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ORIGINAL ARTICLE

Cyclodextrin nanosponges as effective gas carriers

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Abstract Cyclodextrin based carbonate nanosponges were synthetized starting from native β-cylodextrin and active carbonyl compounds i.e. carbonildiimidazole. In this work they were used to form inclusion complexes with three different gases i.e. 1-methylcyclopropene, oxygen and carbon dioxide. The encapsulation of gases were proved by direct reaction to known adduct (1-methylcyclopropene), by gravimetric analysis (CO_2) and by oxymeter (Oxygen). The complexetion of oxygen or carbon dioxide could be useful for many biomedical applications. In particular the oxygen-filled nanosponges could supply oxygen to the hypoxic tissues which are present in various deseases. 1-methylcyclopropene included in β-cyclodextrin nanosponges showed superior antiethylenic performances in long lasting cut flowers in comparison with marketed products.

Keywords Nanosponges · Cyclodextrin · Gas encapsulation · Inclusion compounds

Introduction

Gases compose the atmosphere and play a relevant role in biology, medicine, cosmetic, science technology and agriculture. Chemical and medical industries employ oxygen and carbon dioxide [1]. CO_2 storage is also involved in reducing greenhouse effect on Earth [2]. On the other hand, agrochemistry uses several gases in agricultural field among this 1-methylcyclopropene is a particular useful antiethylenic compound [3].

Several porous materials are employed as gas container: zeolites [4], clathrates [5], calixarenes and carcerands [6], cucurbiturils [7], carbon nano tubes [8] etc.

Cyclodextrins can store gases in their cavity. Molecular encapsulation of gases in cyclodextrins were first studied by Cramer and Henglein [9]. Since gases have low molecular weight and small size consequently α-cyclodextrin was the cyclodextrin of choice for this application. By bubbling various gases such as chlorine, krypton, methane, ethylene, ethane, propane, xenon, oxygen in α-cyclodextrin water solution at 7–120 atm crystalline gas complexes were isolated [10]. The amount of gas complexed vary generally between 0.3 and 1.2 mol gas/mole α-cyclodextrin.

Moreover, crystalline complexes were found to be stable for months at room temperature. On the other hand the gas was easily evolved simply by dissolving the crystalline complex in water.

Due to the higher dimension of the inner cavity, β-cyclodextrin (β-CD) generally do not fit the requirements to host gases. Recently, it was reported the synthesis of new β-cyclodextrin cross-linked polymers called nanosponges [11, 12]. These latter denote superior complexing abilities towards many molecules in comparison with pristine β-cyclodextrin [13]. In this paper we would like to report preliminary results on complexation abilities of cyclodextrin nanosponges in respect to 1-methylcyclopropene, carbon dioxide and oxygen.

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Materials and methods

Materials

β-CD was a kind gift from Wacker Chemie (Germany); Carbonyldiimidazole, decafluoropentane, Pluronic F127, PEG 400, DMF, ethanol and other reagents were purchased by Sigma- Aldrich and used withour further purifications.

SmartFresh^{TM} containing 1-MCP/α-cyclodextrin complex was by AgroFresh Inc. (Rohm and Haas Co., Springhouse, Pa., U.S.A. Carbon dioxide and oxygen was from Siad.

Synthesis of β-CD nanosponges

β-CD nanosponges were synthesized following the procedure reported elsewhere [11]. Briefly, anhydrous β-cyclodextrin was dissolved in dry DMF and allowed to react with carbonyldiimidazole at 90 °C for 3 h. The reaction was carried out using a crosslinker excess, at three different molar ratios, e.g. 1:2, 1:4, 1:8 (β CD:cross-linker). Once the reaction was completed, the solid was ground in a mortar, prolonged washed with deionized water and ethanol and finally, Soxhlet-extracted with ethanol to remove possible low molecular weight by-products. The same procedure as used with α cyclodextrin, but using only 1:4 the cross-linking ratio.

Nanosponge characterization

Particle sizes and surface charge of NS in aqueous suspensions were determined by laser light scattering (LLS) at 25 °C using a 90 Plus instrument (Brookhaven). The NS morphology was evaluated by Trasmission Electron Microscopy (TEM) using a CM10 instrument (Philips) and Scanning Electron Microscopy (SEM) using a Stereoscan (Leica).

