






# Core/Shell Gel Beads with Embedded Halloysite Nanotubes for Controlled Drug Release

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**Abstract:** The use of nanocomposites based on biopolymers and nanoparticles for controlled drug release is an attractive notion. We used halloysite nanotubes that were promising candidates for the loading and release of active molecules due to their hollow cavity. Gel beads based on chitosan with uniformly dispersed halloysite nanotubes were obtained by a dropping method. Alginate was used to generate a coating layer over the hybrid gel beads. This proposed procedure succeeded in controlling the morphology at the mesoscale and it had a relevant effect on the release profile of the model drug from the nanotube cavity.

**Keywords:** halloysite; alginate; chitosan; gel beads; drug release

## 1. Introduction

Researchers have defined hydrogels in many different ways, but nowadays the most accepted definition is the existence of a three-dimensional network, formed by the cross-linking of polymeric chains, that possesses the capability to swell thanks to the presence of hydrophilic groups and to maintain a very high amount of water in its structure [1,2]. Since their discovery, hydrogels have received attention from the scientific community due to the wide range of applications they can be used for: Environmental issues like water remediation, drug delivery systems and tissue engineering, cosmetic and food packaging industry, and oil spill recovery [3–8]. Furthermore, with the evolution of nanotechnology, the challenge to design and prepare hydrogels with specific and requested features at the nano-scale led to the development of nanohydrogels. Among the different polymeric species that can be used to achieve this aim, polysaccharides cover a marked importance, especially in the preparation of the so-called “polysaccharide-based natural hydrogels”, for some of their most peculiar properties such as water solubility and swelling capacity, biocompatibility and biodegradability, self-healing and pH sensitivity that are crucial for their use [9,10]. Moreover, the possibility to modify the structure of the polysaccharides and the adaptability of their networks allows for the development of eco-friendly smart materials [11,12]. To date, the most widely used raw materials include natural biopolymers such as chitosan, alginate, pectin and cellulose. One of the major factors limiting the use of nanohydrogels is their structural instability, thus making necessary the use, among others, of inorganic nanoparticles to overcome them [13–16].

Among clays, halloysite nanotubes (HNTs) have great importance thanks to their own main characteristics [17]. HNTs are a naturally occurring alumino-silicate whose structural formula is

$\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$ , where Al is disposed in an gibbsite-like octahedral organization of Al–OH groups whereas Si–O groups form a tetrahedral sheet [18,19]. Both aluminols and siloxanes layers are overlapped in a kaolinite typical sheet that rolls up due to some structural defects and to the presence of water molecules, thus giving halloysite its peculiar narrow nanotubular structure [20–22].

HNTs dimensions depend on the natural deposit the clay is extracted from. In particular, the internal and external diameters are approximately 10–15 and 50–80 nm respectively, while the nanotubes length can range from 100 nm to 2  $\mu\text{m}$ . [19] Interestingly, it is possible to classify halloysite by considering the distance between interlayers. For instance, it can be 7 or 10 Å depending on the number of water molecules present between the layers, which is namely 0 or 2, respectively [23,24]. Moreover, one of halloysite's most fascinating and important features is the different charge, in the pH interval from 3 to 8, between the outer surface that is mainly composed of Si–O groups and negatively charged, and the inner surface that is mainly composed of Al–OH and positively charged [25,26]. This different charge, due to the chemical composition, allows for selective functionalization, exploiting both the covalent and electrostatic interactions of each surface with other oppositely charged species: Drug molecules, polysaccharides, proteins, lipids, surfactants and so on [27–29]. All these features, and also considering that they are low cost, eco- and biocompatible materials [30], make HNTs suitable for designing hybrid materials for waste water remediation [31–35], cultural heritage treatment [36,37], biotechnological applications [38–44], and packaging [45–50].

