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Loading and release of drugs inside the cavity of halloysite nanotubes

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Abstract. Recent years have witnessed a huge growth of research in pharmaceutical sciences using nanoparticles as drug transport to reduce toxicity and for more specific drug targeting. Conventional application of drugs has major limitation and drawback since they have limited effectiveness, unsustainable release, and poor biodistribution. Thus this paper aims to produce a suitable material loaded with drugs that can provide an efficient effect of the drug at the site of action and maintain them throughout the treatment. Halloysite nanotubes were used as the material since it fulfills all the criteria needed for sustained drug release. Here, benzalkonium chloride was used as an antiseptic along with methylene blue and methyl orange as the cationic and anionic drug models. All three drugs were loaded into the halloysite lumen by vacuum suction. Result shows the most successful loading efficiency is halloysite loaded with methyl orange where the loading is almost 82% and also shows an excellent sustained release for 19 hours when released in 0.3M hydrochloric acid.

1. Introduction

Drugs are the main existing treatment to stop or reverse the effects of diseases. In the recent years, a number of materials have been extensively studied in order to deliver drugs into the body efficiently. A huge interest has been developed in nanoparticle as they are capable of targeting drugs to the site of action while minimizing the toxicity and the side effects. There is an enormous type of nanoparticles available varying from biological substances like albumin, gelatine and liposomes, and substances of chemical nature like polymer and also clay [1]. Halloysite nanotube (HNT) is an ultramicroscopic multi-layered hollow cylinder which its walls are formed from alternating layers of alumina and silica. Halloysite is capable of being loaded with chemically active agents such as drugs, antibiotic, flameretardant, anticorrosion, and protein. HNT becomes one of the favorable excipients because of its biocompatibility and easily available. R. R. Price et al. (2001) reported that halloysite clay could be used as a low-cost alternative to more traditional microencapsulation system [2].

Silica, SiO_2 made up the outermost layer of halloysite nanotubes while alumina, $Al(OH)_3$ construct as the inner lumen [3]. Due to this composition difference of the outer and inner layer, the surface of halloysite has different electrical ξ -potential. The outermost surface is negatively charged while inner lumen is positively charged. This made halloysites unique since most of the typical non-clay nanotubes,

such as carbon nanotubes, have the same inner and outer chemistry. Besides, halloysite nanotubes have porosity of 50 to 60 $cm^2\ g^{-1}$ and cationic exchange capacity of 30 to 50 × 10^{-2} mol kg^{-1} [3]. Studies showed this porosity signifies the availability of the inner and outer tube surfaces for adsorption.

The release of bioactive agents from halloysite tubules usually takes place in two stages: the fast release of drugs from the external surface and the tube ends and slow release from the inner lumen [4]. Work by Dzamukova, M. R., and her colleague (2015) have shown a comparison of the release profile of antiseptic, brilliant green [5]. It dissolves in water within 5 minutes and has limited in time action, while it is released from halloysite during several hours. The initial stage of the release lasts about two hours and constitutes about 60% of the loaded active agents. Complete release of brilliant green is extended over 30 hours, which allows for drastic enhancement in its antiseptic functions adsorption.

The ideal mechanism of a targeted drug delivery is to have the drug releases in a determined manner and degrade within a period of time. However, traditional drugs have limited effectiveness, unsustainable release, and poor biodistribution. Halloysite is believed to be the most promising material for drug loading and sustained release. This project aims to prepare a drug loaded halloysite for drug delivery use. Three model drugs were used; benzalkonium chloride (BAC) as an antiseptic, methylene blue (MB) as cationic drug and methyl orange (MO) as the anionic drug model. Furthermore, this project also aims to have a profound and remarkable knowledge of the loading and release profile of drugs inside halloysite.

2. Materials and Method

2.1. Preparation of HNT-drug

Drug-loaded HNT were prepared according to the following procedures. 0.3 g of HNT powder was suspended in 150 mL of 0.3mg/mL drug in distilled water followed by stirring for 1 hour. The mixture was then transferred to vacuum desiccator for 15 minutes and cycled back to atmospheric pressure for 5 minutes in order to replace the entrapped gas with the saturated solution. This process was repeated two additional times to ensure that the halloysite is filled with the maximum amount of drugs possible. Next, the solution was centrifuged at 3000 rpm for 1 hour to remove the excess drug. The supernatant was collected and analyzed via a UV spectrophotometer. The halloysite-drug powder was washed three times with distilled water and then air dried.

2.2. Characterization

2.2.1. Morphology.

Transmission electron microscopy (TEM) image of the loaded halloysite is taken to see the presence of successful drug loaded inside the tubes.