CO_2 encapsulation in nanosponges

CO_2 adsorption isotherms at 20 °C were obtained by using 5.0 grade CO_2 (99.9999%V; Rivoira). An intelligent gravimetric analyzer (IGA-002) supplied by Hiden Analytical Ltd, UK was used allowing to work in the 0–1 bar range and to get the 20 °C temperature through the use of a circulating water bath system. The samples (120 $\pm$ 10) mg were outgassed at room temperature and at 120 °C until no further weigh loss was observed. Buoyancy corrections were carried out using the weights and densities of all the components of the sample and counterweight sides of the balance and the measured temperature. The density of the samples has been determined by helium (5.5 grade, Rivoira) isotherms conducted on the samples at 293 K in the 0–20 bar range.

1-methylcyclopropene encapsulation in nanosponges

1-methylcyclopropene was synthetized according to the literature [14] with minor modifications. The yield was determined by the Diels–Alder adducts of 1-(trimethyl silyloxy)-1,3-butadiene with 1-MCP. The crude was analyzed by GC-MS. (Agilent 6890 GC system coupled with Agilent 5973 Mass Selective Detector). Online encapsulation of 1-methylcyclopropene in the selected nanosponge was made by directly bubbling 1-methylcyclopropene in water suspension of nanosponge at sub-ambient temperature.

Commercial available SmartFresh^{TM} containing 1-MCP/α-cyclodextrin was used for comparison purpose.

Oxygen encapsulation in nanosponges

Three oxygen encapsulating formulations were developed by using the following procedure. Weighed quantities of. sodium chloride (0.27% w/v), water 27.16% (w/v,) 1:4 β-CD nanosponges (0.67% w/v) were introduced in a three necked round bottom flask. Then decafluropentane (1.34% w/v) and (PEG 400, 0.564% w/v), were added dropwise under continuous stirring for 15 min. After the complete addition the mixture was saturated with oxygen by using an oxygen gas purge. The gas concentration was monitored using an oxymeter (Portamess 913 OXY, Germany) up to an oxygen concentration of 35 mg/l. Then a high-shear mixer (Ultraturrax, Germany) was used for two minutes continuing the O_2 purge to favour oxygen encapsulation into nanosponges. Similar formulations using β-CD nanosponges with 1:2 crosslinking ratio and α-CD-nanosponges with 1:4 crosslinking ratio were prepared using the same procedure.

Considering a possible biomedical application the haemolytic activity of the three formulations was evaluated on human blood. Different percentages (3.8, 7.6, and 11.4% v/v), of oxygen encapsulating nanosponges were added to an erythrocytes suspension (30% v/v) in phosphate buffer pH 7.4. After 90 min of incubation at 37 °C, the samples were centrifuged (at 1000 rpm) and the supernatant was analysed spectrophotometrically at $\lambda = 543$ nm. The haemolytic activity was calculated with reference to blank and complete haemolysed samples induced by addition of ammonium sulfate 20% w/v.

The oxygen release capacity of nanosponge formulations were determined by injecting 10 ml of an oxygen encapsulating formulation into a small Teflon container and dipped into a thermostatic water bath to enable ultrasound (US) propagation. The oxygen release was evaluated at fixed time determining the oxygen content in the solution

using the oxymeter. The oxygen content was also measured after sonication lasting 10, 20, 30, 40 and 60 min using a ultrasound (US) probe having frequency = 2.5 MHz, 260 W peak power.

The oxygen release from nanosponges was also studied combined with a peculiar apparatus consisting of two compartments separated by a silicon membrane in permeation studies. 50 mL of saline solution and 10 mL of oxygen filled nanosponges were placed in the first compartment; 50 mL of saline hypoxic solution (0.4 mg/L) were placed in the second compartment. The concentration of oxygen in the hypoxic compartment was monitored for 60 min by the oximeter in the absence and in the presence of US. To monitor the oxygen concentration, the transducer of the device was held in a fixed position, within the donor compartment. All the experiment were performed in triplicate.

