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Direct chemical grafted curcumin on halloysite nanotubes as dual-responsive prodrug for pharmacological applications

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ABSTRACT

Covalently functionalized halloysite nanotubes (HNTs) were successfully employed as dual-responsive nanocarriers for curcumin (Cur). Particularly, we synthesized HNT-Cur prodrug with a controlled curcumin release on dependence of both intracellular glutathione (GSH) and pH conditions. In order to obtain HNT-Cur produgs, halloysite was firstly functionalized with cysteamine through disulphide linkage. Afterwards, curcumin molecules were chemically conjugated to the amino end groups of halloysite *via* Schiff's base formation. The successful functionalization of halloysite was proved by thermogravimetric analysis, FT-IR spectroscopy, dynamic light scattering and scanning electron microscopy. Experimental data confirmed the presence of curcumin on HNT external surface. Moreover, we investigated the kinetics of curcumin release by UV–vis spectroscopy, which highlighted that HNT-Cur prodrug possesses dual stimuli-responsive ability upon exposure to GSH-rich or acidic environment. *In vitro* antiproliferative and antioxidant properties of HNT-Cur prodrug were studied with the aim to explore their potential applications in pharmaceutics. This work puts forward an efficient strategy to prepare halloysite based nanocarriers with controlled drug delivery capacity through direct chemical grafting with stimuli-responsive linkage.

1. Introduction

Halloysite nanotubes (HNTs) are natural aluminosilicate clays with an hollow tubular structure, with an ideal chemical formula of $Al_2Si_2O_5(OH)\cdot 2H_2O$. Due to its low cost, wide availability, and interesting physico-chemical properties [1], halloysite has attracted considerable attention for applications in many fields [2]. In particular halloysite, being a biocompatible nanomaterial, is perspective as drug carrier and delivery [3–7].

The external surface of halloysite is composed of Si—O—Si groups and the internal surface of a gibbsite-like array of Al—OH groups. The peculiar surface chemistry allows the selective functionalization at the inner or outer side and the formation of a composite with hierarchical nanostructure [8,9]. Halloysite is a natural material with demonstrated low toxicity both *in vitro* [10] and *in vivo* [11]. Incorporation of 5-aminosalicylic acid [12], diphenhydramine hydrochloride [13], diltiazem HCl, propranolol HCl [14], tetracycline hydrochloride [15], dexamethasone, furosemide and nifedipine [16] and cardanol [17] into halloysite was investigated and encouraging results were obtained. Recently, pH 3 and thermo-responsive [18] nanocarriers for curcumin were successfully prepared though the modification of halloysite surfaces.

Curcumin is a naturally active constituent extracted from the plants of the *Curcuma longa*. This molecule presents several biological activities and pharmacological actions [19,20]. The effects and reactions of curcumin have been the subject of investigation in the fields of biology, medicine, pharmacology, and in the food industry yielding a large number of publications for many years. The medical activity of curcumin has been known since ancient times. The beneficial effects can be attributed to the curcumin antioxidant activity, which involves radical and peroxide scavenging as well as metal chelating effect [21–26]; in addition, curcumin shows

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anti-proliferative [27], antiapoptotic [28] and anti-angiogenetic [29] properties.

Pharmacological applications of curcumin are strongly limited by its low solubility in aqueous media. Moreover, curcumin degrades quickly under neutral or alkaline conditions Accordingly, curcumin presents a half-life shorter than 10 min in phosphate buffer solution at pH 7.2 [30], whereas its stability increases in acidic pH conditions [31]. As a result, curcumin bioavailability is poor, particularly after oral or topical administration [32]. Therefore, a carefully designed carrier could significantly facilitate the drug delivery extending the potential pharmaceutical applications of curcumin. In this regard, several drug delivery systems (DDS) were recently developed, such as the curcumin loading into liposomes or nanoparticles [33]. Additionally, both curcumin–phospholipid complexes and structural analog of curcumin were successfully synthesized. However, DDS are not suitable for biomedical applications because of poor circulation stability, toxic side effects and low therapeutic efficacy [34–39].

Similar to other triggered nanocarriers, stimuli-responsive prodrugs are efficient in reducing side effects and enhancing antitumor activity due to tumor-specific drug release. Among them, in recent years acid-responsiveness is the most frequently used approach [40]. Acid-triggered drug release can be realized by the application of pH-sensitive biodegradable chemical linkers, mainly including acid-labile acetal linkage, hydrazine and silyl-ether bonds.

Another stimulus used to achieve triggered drug release from prodrug is the redox gradient existing between the mildly oxidizing extracellular spaces and the reducing intracellular cytoplasm (higher reduction level) [41]. A disulfide bond is the most common chemical linker utilized to bridge polymers and anticancer drugs for reduction-responsive drug release [42,43].