2.2.2. Drug Loading Efficiency.

Drug entrapment efficiency (EE) was determined with the following equation:

$$EE(\%) = C_{HNT} = (V_i C_i - V_s C_s)/V_{HNT} \times 100 \quad (1)$$

where C_{HNT} = concentration of drug associated to halloysite
C_i = initial drug concentration
C_s = drug concentration in the supernatant after incubation
V_i = volume of initial drug solutions
V_s = volume of the supernatant
V_{HNT} = volume of halloysite respectively

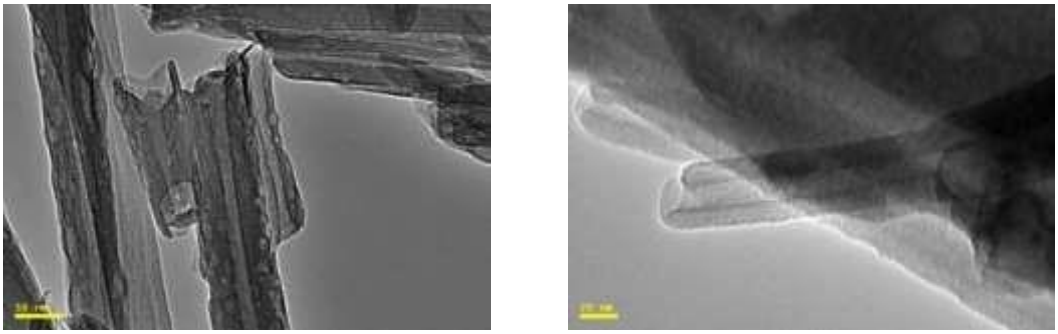
2.2.3. In-Vitro Drug Release.

1 mg of dried drug-loaded halloysite was mixed with 15 ml of 0.3M HCl. The mixture is left to settle and kept in 37°C. At one hour interval, 3mL release media was collected and replaced with the same volume of fresh media. The same steps applied for drug release in Phosphate Buffer Solution (PBS) pH

7.4. Concentration is determined by measuring the adsorption of the solubilized active agents in a UV/VIS spectrophotometer (GENESYS 10S UV-Vis Spectrophotometer) at 662 nm (MB), 463 nm (MO), and 263 nm (BAC).

3. Results

TEM image of HNT-drug is expected to show the presence of drug inside the lumen. Methylene blue is used because it has color compared to BAC which is colorless. However, result from the TEM image does not show any sign of drug presence (Figure 1). Nevertheless**,** there is evidence that able to validate the entrapment of drug inside the HNT. Slight fizzing and bubbling of the suspension during vacuum suction can be seen which signifies air is being removed from the lumen. When the fizzing is seen to stop, the suspension is cycled back to atmospheric pressure to substitute the entrapped gas with the saturated drug solution.



(**a**) (**b**)

Figure 1. (a), (b) TEM image of MB loaded halloysite

Figure 2 shows the entrapment efficiency of three type of drugs. MO shows the highest efficiency entrapment of about 82.29% compared to MB and BAC which are about 70.45% and 66.58% respectively. MO has higher loading efficiency due to the electrostatic attraction between the negative charge of MO and positive charge of HNT inner lumen. Meanwhile, for positively charge MB and BAC, the drug are more attracted to be deposited at the negatively charged HNT surface. According to Vergaro et al., higher loading efficiency also can be contributed by higher solubility of the drug [6].

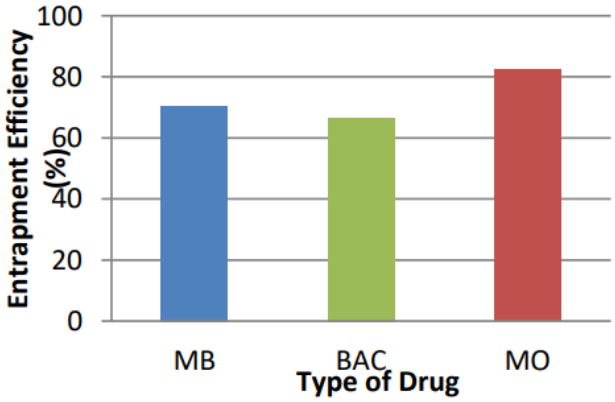


Figure 2. Entrapment efficiency of three type of drugs

Besides, with the aid from vacuum suction technique, it provides a great force to push the liquid drug inside the halloysite cavity. R. R. Price et al., (2001) in his work suggest that vacuum cycling is the recipe for successful entrapment into the very small lumen of the halloysite cylinder [2]. Cycling the suspension from vacuum to atmospheric pressure for multiple times also enhanced the replacement of air in the internal cavities with the drug.

