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Preparation of a novel breviscapine-loaded halloysite nanotubes complex for controlled release of breviscapine

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Abstract. For sustain the release rate and prolong half-life of breviscapine in vivo, the breviscapine-loaded halloysite nanotubes complex was prepared. The breviscapine was encapsulated into halloysite nanotubes (HNTs) using a vacuum process. The complex were investigated by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), transmission electron microscope (TEM), X-ray diffraction (XRD) and fourier transform infrared spectroscopy(FT-IR). The formation of breviscapine-loaded HNTs complex was proved by the test results of SEM, DSC, TEM and IR analysise. The results confirmed that breviscapine was successfully loaded in the halloysite nanotubes. Additionally, the in vitro drug release of breviscapine from breviscapine-loaded HNTs complex was investigated, the result indicated this complex has apparent sustained-release effect.

1. Introduction

Breviscapine (4, 5, 6- tetrahydroxyflavone-7-O-glucuronide, Figure 1), a cerebrovascular drugs extracted from the Chinese herb *Erigeron breviscapinus (Vant.) Hand.-Mazz*, has been frequently used to clinically treat cerebrovascular diseases such as cerebral thrombosis, cerebral infarction, and cerebral circulation insufficiency [1]. However, One of the limitations of breviscapine is its short half-life in vivo, therefore, it was necessary to develop a sustained-release dosage form with good bioavailability and stable efficacy to reduce the frequency of breviscapine administration [2].

Halloysite nanotubes (HNTs) is a natural aluminosilicate nanotube from a clay mineral with a similar structure to kaolinite, which has a predominantly hollow nanotube structure with an inner diameter of 5-10nm [3-4]. HNTs have attracted attentions for their potential as a new type of nanotube container for encapsulating various active agents such as drugs, marine biocides, antifouling paint, cosmetics, and other functional agents [5]. HNTs is a viable and inexpensive nanotube container with a hollow tubular structure, which has high aspect ratio, specific surface area, excellent chemical and thermal stability.

HNTs' special lumen can be used for loading small molecules and monomer [6-7]. Many researches of HNTs as drug carrriers have been reported, indicating the nanocomposites have controlled release properties under certain conditions. Based on this thinking, halloysite as a drug carrrier to load brevescapine, a sustained release solid dispersions loading has been investigated in this work.

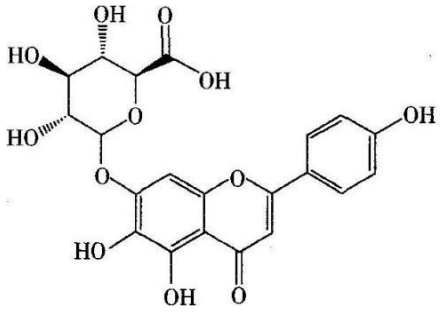

Figure 1. The chemical constituent of breviscapine.

2. Materials and methods

2.1 Materials

The following materials were obtained from their respective suppliers: brevescapine (Shanxi Kingsci Biotechnology Co. Ltd); Reference substances of brevescapine (Shanghai Jin sui Biological Technology Co. Ltd, the purity is 98%); halloysite (SIGMA-ALDRICH); Methanol (HPLC pure; Xilong Chemical Co. Ltd); acetonitrile (HPLC pure; Xilong Chemical Co., Ltd); Potassium hydrogen phosphate anhydrous (AR, Tianjin Damao Chemical Reagent Factory); Disodium phosphate dodecahydrate (AR, Tianjin Daimao Chemical Reagent Factory); Sodium dihydrogen phosphate (AR, Tianjin Daimao Chemical Reagent Factory).

2.2 Preparation of brevescapine-loaded HNT complex

Brevescapine was loaded into HNTs according to the procedure described by Price et. al. [8], with slight modifications. Brevescapine (6mg) was added to 10 mL of absolute methanol and vortexed for 5min. Then, 60mg of HNTs was thoroughly dispersed into the solution with a magnetic stirrer for 20min to form a suspension. The suspension was kept in a vacuum chamber at 25^{o}C. After 20 min, the vacuum was stopped and air was allowed into the chamber for 10 min. This process was repeated four times to increase the encapsulation efficiency. The air in the lumen of HNTs was replaced with brevescapine solution through the vacuum process. Next, brevescapine-loaded HNTs complex were separated from the solution by centrifugation using a high speed centrifuge (Neofuge15R) at 7000 rpm for 15 min. The tubules were washed by using deionic water to remove unloaded brevescapine. Finally, brevescapine-loaded HNTs complex was dried at 45^{o}C in an oven. The dried complex was pulverized in a mortar and left to the next experiment [9].