Notably, halloysite is commonly used as a component in drug delivery systems through exploiting its characteristics in combination with other organic moieties, for example the temperature responsive polymers such as poly(N-isopropylacrylamide) (PNIPAAms) that can selectively interact with the inner/outer surfaces thus influencing the release kinetics by changing their adsorption site [51], or natural occurring biopolymers for the preparation of end capped nanotubes with smart gates, or reverse inorganic micelles for the formation of nanohydrogels inside the HNTs lumen for a triggered absorption or release [52,53].

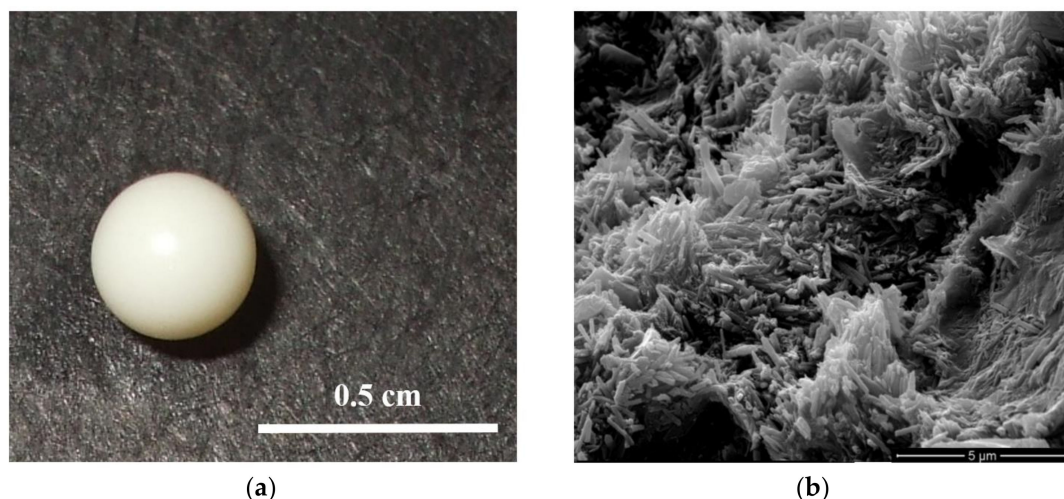
As evidenced in a recent review [17], the combination of polymer hydrogels and hollow inorganic nanotubes represents a perspective strategy for the fabrication of functional carriers in an advanced application.

In this work, we prepared hydrogel beads based on chitosan containing halloysite nanotubes. An alginate layer was introduced by diffusion and immersion of the beads in a sodium alginate solution. The dispersion of nanotubes into the hybrid gel and the localization of the alginate was investigated by SEM and fluorescence microscopy. Doxycycline, an antibiotic of the tetracycline class, was used as the model drug and it was loaded into the halloysite cavity by using a literature protocol [54]. This work represents a promising step for a valid alternative to generate hybrid hydrogels with oppositely charged polysaccharides and nanoclay with specific morphology for controlled drug release.