Results and discussion

Safe, easy, cheap storage, transportation and management of gases is a crucial task of modern chemistry and technology [15]. The encapsulation of gases in nanostructurated materials is of great interest in many applications i.e. hydrogen reservoir for fuel cells, methane chlatrate for hydrocarbon storage etc. Cyclodextrin-based nanosponges obtained by the cross-linking of cyclodextrin with carbonildiimidazole are a nanostructured solid with a good complexation capacity for different types of molecules, even for gases. They showed a rather spherical shape as observed by SEM (Fig. 1). The present work shows three possible applications of nanosponges as gas carriers.

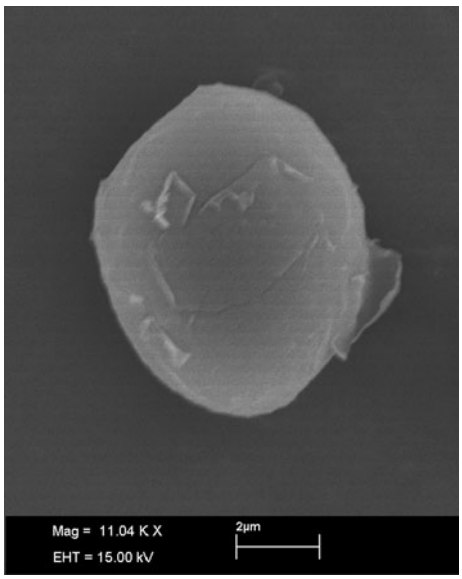

Fig. 1 SEM image of β-CD nanosponges

Senescence in flowers, fruits and vegetables is associated with an increase in ethylene gas production. Among the many compounds proposed as antiethylenic action 1-methylcyclopropene leads to better performance. However, it is rather unstable and has high volatility since 1-methylcyclopropene (1-MCP) can prevent some of the effects of ethylene without any toxicity, but often does so only for a rather short period of time [16].

Due to higher dimension of the inner cavity, native β-cyclodextrin, unlike α-cyclodextrin in SmartFresh^{TM}, unfits well 1-MCP molecule. We optimized the synthesis of 1-MCP and formed the inclusion complex with cyclodextrin nanosponges simply by bubbling on line prepared 1-MCP in a water dipersion of nanosponges in an ice bath. The molecular inclusion was repeated with different nanosponges:sinthesized with a different ratio of CD/crosslinking agent (1/2, 1/4 or 1/8). The content was determined by the Diels–Alder adducts of 1-(trimethyl silyloxy)-1,3-butadiene with 1-MCP. The crude was analyzed by GC-MS. The inclusion compound is rather stable. Table 1 accounts for this statement. The efficacy of this new formulation of 1-MCP was tested on cut flowers and the results related to level 3 of senescence (0-5 scale) that represents the loss of ornamental value. As it is reported in Fig. 2, 1-MCP encapsulated in β -cyclodextrin nanosponges shows superior performace in comparison with marketed Smartfresh^{TM} even at lower concentration of the active molecule.

CO_2 has several beneficial physiological effects namely blood vessel dilation, blood circulation improvement and activation of gastrointestinal movement. In view of that, encapsulation of CO_2 with CDs was earlier patented in Japan in 1987 anticipating its uses in cosmetics, cleansing and personal care products. A bath salt formulation incorporating CO2/α-CD complex was patented by Kao Corporation Japan. CO_2 is of interest even for some food confections.

Beside, CO_2 shows great affinity towards α-cyclodextrin being used to selectively precipitate α-cyclodextrin from the cyclodextrin mixture [17].