2.3 Testing and characterization

2.3.1 Scanning Electron Microscopy. The surface morphology of the pristine HNTs and the brevescapine-loaded HNTs complex was examined respectively through instrument Field Emission Scanning Electron Microscopy (FE-SEM, Zeiss, sigma200，Germany) at an accelerating voltage of 20 kV. Before SEM observation, the sample was deposited on a brass hold and sputtered with gold [10].

2.3.2 Transmission Electron Microscopy. For transmission electron microscopy (Tecnai G2 20 S-TWIN) observation, pristine HNTs and the brevescapine-loaded HNTs complex were immersed in ethylalcohol and dispersed with ultrasonic waves for 40min. A dilute suspension of sample was dropped on the copper grid and left undisturbed for 1 min. Then, excess solution was removed using filter paper. Finally, the sample was detected after the grids was dried.

2.3.3 Fourier transform infrared spectroscopy. Fourier transform infrared spectroscopy can find out the chemical stability of drug in the formulation. Pristine HNTs, pristine breviscapine, physical mixture of HNTs and breviscapine, and the brevescapine-loaded HNTs complex also analyzed respectively using the Fourier-transform infrared (FT-IR, Bruker Vertex 70) at 25^{o}C. Each sample was prepared using the potassium bromide (KBr) pellets method. Briefly, each sample was ground and mixed with KBr, and prepared as a pellet by compression under a force of 10tons in a hydraulic press. All FT-IR spectra's were obtained wavelengths 400 to 4000 cm^{-1} with a resolution of 1cm^{-1}.

2.3.4 Differential scanning calorimetry. Differential scanning calorimetry (DSC) were performed on different samples to determine their composition and predict the thermal stability. DSC measurements of pristine HNTs, pristine breviscapine, physical mixture of HNTs and breviscapine, and the brevescapine- loaded HNTs complex were done using a DSC Q20 (made in USA). The heating-cooling- heating run for all samples were recorded in the temperature range from 0 to 400 ℃ at a scan rate of 10^{o}C/min under nitrogen atmosphere.

2.3.5 X-ray diffraction . XRD of pristine HNTs, pristine breviscapine, physical mixture of HNTs and breviscapine, and the brevescapine-loaded HNTs complex were performed over the XRD-6100 at the incident X-rays (λ=1.54nm) and scanning range of 10–80^{o}. It equipped with a continuous scan attachment and a proportional counter, with Ni-filtered Cu Kα radiation.

2.4 Drug content

Breviscapine loading was determined by dispersing accurately weighed amounts of sample (3mg) in 10mL of absolute methyl alcohol. Then, the samples were broken using an ultrasonic homogenizer for 30 min. After centrifugation was carried out at 7000 rpm for 10 min to separate broken HNTs out of suspension, the supernatant was filtrated through a 0.45μm membrane, and the amount of breviscapine was determined in triplicate for each sample using HPLC. Quantification of breviscapine was carried out using a calibration curve [11].

2.5 In vitro drug release studies

In vitro dissolution of complex was carried out using the dissolution apparatus equipped with six paddles. The dissolution rates were measured at 37^{o}C under 50 rpm paddle speed. Weighed quantities of each sample were placed in buffer solutions. A 100 mL of phosphate buffer solution (pH6.8) was used to simulate the intestinal environment. A 1 mL of aliquot was taken from the vessel at definite intervals of time (5min, 10min, 15min, 30min, 45min, 1, 2, 3, 4, 5, 6 and 8h) and replaced with an equal volume of corresponding dissolution medium. These samples were analyzed using HPLC and concentration of breviscapine was calculated using the calibration curves constructed from the reference standards. The in vitro release studies were performed in triplicate for each of the samples.