## 2. Results and Discussion

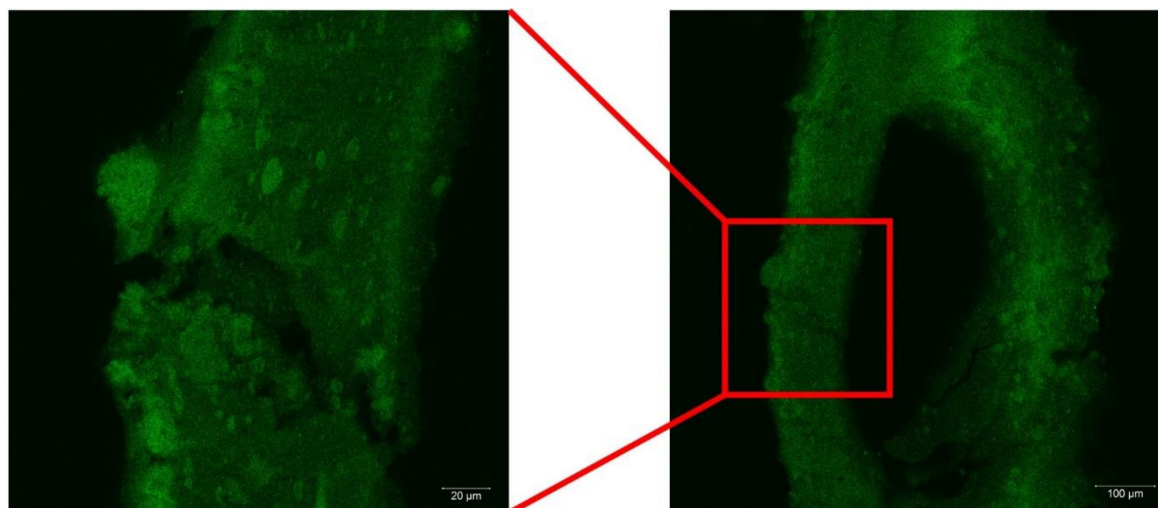
### 2.1. Morphology of the Hybrid Gel Beads

Figure 1 shows the optical image of the prepared chitosan/HNTs gel beads covered with a calcium alginate layer. They had an average diameter of 3.5 mm that shrunk to 0.8 mm when dried. To highlight the halloysite nanotubes distribution in the beads, SEM images were taken and they showed the presence of halloysite nanotubes in a random orientation within the polymer matrix (Figure 1). Similar findings have been reported by us for alginate/halloysite gel beads [55]. The dispersibility of the nanoparticles could be explained by the affinity between the polymer and halloysite and the colloidal stability of the nanoclay in polymer solution [56]. It should be noted that the halloysite cavity did not interact with the chitosan polycation as the inner surface of the nanotubes was positively charged [26]. Therefore, the HNTs lumen was preserved for drug loading.



**Figure 1.** (a) Optical image of wet alginate/chitosan/halloysite nanotubes (HNTs) gel beads and (b) SEM image of the inner part of the dried gel beads.

Neither optical nor SEM imaging were able to identify the alginate location in the beads. We therefore thought to label the alginate polymer with a fluorescent probe 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) that showed fluorescent emission when excited at 490 nm. Firstly, a blank experiment on chitosan/halloysite gel beads was carried out and negligible fluorescence was observed. The laser scanning confocal microscopy images on chitosan/HNTs gel beads covered with a calcium alginate layer clearly showed a fluorescent layer with an average thickness of approximately 130  $\mu\text{m}$ , revealing that the diffusion of alginate into the chitosan/halloysite gel beads occurred up to a certain extent and the core of the beads was alginate free (Figure 2). On this basis, one could conclude that the simple preparation protocol allowed us to prepare a controlled complex architecture in mesoscopic scale that might be suitable for sustained release of active substances.

