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Research paper

Dendrimer-functionalized halloysite nanotubes for effective drug delivery

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ABSTRACT

Halloysite nanotubes functionalized with polyamidoamine dendrimer were prepared and characterized. The material studied was applied as a carrier of three model therapeutic compounds - chlorogenic acid, ibuprofen and salicylic acid. It showed higher adsorption capacity for the drugs studied (123.16 mg/g for chlorogenic acid; 182.72 mg/g for ibuprofen; 39.52 mg/g for salicylic acid) compared to raw halloysite and 3-aminopropyltrimethoxysilane functionalized – halloysite nanotubes. The experimental adsorption data fits the Langmuir model. As a result of surface functionalization of halloysite with the dendrimer, the release rate of chlorogenic and salicylic acid decreased, while the release profile of ibuprofen was similar to that of 3-aminopropyltrimethoxysilane functionalized nanotubes. The release kinetics of chlorogenic acid and salicylic acid followed Higuchi model and the release exponents indicated a Fickian diffusion mechanism. The release mode of ibuprofen followed the first order kinetics and the mechanism was described as non-Fickian (anomalous) transport. The *in vivo* toxicity studies showed that the dendrimer –functionalized halloysite had no effect on the living organisms used in the bioassays.

1. Introduction

Halloysite (Hal), a clay mineral of the kaolin group, is of great interest due to a variety of its potential applications. In contrast to kaolinite ($Al_2Si_2O_5(OH)_4$), halloysite ($Al_2Si_2O_5(OH)_4{\cdot}nH_2O$) contains additional water molecules, and it is composed of aluminosilicate layers. Each layer contains octahedral alumina and tetrahedral silica sheets (1:1 stoichiometric ratio). Tubular morphology, with alumina sheet inside and silica sheet outside, is most commonly found in this clay mineral. The advantages of halloysite nanotubes compared to other materials with the same morphology, are their natural origin, low price, environmental friendliness and biocompatibility (Churchman et al., 2016; Du et al., 2010; Joussein et al., 2005; Rawtani and Agrawal, 2012; Yuan et al., 2015).

Hollow tubular structure and the properties of halloysite influenced development of scientific research on biomedical applications of these nanotubes (Abdullayev and Lvov, 2013; Aguzzi et al., 2007; Fakhrullin and Lvov, 2016; Hanif et al., 2016; Lazzara et al., 2017; Lvov et al., 2008, 2016a, 2016b; Naumenko et al., 2016; Khodzhaeva et al., 2017; Yendluri et al., 2017a, 2017b). There are a number of literature reports on the use of halloysite as a carrier of bioactive compounds. Different treatments are used to improve adsorption and controlled release of active compounds from halloysite material. One of the commonly used methods is acid- and/or heat-treatment of the material (Wang et al., 2014). Under favorable conditions, acid treatment can lead to increased halloysite nanotube lumens and consequently to improved loading efficiency of a drug (Abdullayev et al., 2012). Surface modification of Hal with dopamine can be helpful in more effective immobilization of a desired compound (Chao et al., 2013). The addition of halloysite to other drug carriers, nanocomposite hydrogels, can enhance the mechanical properties of such materials (Tu et al., 2013). Furthermore, hybrid systems based on halloysite nanotubes can also contribute to improvement of loading and release of drugs. A glycoclaster composed of the clay nanotubes and carbohydrate functionalized cyclodextrin can be used for drug transport into living cells (Massaro et al., 2016b).

In addition to the advanced research on the use of raw halloysite as a nanocontainer of drugs, there are numerous works on a modification of Hal surfaces in order to improve the properties of the material (Tan et al., 2016). Chemical modification of inner lumen and outer surface depends on adsorbed compounds and medical application of a designed hybrid system (Massaro et al., 2014; Massaro et al., 2016a; Massaro et al., 2017). 3-Aminopropyl triethoxysilane/trimethoxysilane (APTES/APTS) very often appears as an agent for surface functionalization of different inorganic materials (Yuan et al., 2008; Kurczewska et al., 2009; Narkiewicz et al., 2010). In case of halloysite, it is used to facilitate loading process (Shi et al., 2011), as well as for slowing down of drug release from the material (Tan et al., 2013, 2014). In addition to a simple one-step functionalization, a very interesting concept is to