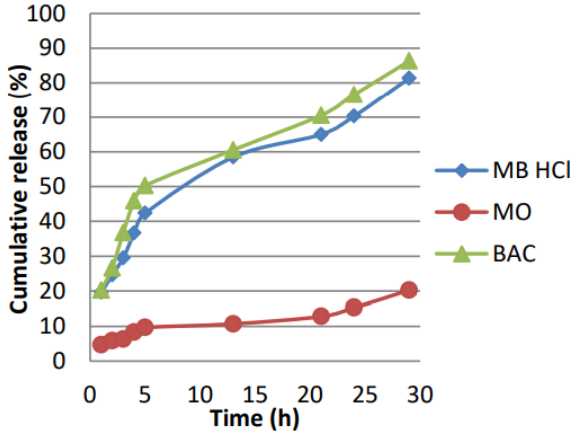


Figure 3. Cumulative release of drugs in 0.3M HCL

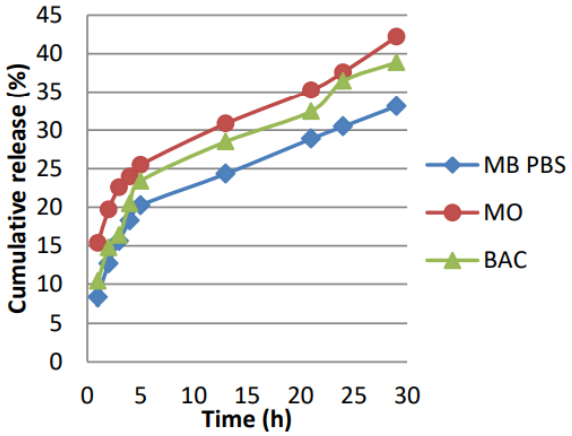


Figure 4. Cumulative release of drugs in PBS

In Figure 3, the extended release profile of three drugs from halloysite nanotubes in 0.3M HCl is illustrated. In 0.3M HCl, MO shows the slowest release with 15.30% release in 24 hours. A number of factors can affect the drug release rate, primarily involving the structure of the container and the chemical properties related to both the clay and the drug.

Chemically, halloysite nanotubes composed of different composition of the outer and inner layer which results in different electrical zeta-potential. In low pH, both HNT lumen and MB/BAC are positively charged. Therefore, a strong electrostatic repulsion accelerates the release of drug from HNT. For anionic MO case, the retention or the slow release could be explained on the basis of strong interaction of opposite charge molecules.

In comparison, Figure 4 shows an extended release of three drugs in PBS pH 7.4. MO gives the fastest release (37.58%) than MB (30.44%) and BAC (36.43%) in 24 hours. These rates contradict with the result in 0.3M HCl due to the chemical properties of the drug molecule in pH neutral. In neutral pH, both MB and MO are in their neutral form. Thus, there is no repulsion or attraction force between the molecules and the HNT charge. Hence, the release kinetics was evaluated using the Korsmeyer-Peppas model:

$$M_t/M_\infty = kt^{1-n} \quad (2)$$

where Mt / M∞ is fraction of drug released at time *t*, *k* is the release constant, and *n* is the release exponent. The value of n indicates the type of diffusion dominating the process.

Table 1 summarized the release kinetics of three drugs. The release exponent (*n*) for all three drugs is up to 0.5 which conclude the drug transport mechanism under Fickian diffusion. Fickian diffusion is associated with concentration gradient, diffusion distance, and the degree of swelling. In fact, MO has the highest solubility of all three drugs and can travel through the pores of halloysite through diffusion and degradation.

Table 1. Drug release kinetics.

Type of drug	Release exponent (*n*)
Methylene blue	0.3691
Methyl orange	0.2716
Benzalkonium chloride	0.3679

4. Conclusion

This project has successfully synthesized halloysite nanotubes as the drug nano-container. Benzalkonium chloride, methylene blue, and methyl orange are used as the drug to be loaded inside the halloysite lumen using the vacuum suction technique. Numerous findings have demonstrated that halloysite nanotubes are proven suitable as drug delivery system. In this study, measurement of drugloading efficiency has highlighted that methyl orange (anionic) interacts preferentially with the HNT lumen, compared to methylene blue and BAC (cationic). It is shown that the loading of drug is affected by chemical properties associated with both HNT and the drug. As for the release of drug, the process is greatly controlled by diffusion and degradation rate.

5. References

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