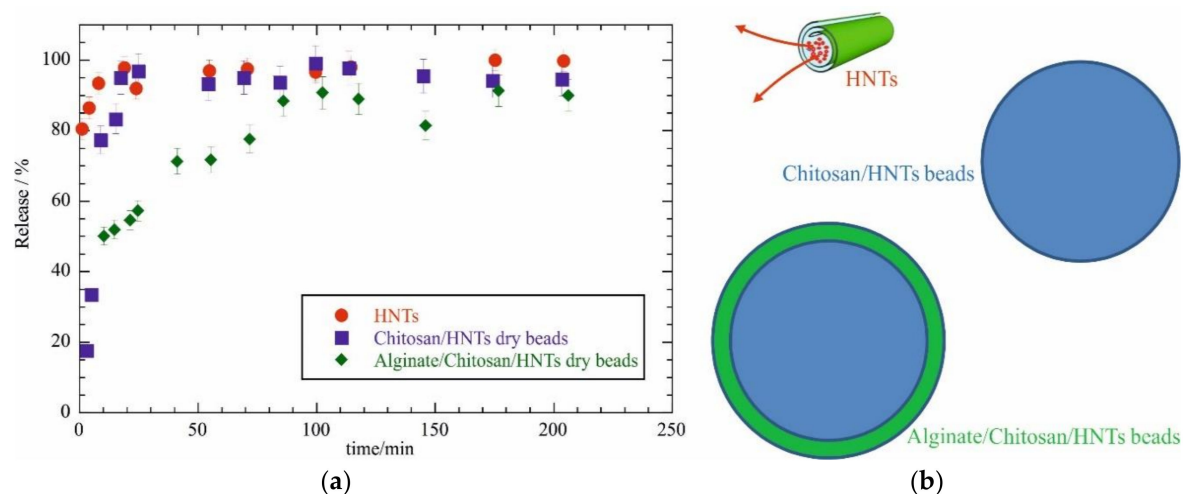


**Figure 2.** Laser scanning confocal microscopy images of alginate/chitosan/HNTs gel beads. Note that Alginate was labelled with 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF).

## 2.2. Drug Release Experiments

Release experiments were carried out by using doxycycline chlorohydrate as a model drug that could be loaded into the halloysite nanotubes [54]. The  $pK$  value for the drug was approximately 3 and the solubility dropped down as soon as the non-ionic form was obtained at  $pH > pK$ . Therefore, under our experimental conditions the drug was always neutral. The release profiles in water are

provided in Figure 3 for doxycycline from halloysite nanotubes, chitosan/HNTs dried beads and alginate/chitosan/HNTs dried beads. It was clearly observed that halloysite incorporation into chitosan gel beds only slightly slowed down the drug release from being fully available in the solvent media in 20 min. The sustained release was due to the slow release of the drug from the nanotube cavity and the subsequent drug diffusion through the polymer matrix to the solvent. The presence of an alginate coating significantly slowed down the doxycycline release from the hybrid beads. In particular after 20 min only 50% of the drug was released into the solvent media while a full release occurs in more than 80 min. These results could be interpreted by considering that the alginate shell in combination with the oppositely charged chitosan could generate a highly viscous layer that further delayed the drug diffusion from the beads to the solvent.



**Figure 3.** (a) Doxycycline chlorohydrate release as a function of time for different carriers. (b) Sketch view of the different release systems.

### 3. Materials and Methods

#### 3.1. Materials

Halloysite, acetic acid, sodium hydroxide, ethylenediaminetetraacetic acid and DTAF, sodium alginate ( $M_w = 70\text{--}100\text{ kg mol}^{-1}$ ), and chitosan ( $M_w = 50\text{--}190\text{ kg mol}^{-1}$ ) were Sigma products. Doxycycline chlorohydrate ( $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8 \cdot \text{HCl}$ ,  $M_w = 480.90\text{ kg mol}^{-1}$ ) was from Alfa Aesar. Halloysite characterization was reported in our recent publication [19].

#### 3.2. HNTs Loading with Doxycycline Chlorohydrate

The drug loading into HNTs cavity was carried out by using a procedure well established in literature [54]. Briefly, 0.5 g of doxycycline chlorohydrate was mixed with 2 g of HNTs in  $20\text{ cm}^3$  of water. The dispersion was kept under vacuum for 30 min. This procedure was repeated three times before centrifugation at 8000 rpm for 20 min to separate the loaded HNTs from the supernatant. The loaded HNTs were rinsed with water and the drug loading of 4.2 wt % was determined by thermogravimetry. The experimental thermogravimetric curves are provided in Supplementary Material. The method used for drug loading calculation was detailed elsewhere [57].