3. Results and discussion

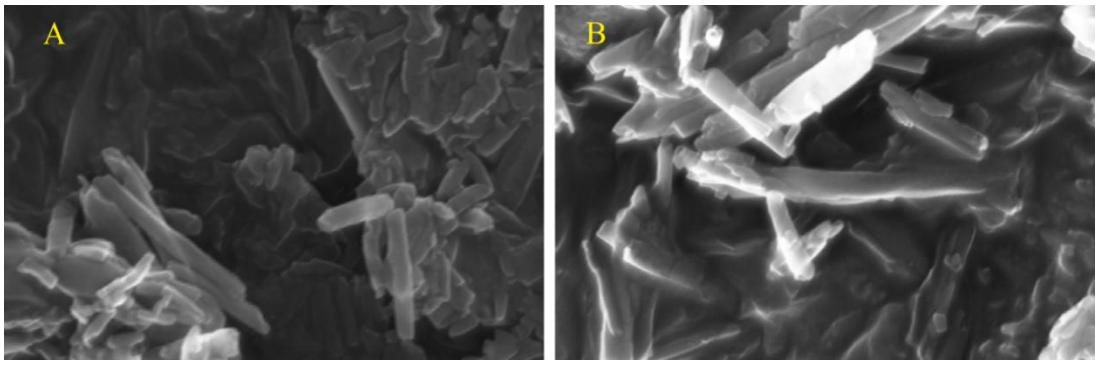

Figure 2. SEM images of (A) pristine HNTs, (B) brevescapine-loaded HNTs complex.

The surface morphology of each sample was investigated by SEM. The image of pristine HNTs in Figure 2(A) showed a rod shape and indicated that HNTs have a length of approximately 1 μm. This result corresponded to those of previous studies. Figure 2(B) showed brevescapine-loaded HNTs complex. The brevescapine-loaded HNTs complex exhibited a smooth surface similar to the pristine HNTs, which proved that breviscapine has no adsorbed on the surface of complex.

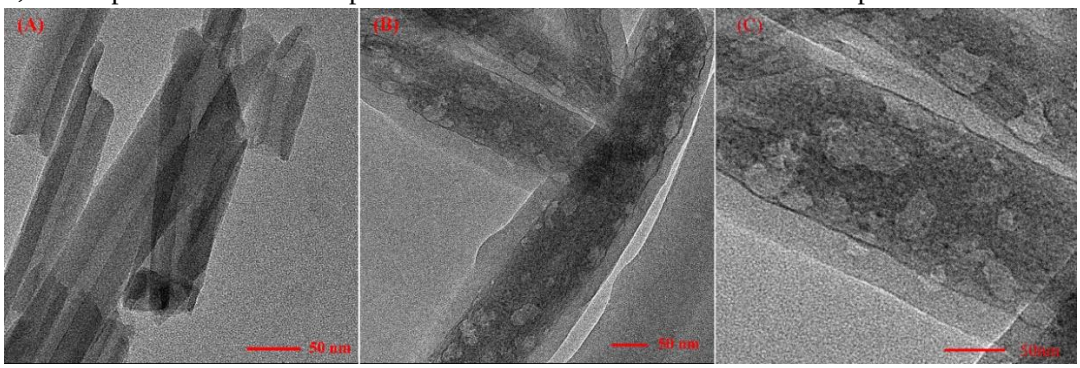

Figure 3. TEM images of (A) pristine HNTs, (B) brevescapine-loaded HNTs complex.

TEM was used to further characterize the structural changes of the pristine HNTs and brevescapine-loaded HNTs complex. Figure 3(A) showed the image of the Breviscapine/HNT particle, indicating that the HNTs have typical hollow structures with an outer diameter of 50–60 nm and a lumen diameter of 10–20 nm. Figure 3(B) showed that brevescapine-loaded HNTs complex. Testifying breviscapine was successfully loaded in halloysite, which was consistent with the SEM images.