Nevertheless, CO_2 is encapsulated in α-cyclodextrin cavity by working with an overpressure i.e. 30 atm. In this

Table 1 Amount of 1-MCP loaded on different nanosponges. Ice bath. 1 atm

Support	% of 1-MCP in NS measured after inclusion (w/w)	% 1-MCP in NS measured after 4 months storage at r.t in open vial (w/w)
NS 1:4	8.1	3.7
NS 1:8	8.2	3.8
NS 1:2	9.1	4.6

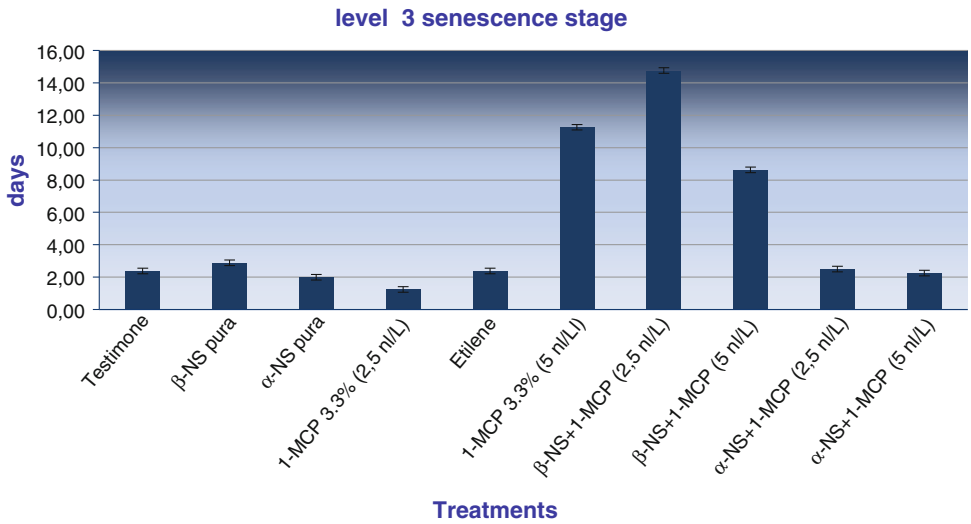

Fig. 2 Long lasting activity of 1-MCP complexed with cyclodextrin nanosponges on cut flowers

case crystalline inclusion compound was achieved with around 1:1 molar ratio between CO_2 and α-cyclodextrin. The moisture content also affect the amount of entrapped CO_2 [18].

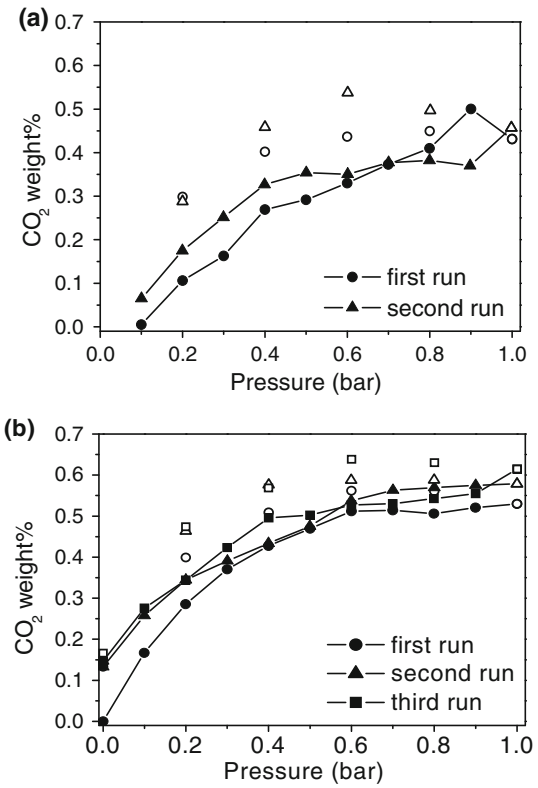

Fig. 3 Excess CO_2 gravimetric isotherms recorded at 20 °C on the *β*-Cyclodextrin nanosponge sample after thermal treatment at (**a**) RT and (**b**) 120 °C. The CO_2 mass% is referred to the mass of the sample after the thermal treatment. Filled and empty scatters refer to the adsorption and desorption branches respectively. Between each adsorption–desorption cycle, a degassing was performed for 5 h at 20 °C

As reported in Fig. 3, *β*-cyclodextrin nanosponges are effective material to retain CO_2 even at atmospheric pressure and room temperature.

