# 활성성분 운반체로 이용되는 벤토나이트에 삽입된 아스코빅산의 특성연구

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# The Characteristic Study of L-ascobic acid intercalated Bentonite using active ingredient carrier

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## Introduction

One class of drug delivery vehicles that has received more attention in recent years is layered materials, which can accommodate therapeutic compounds between their layers and form a variety of intercalated compounds. Because the release of drugs from drug intercalated layered materials is potentially controllable, these new materials have a great potential as a delivery host in the pharmaceutical field. Natural clay minerals are suitable to effectively modulate drug release. High specific surface area, adsorptive capacity, rheological properties, chemical inertness and low or null toxicity make clay minerals futuristic drug delivery carriers. Among the several approaches proposed to achieve controlled release formulations, the ion exchange process has received considerable attention [1-2].

Many studies have been reported on montmorillonite-drug hybrids for controlled drug delivery. Zheng et al. investigated the intercalation of ibuprofen into MMT as a sustained release drug carrier [3]. The $COO^-$ group of ibuprofen interacts with hydroxyl groups of MMT and the release of ibuprofen from ibuprofen-MMT was affected by the pH of the dispersion. Lin et al. studied the intercalation of 5-fluorouracil with MMT as drug carrier [4]. They optimized the intercalation condition and concluded that 5-fluorouracil intercalated into MMT. Fejer et al. reported intercalation and release behavior of promethazine chloride and buformin hydrochloride from MMT, and concluded that both the adsorption and desorption of the small organic molecules differ from those of the larger molecules [5].

L-ascorbic acid (LAA; i.e., vitamin C, a watersoluble vitamin) contains a variety of biological, pharmaceutical and dermatological functions; for example, it can promote collagen biosynthesis, provide photoprotection, scavenge free radical, cause melanin reduction and

enhances the immunity (e.g., anti-viral effect) [6]. From the perspective in biochemistry, these functions are closely related to the so-called antioxidant properties of this compound. However, LAA is very unstable to exposures in air, light, moisture, heat, metal ions, oxygen, and base, since it is easily decomposed into biologically inactive compounds such as oxalic acid, L-xylonic acid, Lthreonic acid, and L-lyxonic acid. Evidently, applications of vitamin C in various fields (e.g., dermatology and pharmacology) are limited unless these limitations have been overcome. To improve chemical stability of LAA, several extensive attentions have been paid on encapsulation and immobilization of LAA using liposome, microemulsions, and liquid crystals.

Herein, we introduced a new method with practical value of intercalating and stabilizing vitamin C within an inorganic layered material MMT. Meanwhile, toxicity assessment also pointed out the margin of safety and toxicity rankings for feasibility of LAA-MMT composites in practical applications.

## Experimental

### Sample preparation

Bentonite contained several kinds of clay that includes chlorite, kaolinite, illite, and montmorillonite. Montmorillonite is about ~45% by weight in unearthed bentonite. The purification process is briefly described as follows: Crude clay, 500g, was dispersed in 4.0 L of distilled water and then maintained at room temperature for 3 days to be swelled up by all clay minerals. The clay solution was then poured into a filtration tank and gently stirred at a rate of ca. 100 rpm. Particles left on the filtration tank were discarded and all the supernatant was collected. The filtered solution was then centrifuged (700 x g) twice. The supernatant was discarded and precipitates were dried by freeze-drying at -40℃ for 3 days. The dried powder (size about 2.5 $\mu$m) was classified as pure montmorillonite for later experimental use.

### Charaterisation

The surface morphology and element component of montmorillonite was examined by scanning electron microscope (SEM) at 15 kV of accelerating voltage. The samples used for morphology examination were also prepared by compacting the nanocomposite powders into a pellet. The powdered samples placed on a microscope holder were coated with gold in a vacuum (ca. $10^{-4}$ torr) chamber.

The XRD patterns for the mineralogy of montmorillonite were obtained by a RIGAKU Geigerflex diffractometer equipped with Cu K X-ray radiation, operating at 40 kV and 30 mA. The diffraction patterns were ranged in between angles ($2\Theta$) of 1-10 running at a scanning rate and counting step size of 0.6 /min and $0.01^{O}$, respectively.

## Results and Discussions

As shown in Fig. 1(a), montmorillonite is one kind of bioinert clay minerals with fine grain and large inter-planar spacing, in particular located in the (001) plane, indicating superior capability to intercalate large molecules into the interlayer space at the (001) plane. It was

shown that the [001] diffraction peak of the montmorillonite appears at $2\theta = 7.2^{O}$.

As shown in Fig. 1(b), the XRD pattern of MMT-LAA composite at pH 3 revealed that the diffraction peak was shifted from $2\theta = 7.2^{O}$ (i.e., MMT alone) to 5.8 and the *d*-spacing increased from 12.4 Å to 15.4 Å, since L-ascorbic acid particles were intercalated within montmorillonite layers. Fig. 1(c), (d) also showed a layer with the basal spacing of 15.5 A ($2\theta = 5.7^{O}$) and 19.3 Å ($2\theta = 4.7^{O}$) at pH 7 and 10, respectively. These results suggested that L-ascorbate anions might be absorbed or intercalated within the inorganic layers, particularly in wider basal *d*-spacing at higher pHs (e.g., from pH 7 to 10). Due to such a specific characteristics, it is appropriate to be selected as the drug carrier at pH alterations.