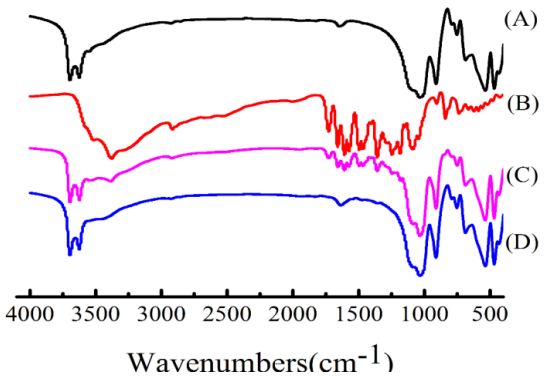

Figure 4. FT-IR spectra of (A) pristine HNTs, (B) pristine breviscapine, (C) physical mixture of HNTs and breviscapine, and (D) brevescapine-loaded HNTs complex

To confirm the structure of the HNTs and the existence of brevescapine-loaded HNTs complex, FT-IR studies were conducted (Figure 4). In the FT-IR spectrum of the pristine HNTs (Figure 4A), the peaks appeared at 3696 and 3621 cm^{-1} were assigned to the OH stretching vibration of the inner Al-OH groups of the HNTs. The peaks at 1645, 911, and 470 cm^{-1} are assigned to the stretching vibrations of OH groups in absorbed water, the OH deformation vibration of the inner hydroxyl groups, and Si-O-Si deformation vibration, respectively. Figure 4(B) displayed the FT-IR spectrum of the breviscapine. The characteristic band at 3379cm^{-1} was observed and was due to OH stretching. A sharp peak observed at 2916 and 1148cm^{-1} was due to CH stretching. The FT-IR spectra of breviscapine showed a peak at 1727cm^{-1}, which was ascribed to C=O stretching. The peak observed at 1660cm^{-1} was C=C aromatic stretching. The peak at 1247 cm^{-1} characterizes the stretching vibration of the epoxy. Figure 4(C) depicted the FT-IR spectrum of the physical mixture of breviscapine and halloysite, it can be seen from the figure that the spectrum of the mixture is the overlap of the infrared spectra of halloysite and breviscapine. The peaks of breviscapine from 4000cm^{-1} to 400cm^{-1} can be observed in the spectrum. No additional peaks were seen, indicating there was no interaction of breviscapine either with halloysite. Figure 4(D) showed the FT-IR spectrum of brevescapine-loaded HNTs complex after encapsulated breviscapine. As can see in the spectrum, the peak of brevescapine-loaded HNTs complex is almost similar to the HNTs peak. The characteristic peak of breviscapine has no test in the complex, indicating that the surface of HNTs almost no residual breviscapine, breviscapine were almost loaded to the HNTs cavity [12].

In order to further verify the formation of the drug loaded, the DSC thermograms of pristineHNTs (A), pristinebreviscapine (B), physical mixture of HNTs and breviscapine (C), brevescapine-loaded HNTs complex (D) were obtained (Figure. 5).

Differential scanning calorimetry (DSC) was used to study the crystalline behaviour of HNTs and brevescapine-loaded HNTs complex. Figure 5(A) shows the crystallization exotherms of breviscapine. The drug of breviscapine showed a sharp peak at 186.59^{o}C and 354.14^{o}C due to its exothermic peak and melting peaks. Figure 5(B) shows the crystallization

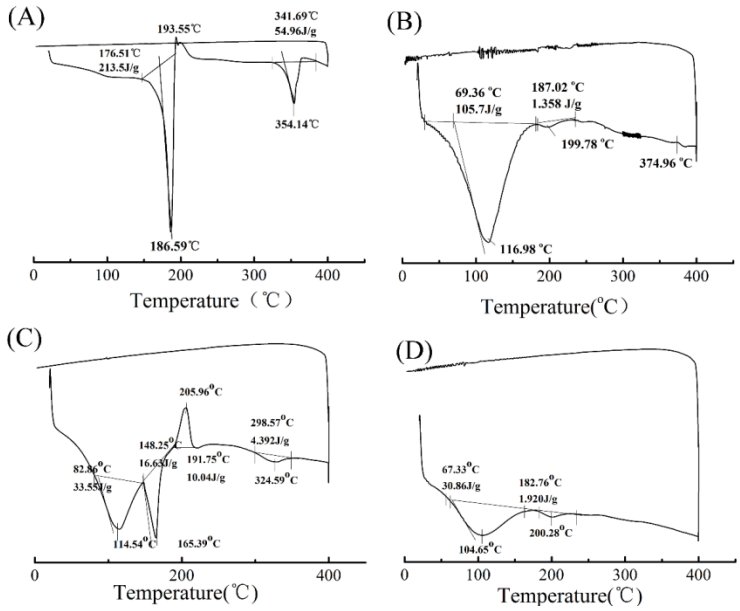

Figure 5. DSC thermograms of (A) pristine breviscapine,(B) pristine HNTs, (C) physical mixture of HNTs and breviscapine, and (D) brevescapine-loaded HNTs complex.

