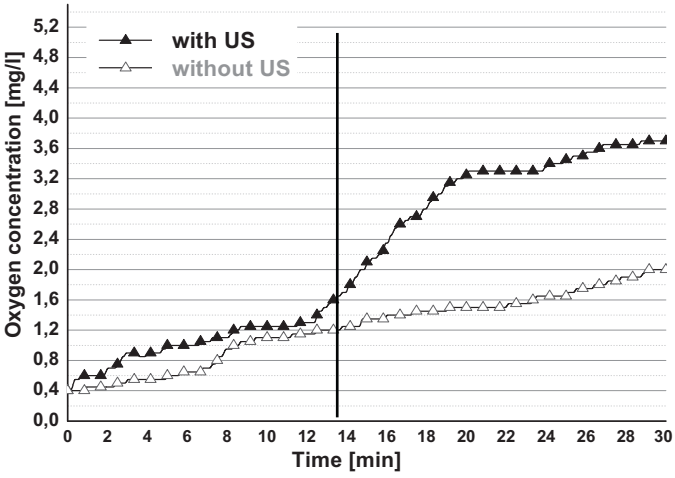

Fig. 8. Oxygen permeation using the BNS gel in the absence and in the presence of US.

Consequently we focused our attention on βNS because of their higher capacity to promote gas permeation through a silicone membrane.

The application of US on βNS aqueous suspension produced an oxygen permeation increase of about 192% ± 2% after 15 min (Fig. 7) with an initial peak of the gas permeated. This profile was not observed using an oxygen saturated saline solution as control.

To improve the topical application we developed a formulation using the combination of oxygen-encapsulating NS and Pluronic F127 hydrogel. This NS gel produced a regular sustained oxygen release in the presence and in the absence of US (Fig. 8). The presence of US increases the oxygen release of 89.7 ± 2% after 30 min.

In conclusion NS were able to encapsulate, store and release oxygen for prolonged period.

US enhanced the *in vitro* release and the permeation of oxygen. The NS/hydrogel system produces a slower sustained release. Therefore NS might be suitable carrier for oxygen topical delivery in the presence and in the absence of US and might act as oxygen reservoir.

Acknowledgements

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