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Cyclodextrin-based Nanosponges for Drug Delivery

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Abstract

Nanosponges were prepared from β-cyclodextrins as nanoporous materials for possible use as carriers for drug delivery. The structure of β-cyclodextrin-based nanosponges was principally investigated by FT-IR, DSC and RX analyses. Sizes, morphology and toxicity were also examined. The capacity of the nanosponges to incorporate molecules within their structure was evaluated using drugs with different structures and solubilities. The nanosponges were found capable of carrying both lipophilic and hydrophilic drugs and of improving the solubility of poorly water-soluble molecules.

Introduction

Nano-sized colloidal carriers have recently been developed and proposed for drug delivery, since their use can solubilize poorly water-soluble drugs and provide prolonged release, as well as improving a drug's bioavailability and in some cases modifying its pharmacokinetic parameters. They can also decrease side-effects and protect drugs from degradation. Among colloidal carriers, liposomes, microparticles and nanoparticles have in particular been described as a new technological approach to drug administration. Cyclodextrins and their derivatives have been used as solubilizers to enhance the loading capacity of liposomes, microparticles and nanoparticles [1]. Entrapment of cyclodextrin inclusion complexes in liposomes [2] increases the drug-to-lipid mass ratio and enlarges the number of insoluble drugs that can be incorporated. The first studies on the role of cyclodextrins in microparticle preparation were by Loftsson and co-workers [3]. Solid lipid nanoparticles (SLN) have since been prepared as carriers of drug complexes of β-cyclodextrin, and showed good loading capacity and slower drug release [4].

Polymeric nanoparticles can also contain cyclodextrins:nanoparticles of poly (butylcyanoacrylate) have been prepared in the presence of cyclodextrins [5], and modified cyclodextrins have been used as matrices to obtain nanoparticulate systems. Particularly, amphiphilic cyclodextrins have been used to form nanoparticles; SLN based on amphiphilic supramolecular derivatives of cyclodextrins and loaded with drugs have been recently studied [6] Nanospheres and nanocapsules have also been made from cyclodextrins modified on the secondary face with C_6 aliphatic esters using the nanoprecipitation technique [7].

The aim of this research was to prepare and characterize a new nanoparticulate system, i.e. cyclodextrin-based nanosponges, which behave as innovative drug carriers.

Experimental

β-CD was a kind gift from Wacker Chemie (Germany); dexamethasone and flurbiprofen were purchased from Fluka (Buchs, CH). Doxorubicin hydrochloride was a kind gift from Pharmacia. All reagents (ACS grade) were from Sigma (USA) and were used without further purification.

β-CD nanosponges were synthesized following the procedure reported elsewhere [8]. Briefly, anhydrous β-cyclodextrin was put to react in melted diphenylcarbonate at 90 °C for 5 h. Once the reaction was complete, the solid was ground in a mortar and Soxlet-extracted with ethanol to remove unreacted diphenylcarbonate and the phenol produced.

FT-IR analyses of nanosponges were carried out on a Perkin-Elmer system 2000 spectrophotometer. Thermal analyses were performed using a DSC 7 system (Perkin Elmer); for this purpose samples of 3–5 mg were weighed in aluminum sample pans and then heated at 10 °C/min under nitrogen purge in the range 20–350 °C. The powder X-ray diffraction patterns were obtained

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using a Huber diffractometer. The nanosponge samples were analyzed in the 2θ angle range of 5–50°.

The sizes and polydispersity indices of nanosponges were determined after their dispersion in water and in physiological phosphate buffer (pH = 7.4) by photon correlation spectroscopy (PCS) using an N4 MD instrument (Coulter) at a fixed angle of 90° and at a temperature of 25 °C. Each sample was analyzed in triplicate.

The nanosponge morphology was evaluated by optical microscopy by using a Leitz inverted microscope. The particle size and shape of nanosponges were also confirmed by Transmission electron microscopy (TEM) analyses (Philips CM10 instrument). The electrophoretic mobility and zeta potential were measured using a 90 PLUS instrument (Brookhaven Instrument Corporation, NY, USA). To determine the zeta potential, samples of nanosponge dispersions in water were diluted with KCl (0.1 mM) and placed in the electrophoretic cell, where an electric field of about 15 V/cm was applied. Each sample was analyzed in triplicate.

