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Paclitaxel Encapsulated in Halloysite Clay Nanotubes for Intestinal and Intracellular Delivery

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ABSTRACT

Naturally formed halloysite tubules have a length of 1 μm and lumens with a diameter of 12-15 nm which can be loaded with drugs. Halloysite's biocompatibility allows for its safe delivering to cells at a concentration of up to 0.5 mg/mL. We encapsulated the anticancer drug paclitaxel in halloysite and evaluated the drug release kinetics in simulated gastric and intestinal conditions. To facilitate maximum drug release in intestinal tract, halloysite tubes were coated with the pH-responsive polymer poly(methacrylic acid-*co*-methyl methacrylate). Release kinetics indicated a triggered drug release pattern at higher pH, corresponding to digestive tract environment. Tablets containing halloysite, loaded with paclitaxel, as a compression excipient were formulated with drug release occurring at a sustained rate. *In vitro* anticancer effects of paclitaxel-loaded halloysite nanotubes were evaluated on human cancer cells. In all the treated cell samples, polyploid nuclei of different sizes and fragmented chromatin were observed, indicating a high therapeutic effect of halloysite formulated paclitaxel.

Introduction

Halloysite clay is a natural, abundantly available tubular material formed by rolled kaolin sheets and its chemical formula is $Al_2Si_2O_5(OH)_4$. The diameter of halloysite tubes is 50-70 nm with an inner lumen of 12-15 nm and length of 500-1000 nm (Fig. 1). The tube wall contains approximately 15 closely packed aluminosilicate sheets with a spacing of approximately 0.72 nm and has a density of 2.53 g/cm^3. The surface charge polarity of halloysite tubes is negative and its inner lumen is positively charged due to the presence of silica and alumina on the outside and inside, respectively. Halloysite is a safe, biocompatible nanomaterial.[1-4] All these properties advocated the use of halloysite nanotubes (HNT) as natural nanocontainers for loading and sustained release of proteins, DNA, and drugs.[5-10]

Halloysite was utilized for the loading of tetracycline, kellin, ofloxacin, gentamicin, aspirin, dexamethasone, nifedipine, curcumin, doxorubicin, and acetaminophen at 10-15 wt.% from aqueous/nonaqueous polar solvents.[6-15] The prepared composites remained stable over extended periods in dry conditions and then released the captured drugs in aqueous or serum/blood for 5-10 h. Biomacromolecules, such as enzymes immobilized on halloysite surface and in lumen, enhanced stability at extreme environmental conditions of pH and temperature.[16] Owing to halloysite's different charge polarity on the outside and inside, dual enzyme composites were prepared in media of different pH, depending on the isoelectric point of enzyme. Halloysite was also utilized to impart mechanical strength to polymer-based scaffolds for tissue engineering applications.[17]

Halloysite is a safe, biocompatible material, allowing for efficient removal from an organism with macrophages. It was shown for many biological cells and tissues that halloysite is safe at concentrations of up to 0.5 mg/mL, an exceptionally high safe concentration for inorganic inclusions into cells.[3,4] Apart from cell toxicity studies, HNTs were used as an oral feed for chickens and piglets to remove toxins.[18] Halloysite was suggested as a novel tablet compression excipient for oral drug delivery and was used in the

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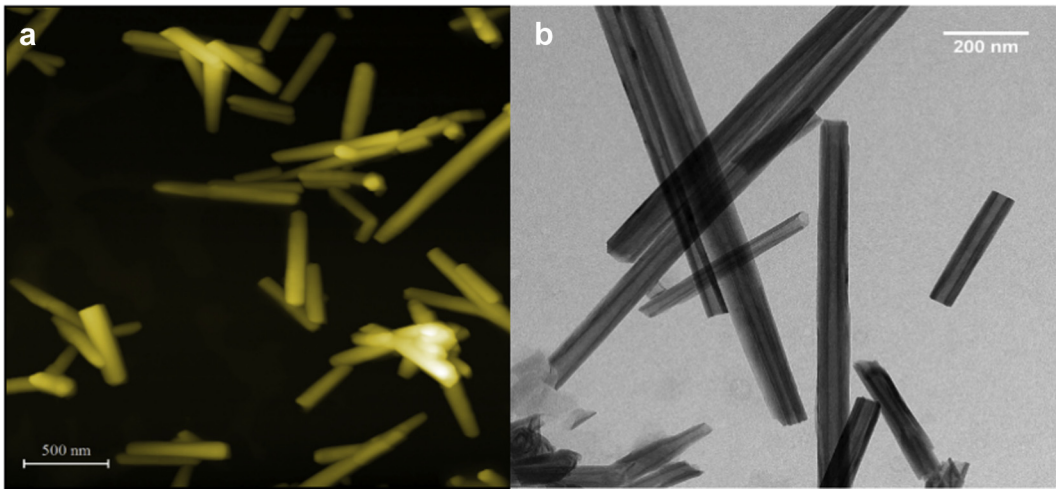