exotherms of HNTs. The apparent endothermic peaks at 116.98°C and 374.96°C are the dehydration peak and the melting peak of halloysite. Figure 5(C) shows the crystallization exotherms of HNTs and breviscapine mixture. The peaks of breviscapine can be observed in the DSC thermogram. No extra peaks were seen, indicating that there was no interaction of breviscapine either with halloysite. Figure 5(D) shows the crystallization exotherms of brevescapine-loaded HNTs complex. The drug of breviscapine showed a sharp peak at 186.59 °C and 354.14°C due to its exothermic peak and melting peaks, which did not appear in the DSC thermogram of brevescapine-loaded HNTs complex. The DSC results prove that the breviscapine were almost in the cavity of HNTs [13].

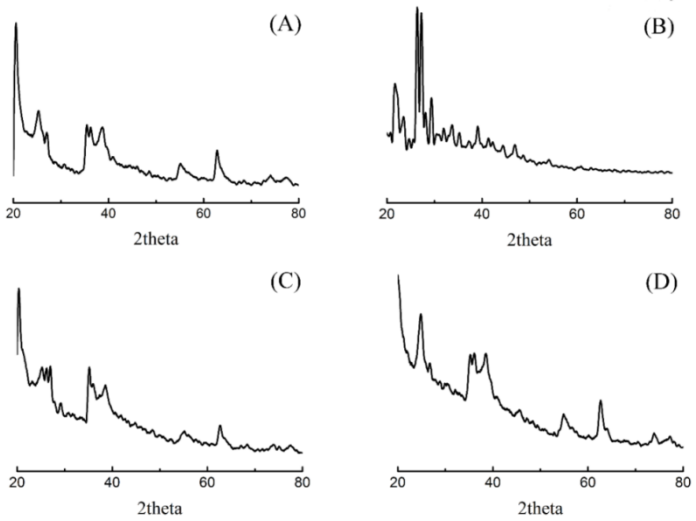

Figure6. XRD spectra of (A) pristine HNTs,(B) pristinebreviscapine , (C) physical mixture of HNTs and breviscapine, and(D) brevescapine-loaded HNTs complex.

X-ray diffraction method commonly used in the identification of crystalline compounds, each having a different crystalline material interplanar spacings at the same angle, thus showing different diffraction peaks. XRD spectrum of HNTs is shown in Figure 6(A). The main absorption peak of halloysite nanotubes is about 20.06, 29.92 at 2θ. XRD spectrum of breviscapine is shown in Figure 6(B). The main absorption peak of brevescapine is about 27, 28 at 2θ. XRD spectrum of HNTs and breviscapine mixture is shown in Figure 6(C), The diffraction angle of halloysite and breviscapine are both shown in the figure 6(C), that can see that halloysite and breviscapine is physical mixture. Figure 6(D) shows the XRD spectrum of brevescapine-loaded HNTs complex, its diffraction angle is similar to halloysite, it has no breviscapine's diffraction angle. It can be determined that the load was formed [14].

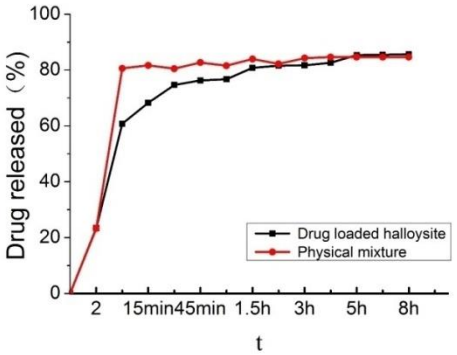

Figure 7. Cumulative release of breviscapine from (a) physical mixture of HNTs and breviscapine, (b) brevescapine-loaded HNTs complex at 37°C.