Haemolysis experiments were done on human erythrocytes. Briefly, increasing concentrations of nanosponges, up to 20 mg/mL, were added to blood and the mixture incubated for 90 min at 37 °C and then centrifuged at 2000 rpm for 5 min. The supernatants were analyzed spectrophotometrically at 543 nm and the percentage of haemolysis was determined against a sample completely haemolysed. Cytotoxicity experiments were performed on HT29 cell lines and lasted 24 h. Cell viability was determined at two nanosponge concentrations (7.5 and 15 μg/mL) using the Tryphan Blue Assay.

For the loading experiments, an excess of drug was incubated with a water dispersion of cyclodextrin nanosponges (10 wt%). After shaking for 24 h at room temperature, nanosponge aliquots were washed, filtered and freeze-dried. The lyophilized product was used to determine the amount of drug present by suitable HPLC methods.

In vitro release kinetics experiments were performed using a multi-compartment rotating cell: 1 mL of aqueous dispersion of nanosponges containing one of the three drugs was placed in the donor compartment, while the receptor compartment, separated by a hydrophilic dialysis membrane (cut-off 12000 D Dialysis tubing, Sigma Chem. Co., St. Louis, USA), was filled with phosphate buffer at pH 7.4 or at pH 1.2. Each experiment lasted for 2 h. At fixed times, the receptor buffer was completely withdrawn and replaced with fresh buffer. The amount of drug in the nanosponge dispersion was determined by suitable HPLC methods.

Results and discussion

In the recent years, β-cyclodextrin-based nanosponges have been synthesized and studied for their potential application in water purification processes. They have shown superior decontamination capability *versus* classical active carbon, in particular for removing polychlorinated biphenyls (PCBs) and persistent organic pollutants (POPs), but they can also be used in other applications such as cosmetics (fragrance release) agriculture (controlled release of crop products) analytical (HPLC stationary phase) etc. In the pharmaceutical field, in particular, they could be employed as solubilizing agents or nanocarriers.

In this research a new drug-delivery system, based on cyclodextrin nanosponges, was prepared and characterised as an innovative nanoporous material. The nanosponges contain β-cyclodextrins as building-blocks, linked with carbonate groups to form a high cross-linked network. The reaction is very simple and carried out under relative mild conditions. The final nanosponge structure contains both cyclodextrin lyphophilic cavities and carbonate bridges, leading to a network of more hydrophilic channels.

FT-IR results confirmed the presence of carbonate groups in the nanosponge structure at 1700 cm^{-1}.

Nanosponges are solid, insoluble in water, and rather crystalline. The diffractogram reported in Figure 1 clearly shows that they present a crystal structure alongside an amorphous phase. Moreover, the DSC analysis of nanosponges showed no peak before 320 °C, meaning that this material has high thermal stability. If ultrasound and a suitable cyclodextrin/cross-linker agent ratio are applied it is even possible to prepare nanosponges with average diameter below 1 μm and narrow size distribution, as well as being spherical in shape, as clearly appears in the optical microphotograph in Figure 2. TEM analyses also support these observations.

Zeta potential of nanosponges was also determined, as a measure of surface charge. The value obtained was about −30 mV, which means that the particles have little tendency to aggregate.

They were not haemolytic up to 15 mg/mL, and preliminary results revealed no significant cytotoxic effect of nanosponges at two selected concentration on cell HT29 cultures after 24 h incubation (Figure 3).

The effect of cyclodextrin nanosponges on drug solubilization was evaluated on both lipophilic and hydrophilic molecules Three model drugs were selected, dexamethasone, flurbiprofen and doxorubicin hydrochloride, with different structure and solubilities (Figure 4). Dexamethasone and flurbiprofen are lipophilic drugs whose log$P$ values are 1.9 and 4.1, respectively, while doxorubicin hydrochloride is a hydrophilic drug with a log$P$ value of −0.25.

