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Pharmaceutical properties of supramolecular assembly of co-loaded cardanol/triazole-halloysite systems

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ABSTRACT

Halloysite nanotubes were explored as drug carrier for cardanol, which is considered as a promising natural anticancer active species. To this aim, besides the pristine nanoclay, a chemical modification of the nanocarrier was performed by attaching triazolium salts with different hydrophobicity at the outer surface of the hollow nanotubes. The interaction between cardanol and nanotubes was highlighted in solution by HPLC. This method proved the loading of the drug into the nanotubes. The solid dried complexes formed by pristine and modified halloysite with the cardanol were characterized by IR spectroscopy, thermogravimetric analysis as well as water contact angle to evidence the structure, thermal properties and wettability of the obtained materials. The kinetics of cardanol release as well as cell viability experiments provided promising results that put forward a new strategy for potential applications of cardanol as active antiproliferative molecule and clay nanotubes as drug carrier.

1. Introduction

Liver cancer is a major malignant tumor worldwide and is the third most common cause of cancer-related mortality (Feng et al., 2013). Over 80% of liver cancer patients are diagnosed with hepatocellular carcinoma, which is resistant to most conventional chemotherapeutic agents (Wilson et al., 2012). Moreover, the use of chemoprevention agents is typically associated with side effects that lead to the destruction of normal tissues, such as those of the digestive, hematopoietic and nervous systems (Meyskens and Gerner, 1999; Suzuki et al., 2008; Florea and Büsselberg, 2011). The development of drugs that specifically target tumor cells, but not normal cells, represents a common goal. Natural compounds contain various types of medicinal ingredients, including vitamin derivatives, phenolic and flavonoid agents, organic sulfur compounds, isothiocyanates, curcumins, fatty acids and D-limonene (Amin et al., 2009; Gullett et al., 2010; Tsuda et al., 2004), and sufficient evidences have demonstrated that these components can substantially inhibit tumor formation (Amin et al., 2009).

Cardanol is one of the promising renewable natural resources, obtained as the main fraction from the distillation of cashew nut shell liquid (Fig. 1). The presence of a C15-long aliphatic chain attached to the meta position of the phenolic ring confers exclusive properties to cardanol derivatives, such as high solubility in nonpolar environments and good processability.

Current studies about the antiproliferative/cytotoxic activity on cancer cell lines of extracts of Thai *Apis mellifera* propolis containing cardanol and cardol as main bioactive components have established potential anticancer bioactivity (Teerasripreecha et al., 2012).

However a crucial drawback in the use of natural compounds as drug candidates is their very low solubility in physiological media; therefore, a number of drug carriers have been developed to overcome this problem.

In the last year the use of porous materials as carriers for the encapsulation and delivery of drugs has received considerable attention because of their stable structure and controllable surface

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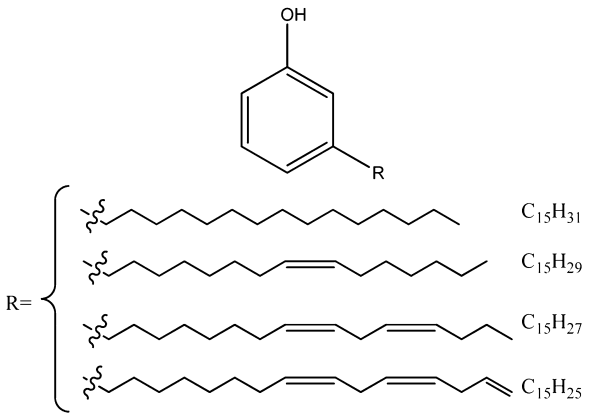

Fig. 1. Molecular structure and composition of cardanol.

reactivity (Horcajada et al., 2006; Ito et al., 2005; Jain et al., 2005; Martucci et al., 2012; Mizushima et al., 2006; Song et al., 2005; Streubel et al., 2002; Vallet-Regi et al., 2001).

Among the materials that have porous structure, halloysite (HNT), a natural aluminosilicate clay with a hollow tubular structure, is enable to load and to release biomacromolecules and drugs (Joussein et al., 2005; Price et al., 2001; Shchukin et al., 2005).

Intracellular uptake by cells of different origins (cervical adenocarcinoma or breast cancer cells) and cytoviability tests demonstrated relative halloysite cytocompatibility and potential as a bio-friendly cargo nanocontainer for biomaterials (Vergaro et al., 2010).

Modification of the halloysite tube outer surface via covalent bond formation may open up new applications based upon molecular recognition, such as molecular separation, molecular storage, catalysis, and drug delivery (Abdullayev and Lvov, 2010; Abdullayev et al., 2011; Lvov et al., 2008; Massaro et al., 2014a,b,c).

In our previous research we demonstrated that modified HNT with triazolium salts (f-HNT) (Fig. 2) improves the antitumor effect of curcumin in physiological medium when compared with the use of curcumin alone. It is interesting to note that the presence of the biological active triazolium moiety exerts a synergic effect with curcumin that carry out in an increased cytotoxic activity of the new nano-formulation, HNT-triazolium salts/Curcumin, against several tumor cell lines (Riela et al., 2014).