Here, we report the synthesis and characterization of dual-stimuli-responsive halloysite-curcumin prodrug. Curcumin was covalently linked to halloysite precursor through GSH- or pH-responsive bonds. Moreover, stability of curcumin could take advantage from the halloysite surface that is slightly acid. Potential pharmacological applications of HNT-Cur prodrug were explored by studying the kinetics release of curcumin in aqueous media at different pH conditions. Release experiments were also conducted in the presence of glutathione (GSH). Moreover, *in vitro* antiproliferative and antioxidant properties of functionalized HNT were investigated.

2. Results and discussion

We synthesized stimuli-responsive prodrugs based on halloysite and curcumin, which is a well-known antioxidant and anticancer compound. Specifically, curcumin molecules were covalently conjugated with halloysite scaffold *via* pH-responsive imine bond. In order to obtain dual stimuli-responsive prodrugs, halloysite scaffold bearing a GSH-responsive disulphide bond was synthesized (Scheme 1). As described elsewhere [44], pristine halloysite was modified in f-HNT-SH **1**, which was covalently linked to cysteamine hydrochloride **2** (Scheme 1).

Firstly, we investigated the synthetic procedure reported in literature for disulphide bond formation [45]. The obtained filtered material was analysed by TGA with the aim to determine the f-HNT-SH loading percentage, which was 3.7 wt% (Table 1, Entry 1).

Afterwards, we investigated the same reaction performed by microwave irradiation (MW) in methanol or in solvent free conditions. As reported in Table 1, loading values obtained just after 1 h of preparation are comparable to those of f-HNT-SH synthesized by using the traditional heating (Entries 2–3). These results highlighted the importance of microwave irradiation in improving the synthesis efficiency.

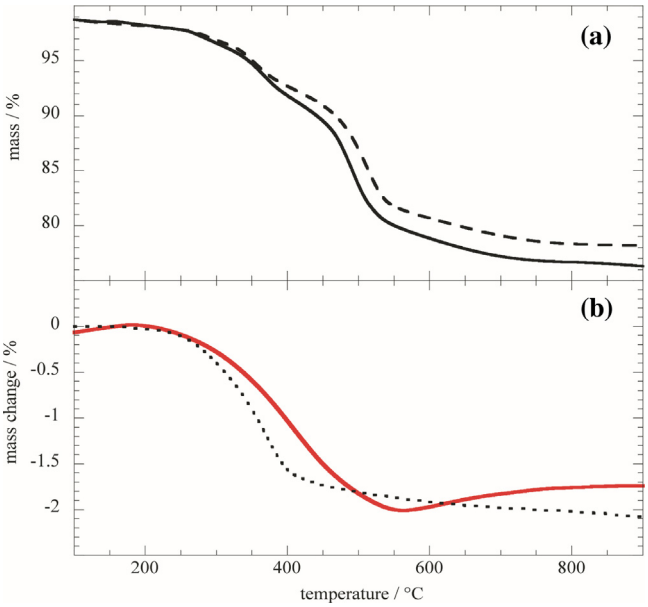

Fig. 1. TG curves of (a) compounds **3** (dashed line) and **4** (continue line); (b) dependence on temperature of experimental mass loss difference between compounds **3** and **4** (solid line) and that calculated taking into account the Cur loading (dashed line).

Namely, the preparation in solvent free conditions under microwaves action increased the linkage loading on the HNT external surface with respect to that traditional synthesis. In addition, the time requested for the HNT functionalization was reduced. It should be noted that the reaction may be considered "green" because of the solvent-free conditions.

The covalent linkage of curcumin on compound **3** was carried out according to the experimental conditions reported in Table 1. Firstly, we investigated the procedure reported in literature for Schiff base synthesis [46]. Following this (reaction time of 24 h, temperature of 78 °C and ethanol as solvent), we obtained a loading of 0.88% respect to compound **3**. Therefore, in order to improve the loading of curcumin on HNT surface, we performed the reaction under MW and in solvent-free condition (Entry 5); also in this case no improvement in loading was observed. Subsequently the reaction was performed using several solvents, with different ability to interact with microwaves. In particular we studied the effect of water, ethanol, THF and a mixture of water/ethanol (1:1).

The results are reported in Table 1 (Entries 6–9), which show that the best experimental conditions were obtained in the presence of ethanol for an irradiation time of 1 h (Entry 8). The different percent loading obtained with the different solvents could be explained as a consequence of the different ability of the solvents to interact with microwaves and their different ability to solubilize curcumin. Particularly, ethanol and THF possess different capacity to convert microwave energy into heat as expressed by their tan δ values (0.941 and 0.047, respectively) [47].

TGA experiments (Fig. 1) provided the curcumin loading onto the HNT-Cur hybrid as well as the thermal behavior of the organic moieties attached onto the nanoclay surface. As Fig. 1a shows, the thermogram of compound **4** presents an excess of mass loss with respect to compound **3** in agreement with the attachment of the organic moiety.

In order to highlight the thermal stability of attached Cur in the composite material, TG curve of compound **3** was subtracted to that for compound **4**. Fig. 1b highlights that the obtained mass change compared to that calculated for Cur pure sample scales down for the loading in the composite (Table 1, Entry 8). From such a comparison