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combine by chemical bonding of different drug carriers. Dendrimers, including polyamidoamine (PAMAM) dendrimers, form host-guest complexes with small molecules through electrostatic and hydrophobic interactions. This class of compounds is also widely studied for their biomedical and biotechnological applications (Sato and Anzai, 2013). Therefore, chemical functionalization of halloysite surface with a dendrimer should significantly affect both the adsorption of drug molecules and their release from such a carrier. At the moment, there are reports in a literature concerning the use of similar hybrid systems only for environmental applications (Shahamati Fard et al., 2016). However, there are few works showing the use of other nanostructured materials which, in combination with PAMAM dendrimers, can slow down the release rate of small molecules compared to unmodified materials (Torres et al., 2016).

The objective of this study was to obtain and characterize a drug delivery system, PAMAM-dendrimer functionalized halloysite nanotubes. The material studied was investigated as a carrier of three model drug compounds – chlorogenic acid, ibuprofen and salicylic acid. All the small drug molecules have carboxyl groups. We suspect that it should promote a formation of stronger ionic bonds between the drug molecules and the organic unit in the carrier studied compared with systems based only on hydrogen bonding. We present the evaluation of drug adsorption isotherms and drug release kinetics, as well as the toxic effect of the material studied on living organisms. Based on our knowledge, this is the first report presenting an application of PAMAM dendrimer-functionalized halloysite nanotubes for loading and release of drug molecules.

2. Materials and methods

2.1. Materials

The clay mineral - halloysite (Hal) with tubular structure was purchased from Sigma-Aldrich (product of Applied Minerals, Inc., Dragon Mine, USA). According to the supplier, the diameters of the halloysite nanotubes were 30–70 nm with a length of 1–3 μm, pore volume – 1.26-1.34 mL/g, specific surface area – 64 $m^2\ g^{-1}$, cation exchange capacity – 8.0 meq/g and relative density – 2.53 g/cm^3. The chemical composition of the halloysite from Dragon Mine was 43.50% SiO_2, 0.02% TiO_2, 38.88% Al_2O_3, 0.33% Fe_2O_3, 0.12% MgO, 0.26% CaO, 0.07% Na_2O, 0.07% K_2O, 0.83% P_2O_5, 0.26% SO_3 and 15.70% others (Pasbakhsh et al., 2013). Chlorogenic acid (CHLG), ibuprofen (IBU), salicylic acid (SAL) and all other chemicals were obtained from Sigma-Aldrich and used as received without further purification. All solvents were of the p.a. grade, purchased from POCH (Poland). Demineralized water was used for aqueous solutions preparation.

2.2. Synthesis of polyamidoamine (PAMAM) dendrimer

To a stirred solution of methyl acrylate (55 g, 638.87 mmol) in methanol (50 mL) cooled in an ice-water bath a solution of tris(aminoethyl)amine (10 g, 68.38 mmol) in methanol (50 mL) was added dropwise. After addition of tris(aminoethyl)amine solution the resulting mixture was allowed to warm to room temperature and was stirred for further six days. The solvent and excess methyl acrylate were removed under reduced pressure using rotary evaporator, yielding intermediate dendrimer (Fig. 1a).

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 2.45 (12H, t, CH_2), 2.51 (12H, bs, CH_2), 2.78 (12H, t, CH_2), 3.67 (18H, s, CH_3); MS (ESI, positive) *m/z* 663.1.

Then, to a stirred solution of ethylenediamine (13.6 g, 226 mmol) in methanol (50 mL) cooled in an ice-water bath a solution of intermediate dendrimer (5 g, 7.5 mmol) in methanol (50 mL) was added dropwise. The resulting solution was allowed to warm to room temperature and was stirred for further seven days. Methanol and excessive ethylenediamine were removed in vacuum, yielding a yellow oil (PAMAM dendrimer, Fig. 1b).