The *β*-Cyclodextrin nanosponges were degassed at 20 °C and at 120 °C in order to verify the effect of the treatment temperature on the CO_2 adsorption process. The isotherms obtained at 20 °C are reported in Fig. 3. The stored CO_2 amounts to 0.4 ± 0.1 mass% for the 20 °C treated sample and to 0.6 ± 0.1 mass% for the one treated at 120 °C. In both cases, the kinetics of the CO_2 adsorption was rather slow, making necessary the use of an equilibration time of 4 h for each point of the isotherms. The adsorption/desorption run was repeated twice for the 20 °C treated sample and three times for the one treated at 120 °C. In fact, whereas for the former a good coincidence of the first and second runs indicates the good reverisibility of the adsorption process, for the sample treated at 120 °C,

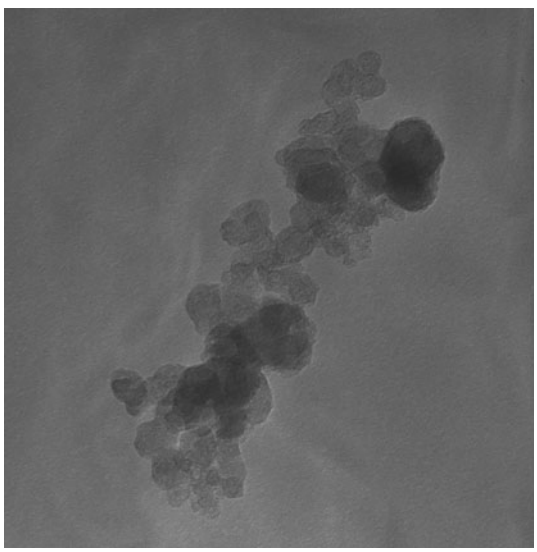

Fig. 4 TEM image of oxygen-encapsulating *β*-cyclodextrin nanosponges (magnification 46000)

Table 2 Characteristics of the oxygen-loaded nanosponges

Formulation	Diameter ± SD	Polidispersity index	Zeta potential
β-CD nanosponges 1:4	409 ± 60.9	0.19	15 ± 1.6
β-CD nanosponges 1:2	523 ± 32.5	0.23	19 ± 1.3
α-CD nanosponges 1:4	602 ± 48	0.20	−23 ± 1.0

a partial hysteresis between the first and the second cycle indicate that 0.1 mass% of CO_2 was irreversibly stored in the sample. The coincidence of the second and third cycle is a further indication of that. CO_2 entrappement in nanosponges is particularly strong and a significant amount of CO_2 is retained even at 373°K for 36 h under vacuum.

Although the entrapped amount is 1/5 of that reported for α-cyclodextrin, we underline that in the case of nanosponges milder reaction conditions are used and higher pressure experiments are in progress.

All types of nanosponges showed the capacity of encapsulate and store oxygen. Previously we evaluated the behaviour of oxygen filled nanosponges in the presence and in the absence of US observing [19] that NS can act as oxygen reservoir. In the present work perfluorodecan was added to the NS formulation to increase the amount of gas stored. Perfluorocarbon have the capacity of dissolve and retain oxygen [20].

Considering the potential in vivo application of this type of NS formulation the sizes and biocompatibility of the nanosponge were initially determined. Nanosponge sizes were previously reduced using a ball mill (PM 100, Retsch). The size analysis of oxygen encapsulating nanosponges showed that their average diameter is between about 400 and 600 nm. The particle size distribution was unimodal with a narrow distribution with a spherical morphology as show by TEM analysis (Fig. 4). The nanosponge formulations showed sufficiently high Zeta potential values, which prevent the formation of nanosponge aggregates, as reported in the Table 2.

The haemolytic activity of all the formulation was negligible proving their safety. The oxygen is released from β-CD nanosponges formulations, over a prolonged period, i.e. 60 min, starting from hypoxic condition (0.4 mg/L). The oxygen release profiles indicate that there is an initial burst effect and then the oxygen release is maintained with a sustained profile for 60 min (Fig. 5). In the presence of US an increased release was observed reaching a concentration of 7 mg/L after 1 h. The same release behaviour was observed in the presence of α-CD nanosponge formulation and the other β-CD nanosponge (1:2 ratio).