Figure 1. (a) Atomic force microscopy and (b) TEM images of halloysite nanotubes precipitated from aqueous dispersion.

formulation of tablets. The potential of HNT for oral drug delivery was demonstrated by the successful preparation of tablets, where it was utilized as a pharmaceutical excipient and drug delivery agent.[1,19] The drug release profile from these tablets followed a sustained rate for 250 h. Anticancer activity was studied in HeLa (cervical cancer) and A549 (lung cancer) cells, using various concentrations of dextran-capped halloysite-paclitaxel composites which showed improved therapeutic effect.[20]

We also explored oral delivery of paclitaxel from tablets. For this purpose, we encapsulated the anticancer drug paclitaxel in halloysite and incorporated them into a tablet formulation. Paclitaxel release profile was studied in simulated gastric and intestinal conditions to utilize this formulation for oral drug delivery. To employ these composites, especially for colon cancer therapy, it is required to reduce paclitaxel release in acidic pH (gastric) conditions and to facilitate higher drug release in basic pH (intestinal) conditions. This objective was achieved by coating paclitaxel-loaded halloysite tubes with a polymer which is soluble in basic media (pH > 6.8), that is, poly(methacrylic acid-*co*-methyl methacrylate) or PMMM.[21] Drug release studies indicated a hindered release in acidic pH and higher release in basic pH. In this work, we have shown paclitaxel encapsulation into HNTs, its pH-triggered sustained release due to coating of the nanotubes with dextrin and PMMM, as well as significant intracellular penetration of the halloysite carriers, showing high anticancer efficiency.

Materials and Methods

Materials and Instruments

Halloysite clay was provided by Applied Minerals Inc. (New York City, NY). Paclitaxel was purchased from LC Laboratories. Excipients microcrystalline cellulose, magnesium stearate, croscarmellose, colloidal silica, PMMM, dextrin, paclitaxel, paraformaldehyde solution, and L-glutamine were supplied by Sigma-Aldrich (St. Louis, MO). Fetal bovine serum was obtained from PAA

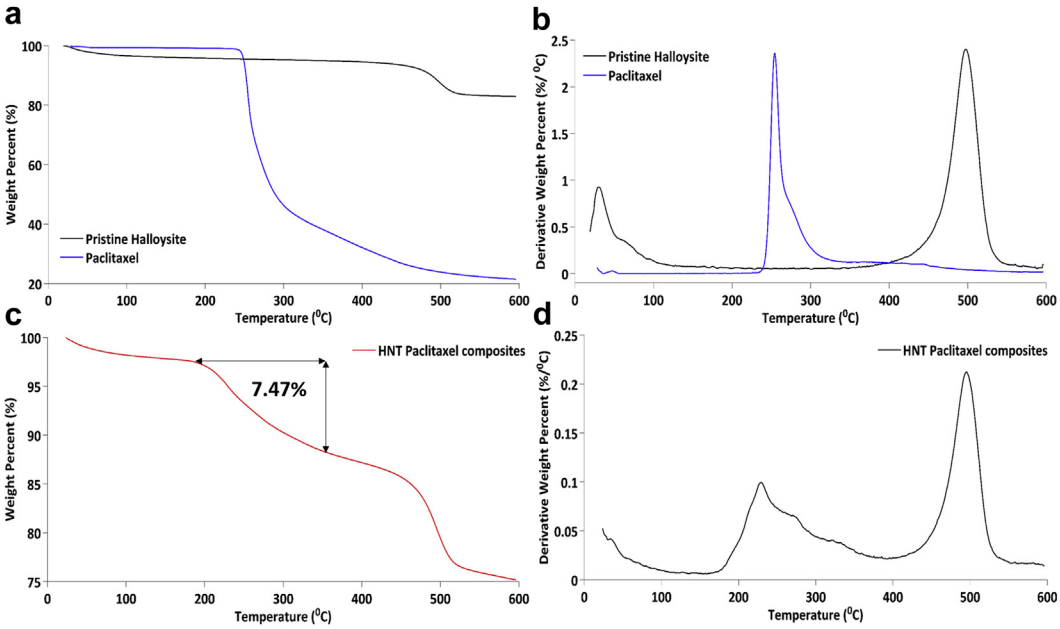

Figure 2. TGA curves and their derivatives for pure paclitaxel and halloysite nanotubes (a, b) and for the halloysite loaded with paclitaxel (c, d), heating rate 10°C/min.