The results of loading experiments showed that nanosponges may be used as drug carriers. Due to their structure they can include either lipophilic drugs (e.g. dexamethasone or flurbiprofen) or hydrophilc drugs (e.g. doxorubicin) showing good solubilization capacity. They particularly improved the aqueous solubility of the lipophilic drugs: the percentage

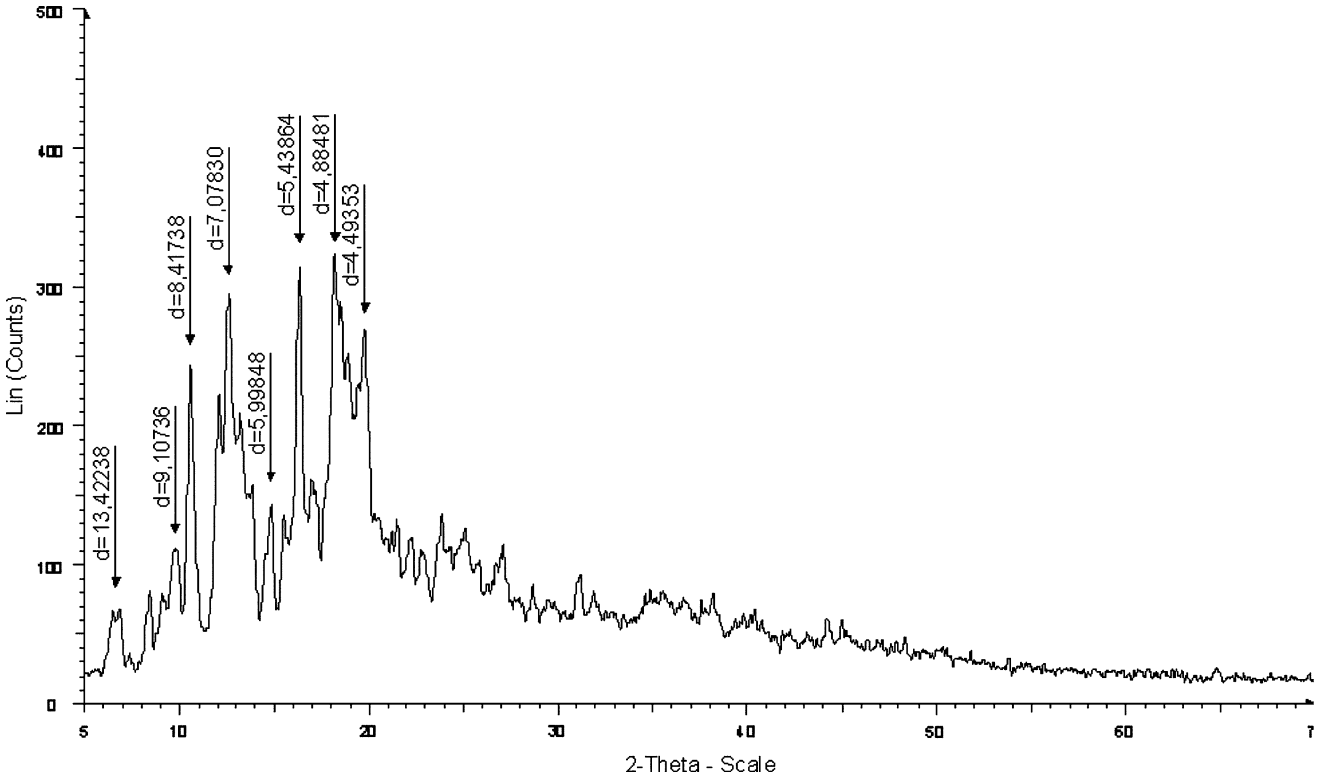

Figure 1. Rx-diffractogram of β-cyclodextrin nanosponges.

incorporated was about 15 wt%, whereas it was only 4 wt% for doxorubicin hydrochloride. This different behavior could be ascribed to the higher number of lipophilic sites available for the complexation of lipophilic drugs in comparison with the hydrophilic sites. The nanosponge–drug interaction was confirmed by DSC analyses, since the thermograms did not contain the drug's melting peak. We speculate that the drugs might be molecularly dispersed in the nanosponges, the lipophilic molecules preferably in the cyclodextrin hydrophobic cavities and the hydrophilic molecules completely or partially in the surrounding network.

Drug release from the nanosponges was slow, below 10% for flurbiprofen (Figure 5) and below 20% for dexamethasone (Figure 6) after 2 h, confirming the strong interaction between the drug and nanosponge structure. The drug release profiles also depended on the physiological medium, as shown in Figure 7 for doxorubicin: in this case the drug was released very slowly at pH 1.1, while release was faster if the pH was increased to 7.4. This might mean that the drug would not be released or only slightly released during passage of the formulation through the stomach, but could be delivered directly to the duodenum, thus being available for absorption but protected from the gastric environment.