Figure 7(a) is a graph of the release profile of breviscapine from the physical mixture of HNTs and breviscapine. In this sample, the breviscapine is observed to release in a fairly rapid manner over a period of 3min, and the breviscapine has been completely released rapidly. Figure 7 (b) is a graph of the release profile of breviscapine from the HNTs complex. Breviscapine from the halloysite nanotubes in the 30min release of 80%. Compared with the physical mixture, the drug loaded halloysite has a apparent sustained-release effect [15].

4. Conclusion

In summary, a novel brevescapine-loaded HNTs complex was successfully prepared by using a vacuum process. The morphology and structure were characterized by SEM and FT-IR. Further characterizations were verified by TEM, DSC, XRD. The in vitro release experiment confirmed the brevescapine-loaded HNTs complex can develop a sustained-release and stable efficacy. In addition, HNTs are environmental friendly and low cost materials that are very easy to handle. Therefore, HNT containing breviscapine have great potential for drug in the nanoscopic drug carriers.

Acknowledgments

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Reference

[1] Jiang L, Xia Q, Dong X, et al. Neuroprotective effect of breviscapine on traumatic brain injury in rats associated with the inhibition of GSK3β signaling pathway[J]. *Brain Research*, 2017, 1660: 1-9.

[2] Guo C, Zhu Y, Weng Y, et al. Therapeutic time window and underlying therapeutic mechanism of breviscapine injection against cerebral ischemia/reperfusion injury in rats[J]. Journal of ethnopharmacology, 2014, 151(1): 660-666.

[3] Lvov Y M, Shchukin D G, Mohwald H, et al. Halloysite clay nanotubes for controlled release of protective agents[J]. AcsNano, 2008, 2(5): 814-820.

[4] Rooj S, Das A, Thakur V, et al. Preparation and properties of natural nanocomposites based on natural rubber and naturally occurring halloysite nanotubes[J]. Materials & Design, 2010, 31(4): 2151-2156.

[5] Abdullayev E, Price R, Shchukin D, et al. Halloysite tubes as nanocontainers for anticorrosion coating with benzotriazole[J]. ACS applied materials & interfaces, 2009, 1(7): 1437-1443.

[6] Marney D C O, Russell L J, Wu D Y, et al. The suitability of halloysite nanotubes as a fire retardant for nylon 6[J]. Polymer degradation and stability, 2008, 93(10): 1971-1978.

[7] Handge U A, Hedicke-Höchstötter K, Altstädt V. Composites of polyamide 6 and silicate nanotubes of the mineral halloysite: influence of molecular weight on thermal, mechanical and rheological properties[J]. Polymer, 2010, 51(12): 2690-2699.

[8] RR Price, BP Gaber, Y Lvov. In-vitro release characteristics of tetracycline HCl, khellin and nicotinamide adenine dineculeotide from halloysite; a cylindrical mineral[J]. Journal of microencapsulation, 2001, 18(6):713-722.

[9] Yendluri R, Otto D P, De Villiers M M, et al. Application of halloysite clay nanotubes as a pharmaceutical excipient[J]. International Journal of Pharmaceutics, 2017, 521(1):267-273.

[10] Liu H Y, Du L, Zhao Y T, et al. In vitro hemocompatibility and cytotoxicity evaluation of halloysite nanotubes for biomedical application[J]. Journal of Nanomaterials, 2015, 16(1): 384.

[11] Ding L, Stilwell J, Zhang T, et al. Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast[J]. Nano Letters, 2005, 5(12): 2448-2464.

[12] Kulkarni V H, Kulkarni P V, Keshavayya J. Glutaraldehyde‐crosslinked chitosan beads for controlled release of diclofenac sodium[J]. Journal of Applied Polymer Science, 2007, 103(1): 211-217.

[13] Kubade P, Tambe P. Influence of halloysite nanotubes on morphology, crystallization, mechanical and thermal behaviour of PP/ABS blends and its composites in presence and absence of dual compatibilizer[J]. Composite Interfaces, 2016, 23(5): 433-451.

[14] Gorrasi G. Dispersion of halloysite loaded with natural antimicrobials into pectins: Characterization and controlled release analysis[J]. Carbohydrate polymers, 2015, 127: 47-53.

[15] Lee M H, Park H J. Preparation of halloysite nanotubes coated with Eudragit for a controlled release of thyme essential oil[J]. Journal of Applied Polymer Science, 2015, 132(46).