^{1}H NMR ($CDCl_3$, 400 MHz), δ: 2.21 (12H, m, CH_2), 2.38–2.73 (48H, m: overlapping CH_2, NH_2), 3.05 (12H, m, CH_2), 8.07 (6H, bs, CONH); MS (ESI, positive) *m/z* 831.72.

2.3. Synthesis of PAMAM-functionalized halloysite nanotubes

The synthesis of polyamidoamine dendrimer-functionalized halloysite nanotubes (Hal_PAMAM) consisted of several steps (Fig. 2). The nanotubes were initially functionalized with 3-aminopropyltrimethoxysilane (APTS) following the procedure previously reported (Kurczewska et al., 2017). In the next process, Hal_APTS (10.0 g) was dispersed in chloroform (100 mL) and reacted with suberic acid bis (*N*-hydroxysuccinimide ester) (DSS; 1.3 mmol *per* 1.0 mmol of APTS). Then the mixture was stirred and heated for 72 h. The solid was filtered, washed and dried. The intermediate product, labeled as Hal_DSS, was reacted with polyamidoamine dendrimer (PAMAM; 1.1 mmol per 1.0 mmol of DSS) in methylene chloride. The mixture was stirred for 72 h at room temperature. The obtained solid – Hal_PAMAM - was centrifuged, washed several times with methanol and demineralized water and dried for 24 h at 40 °C.

2.4. Characterization

The infrared spectra were taken on an IFS 66v/s Fourier transform infrared (FTIR) spectrophotometer from Bruker, equipped with an MCT detector (125 scans, resolution 2 cm^{-1}). The spectra were recorded in the 400–4000 cm^{-1} range for KBr pellets. The thermogravimetric studies were carried out in a Setsys 1200 apparatus (Setaram) at a heating rate of 5 °C/min under helium atmosphere. X-ray diffraction (XRD) was measured using Brucker AXS D8 Advance powder diffractometer equipped with Johansson monochromator (λCu $K_{\alpha1}$ = 1,5406 Å). The surface morphology was studied in a Carl Zeiss EVO-40 scanning electron microscope, SEM (resolution 2 nm, operating voltage 80 kV). Transmission electron microscope (TEM) images were recorded on a Hitachi HT7700 microscope, operating at accelerating voltage of 100 kV. NMR spectra were recorded on a Bruker (Billerica, MA, USA) NanoBay 400 MHz spectrometer. Elemental analysis of Hal_APTS was carried out on a Vario ELIII (Elementar, USA) analyzer.

Nitrogen adsorption-desorption isotherms were measured on a sorptometer Quantachrome Autosorb iQ (Boynton Beach, Florida, USA). The samples were degassed at 150 °C (Hal) and 40 °C (Hal_PAMAM). The specific surface area (S_{BET}) and the pore size distribution were calculated by Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively.

The ESI mass spectra were obtained on an amaZon SLBruker mass spectrometer equipped with an electrospray ion (ESI) source in infusion mode. The sample solution was introduced into the ionization source at a flow rate of 5 μL min^{-1} using a syringe pump. The apparatus was operated using the so-called “enhanced resolution mode” (mass range: 50–2200 *m/z*, scanning rate: 8100 *m/z* per second). The capillary voltage was set at − 4.5 kV and the endplate offset at − 500 V. The source temperature was 80 °C and the desolvation temperature was 250 °C. Helium was used as the cone gas and desolvating gas (nitrogen) at flow rates of 50 L h^{-1} and 800 L h^{-1}, respectively. The mass spectrometer was operated in the ESI positive and negative ionization mode. For all the experiments, 0.1 mM water/methanol solutions of the dendrimer and the complexes of the dendrimer with the drugs studied were used.

2.5. Drug adsorption experiments

Adsorption isotherm studies were performed for three model drugs following the general procedure: 5 mg of halloysite samples (Hal, Hal_APTS and Hal_PAMAM) were introduced into 5 mL of drug solution (0.2–1.0 mM in ethanol for CHLG and IBU; methanol for SAL). The mixtures were stirred for 24 h at constant temperature (298 ± 1 K) to