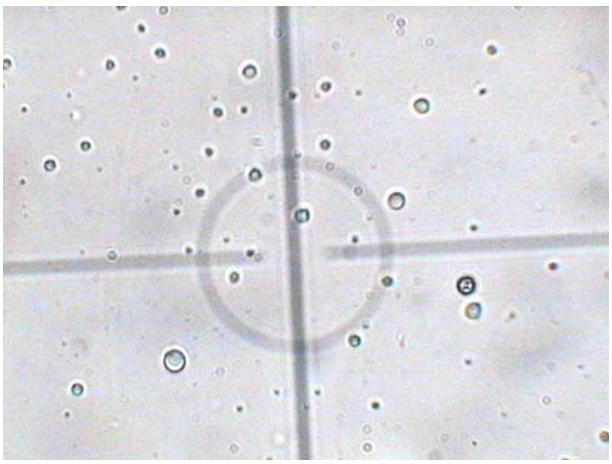

Figure 2. Microphotograph (×100) of β-cyclodextrin nanosponges.

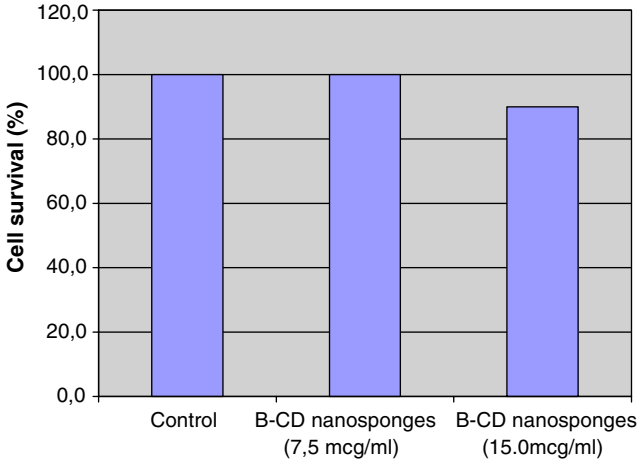

Figure 3. Cytotoxicity of β-cyclodextrin nanosponges on HT29 cells after 24 h incubation.