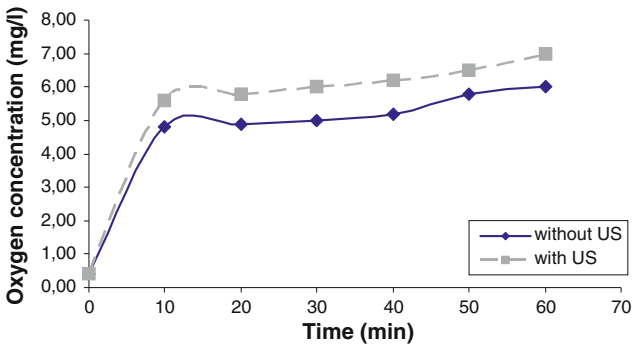

Fig. 5 Profile of Oxygen release from β-cyclodextrin nanosponges

It was possible to modulate the gas release coating the gas filled nanosponges. A decrease of the oxygen release was obtained by the coating of nanosponges with Pluronic F127 (data not shown).

Oxygen permeation through a silicone membrane was observed after the application of the nanosponge formulation in the donor compartment (Fig. 6). To increase the kinetics release Ultrasounds (US) were applied. The

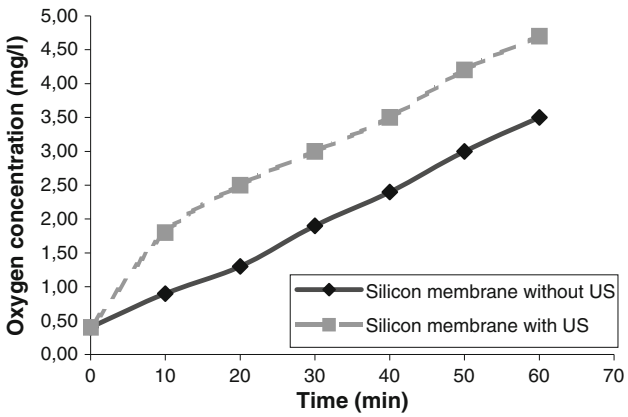

Fig. 6 Oxygen release from β-cyclodextrin nanosponges through Silicon membrane

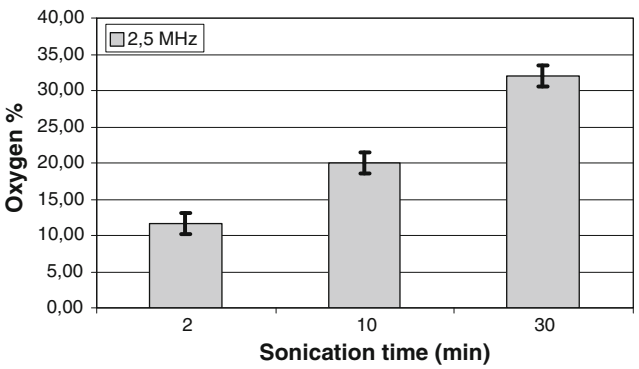

Fig. 7 Effect of sonication (US) on O_2 release from β-cyclodextrin nanosponges

oxygen concentration in the receiving compartment increased from 0.4 mg/L to about 3.5 mg/L after 60 min in the absence of US, while in the presence of US reached a concentration of 4.7 mg/L. The use of Ultrasounds (US) enhanced the oxygen permeation. US sonication applied on the surface of the siliconic membrane produced an increase of approximately 30% in oxygen release from the β-CD nanosponges formulations as shown in Fig. 7.

Conclusions

Newly synthetized cyclodextrin carbonate nanosponges are able to host some gases. Among them 1-methylcyclopropene, carbon dioxide and oxygen.

1-methylcyclopropene/NS complex show superior performance in long lasting cut flowers even in comparison with commercially available products.

CO_2 could be entrapped in β-cyclodextrin carbonate nanosponges even at atmosheric pressure and room temperature. This latter entrappement is particularly strong and a significant amount of CO_2 is retained even at 373°K for 36 h under vacuum. Moreover nanosponge formulations were able to encapsulate oxygen and release it for long period. The oxygen delivery can be increased using US as external stimulus.

This peculiar property of nanosponges might be a promising tool for gas delivery.

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