#### 3.3. Preparation of Gel Beds

The chitosan based gel beads were prepared by using the dropping technique [58]. Chitosan (2 wt %) was dissolved in water containing 0.5 wt % of acetic acid. A peristaltic pump was used to drop the chitosan solution into an aqueous solution of NaOH 1.5 M. The needle diameter was 0.4 mm and the distance from the needle to the liquid surface was 2 cm. The obtained gel beads stood in the NaOH solution overnight, and afterwards they were rinsed with water three times. The preparation



of the hybrid HNTs/Chitosan gel beads was carried out by using the same methodology. In this case, HNTs loaded with doxycycline chlorohydrate were dispersed into the chitosan solution with a polymer: HNTs weight ratio of 1:1. Some of the beads were in contact with a sodium alginate solution (2 wt %) for 10 mins and then with  $\text{CaCl}_2$  0.1 M to cross-link the alginate polymer. Beads were dried out at 40 °C overnight.

### 3.4. Doxycycline Chlorohydrate Release Experiments

The release profiles in water were determined by measuring UV-VIS spectra in a quartz cuvettes without stirring. In particular, one dried bead or the equivalent amount of loaded HNTs was weighted and directly placed into a cuvette. An amount of 2 cm<sup>3</sup> of distilled water was added and the spectra was recorded for 200 min.

### 3.5. Synthesis of DTAF Labeled Sodium Alginate

Alginate fluorescent labelling was carried out following the literature [59]. Sodium alginate (10 mg cm<sup>-3</sup>) was solubilized in sodium bicarbonate (50 mM) and 1.0 M NaOH was used to adjust the pH to 9. DTAF (concentration of 10 mg mL<sup>-1</sup> in dimethyl sulfoxide) was added at room temperature with an alginate: DTAF solutions volume ratio of 1:0.4. After one night of stirring, the mixture was dialysed in a 10 kDa cut-off dialysis tubing against phosphate-buffered saline (PBS) until the DTAF was not detected in the dialysate by UV at 490 nm.

### 3.6. Experimental Methods

UV-VIS spectra of doxycycline chlorohydrate were recorded by a Specord S600 (Analytik, Jena, Germany). Doxycycline chlorohydrate in water had a peak at 362 nm with an extinction coefficient of  $23.6 \pm 0.3 \text{ cm}^3 \text{ mg}^{-1}$ . SEM images were obtained by using a microscope ESEM FEI QUANTA 200F (FEI, Hillsboro, OR, USA) in high vacuum mode ( $<6 \times 10^{-4}$  Pa). Before each SEM experiment, the surface of the sample was coated with gold in argon by means of an Edwards Sputter Coater S150A (Edwards Lifesciences, Milan, Italy) to avoid charging under an electron beam. Laser scanning confocal microscopy images were obtained using a LSM 780 instrument (Carl Zeiss, Jena, Germany) equipped with apochromatic 20× and 40× objectives and argon laser (488 nm). Images were processed using ZEN Black software (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Thermogravimetry (TG) measurements were performed by means of a Q5000 IR apparatus (TA Instruments, Milan, Italy) under nitrogen flows of 25 and 10 cm<sup>3</sup> min<sup>-1</sup> for the sample and the balance, respectively. The sample (approximately 3 mg) was heated from room temperature to 700 °C at 10 °C min<sup>-1</sup>. Calibration was carried out by following the procedure reported in literature [60].

## 4. Conclusions

In summary, we prepared hybrid gel beads with a chitosan rich core and an alginate rich shell containing halloysite nanotubes. The clay nanoparticles were loaded with a model drug and showed a good dispersion within the beads. The kinetics of the drug release was controlled by a core/shell structure. This work opens new perspectives into the preparation of hybrid biopolymer/nanoclay structures for drug delivery applications, and proposes a new strategy for obtaining tuned drug release.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2079-6412/9/2/70/s1>, Figure S1: Thermogravimetric curves for HNTs loaded with Doxycycline chlorohydrate and their pure components (HNTs and Doxycycline chlorohydrate).

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**Conflicts of Interest:** The authors declare no conflict of interest.

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