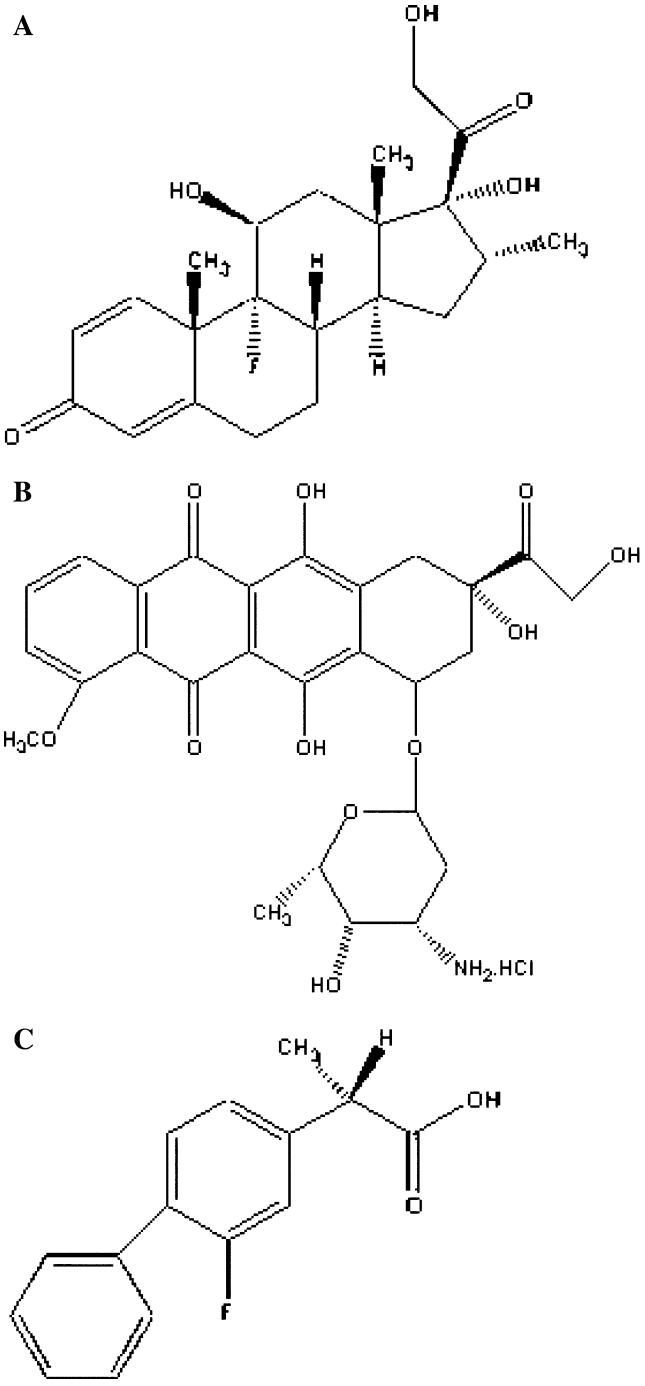

Figure 4. Molecular structures of dexamethasone (a), doxorubicin hydrochloride (b), flurbiprofen (c).

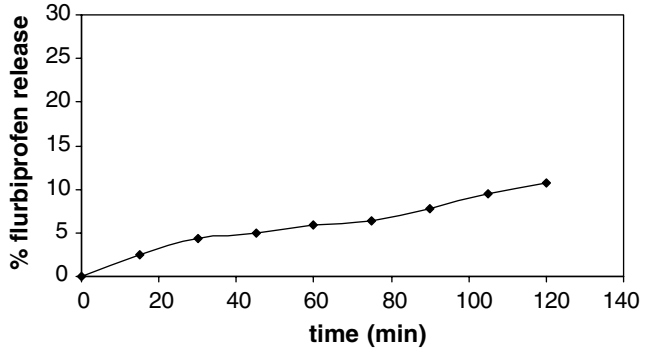

Figure 5. Flurbiprofen release profile from β-cyclodextrin nanosponges at pH 7.4.

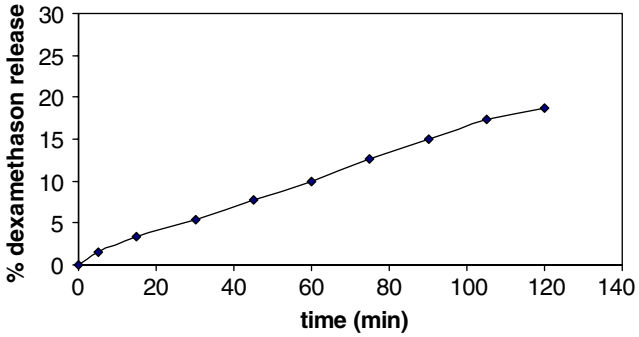

Figure 6. Dexamethasone release profile from β-cyclodextrin nanosponges at pH 7.4.

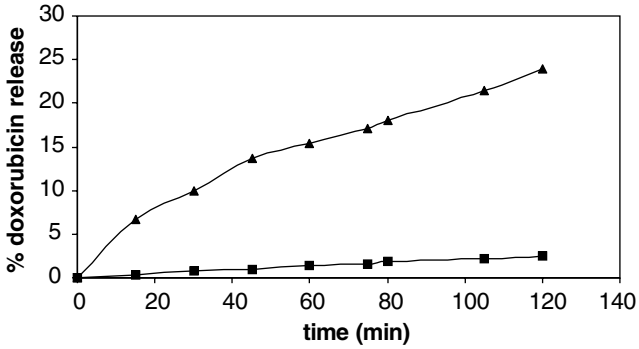

Figure 7. Doxorubicin release profiles from β-cyclodextrin nanosponges at two different pH values triangle: pH = 7.4, square: pH = 1.1.

Conclusions

Cyclodextrin-based nanosponges have the ability to include either lipophilic or hydrophilic drugs and to release them slowly into physiological media. They could be used to improve the aqueous solubility of lipophilic drugs or to protect degradable molecules. They are also small in size and spherical in shape, and would thus be suitable to formulate drug delivery systems for various administration routes beside the oral one.

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