In this work, for the first time, reverse-phase HPLC technique was used to analyze if the halloysite nanotubes could be a suitable carrier for drug molecules. HPLC equipped with Diode-Array/UV detector offers the remarkable advantage to record in real time the UV–vis spectrum of the chromatographic eluate (in the range 200–600 nm), and therefore it allows the simultaneous monitoring of different species/analytes.

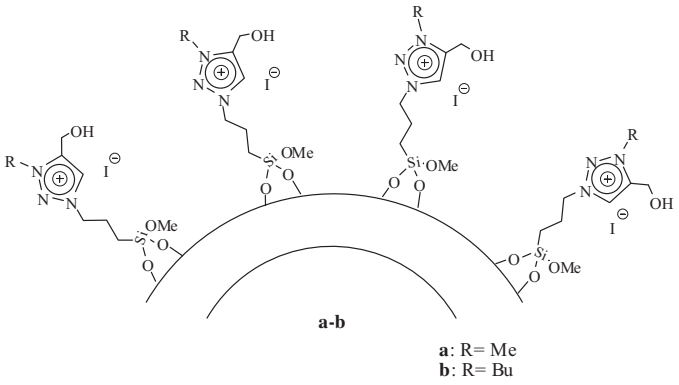

Fig. 2. Schematic representation of functionalized triazolium salt HNT (f-HNT).

We report a study of the supramolecular interaction between pristine halloysite (p-HNT) and cardanol by thermogravimetric analysis (TGA), IR spectroscopy, dynamic light scattering (DLS), contact angles measurements and SEM investigations. Moreover, the effect of the surface functionalization of halloysite with triazolium salts (f-HNT), the synergic effect, on the loading of cardanol was also investigated. Finally the cytotoxic effect on three different hepatocarcinoma cell lines, HA22T/VGH, Hep3B and HepG2, was evaluated, too.

2. Experimentals

All reagents needed were used as purchased (Aldrich), without further purification.

Cardanol was provided by Prof. Attanasi, University of Urbino.

Halloysite was supplied by Applied Minerals. This material has an average tube diameter of 50 nm and inner lumen diameter of 15 nm. Typical specific surface area of this halloysite is 65 m^2/g; pore volume of ~1.25 mL/g; refractive index 1.54; and specific gravity 2.53 g/cm^3.

Functionalized triazolium salt halloysite was prepared as previously reported (Riela et al., 2014).

The UV–vis absorbance spectra were recorded with a Beckmann DU 650 spectrometer.

The microscope ESEM FEI QUANTA 200F was used to study the morphology of the functionalized HNTs. Before each experiment, the sample was coated with gold in argon by means of an Edwards Sputter Coater S150A to avoid charging under electron beam.

Thermogravimetric analyses were performed by a Q5000 IR apparatus (TA Instruments) under a nitrogen flow of 25 $cm^3\,min^{-1}$ for the sample and 10 $cm^3\,min^{-1}$ for the balance. The weight of each sample was ca. 10 mg. The measurements were carried out by heating the sample from room temperature to 900 °C at a rate of 10 °C min^{-1}.

The DLS measurements were performed at 22.0 ± 0.1 °C in a sealed cylindrical scattering cell at a scattering angle of 90° by means of a Brookhaven Instrument apparatus composed of an BI-9000ATcorrelator and a He–Ne laser (75 mW) with a wavelength (λ) of 632.8 nm. The solvent was filtered by means of a Millipore filter with 0.45 μm pore size. For all systems, the field-time autocorrelation functions were well described by a mono-exponential decay function, which provides the decay rate (Γ) of the single diffusive mode. For the translational motion, the collective diffusion coefficient at a given concentration is $D_t = \Gamma/q^2$ where q is the scattering vector given by $4\pi R_I \lambda^{-1} \sin(\theta/2)$ being R_I the water refractive index and θ the scattering angle.

Contact angle measurements were performed by using an optical contact angle apparatus (OCA 20, Data Physics Instruments) equipped with a video measuring system having a high-resolution CCD camera and a high-performance digitizing adapter. SCA 20 software (Data Physics Instruments) was used for data acquisition. To obtain a tablet, the powder like material was pressed under 10^4 kg cm^{-2} for 10 min. The contact angle of water in air was measured by the sessile drop method. The water droplet volume was 10.0 ± 0.5 μL. Temperature was set at 25.0 ± 0.1 °C for the support and the injecting syringe as well. Images were collected 25 times per second. From the data analysis the contact angle, the volume and the contact area of the drop were calculated. The volume of the droplet was constant within the time of the experiment.

The dispersions were sonicated with an ultrasound bath VWR Ultrasonic Cleaner (power 200 W, frequency 75 MHz).

The chromatographic measurements were performed using a Shimadzu Class VP (Shimadzu, Japan) which consist of a pump (LC-10AD VP Shimadzu), an injection valve equipped with a 20 μL injection loop, an UV–vis Diode-Array (SPD-M10A VP) detector and an acquisition data software Class-VP. After optimization of