After MMT purified from the crude clay of bentonite, it still kept its layer structure and uniformed fine grains to be observed from scanning electron micrograph (SEM) as shown in Fig.2 (a). This photograph showed crumpled platelets that looked like gypsum flower. In addition, as shown in Fig. 3(b)-(d), SEM images of MMT-LAA powders at different pH value (i.e., 3, 7, 10) indicated spherical aggregates on the surface of MMT. At pH 3, XRD patterns showed that owing to the tightly closed structure in MMT, fewer particles could penetrate into the interlayer. At pH 10, the layers of MMT opened widely and thus much fewer particles covered on the surface of MMT due to significant intercalation within layers. These also pointed out a marked rise in the capacity for intercalation over pH changes. The most abundant chemical composition of purified MMT analyzed by EDS are metallic Si (34.42%) and O (25.26%), suggesting $SiO_2$ as a major building block in structure. The chemical formula of the purified MMT could be termed $(Al_{0.61}Fe_{0.04}Mg_{0.12})Si_{0.88}O_{2.68}Na_{0.10}K_{0.05.}$

## Conclusion

SEM and XRD results showed that as the pH increased from 3 to 10, the interlayer of montmorillonite gradually expanded allowing more Lascorbic acid molecules to penetrate within the interlayer.

Dose-response analysis revealed that once MMT was combined with LAA, the EC50 of MMT-LAA was significantly larger than that of MMT and LAA, implying that MMT-LAA was much less toxic than LAA and MMT. In conclusion, MMT might be scientifically feasible to be developed a new form of nanocomposites for further applications in various fields.

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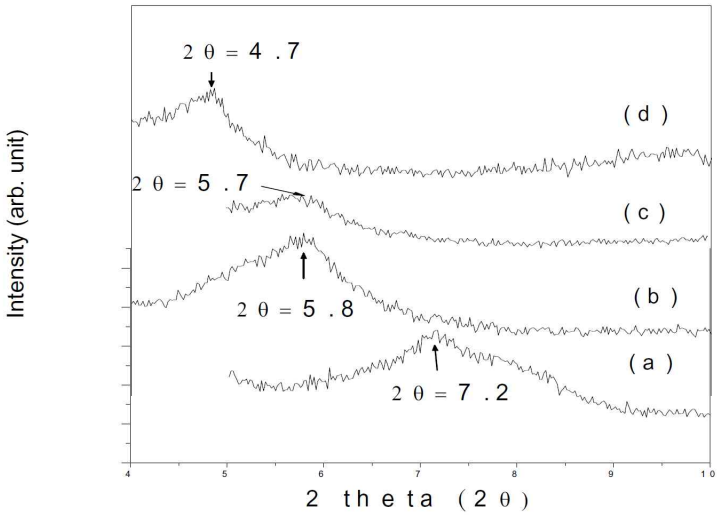


Fig 1(a). X-ray diffraction pattern of montmorillonite (MMT) alone at pH 7 (b) MMT-LAA at pH 3; (c) MMT-LAA at pH 7; (d) MMT-LAA at pH 10.

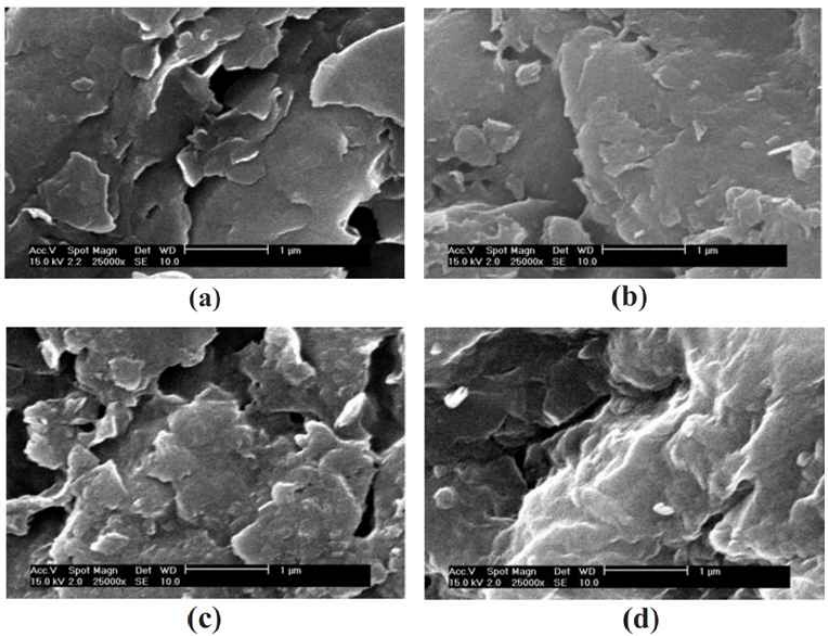


Fig 2. SEM image of (a) montmorillonite surface (25,000X).at pH 7;(b) MMT-LAA surface (25,000X) at pH 3; (c) MMT-LAA surface (25,000X) at pH 7; (d) MMT-LAA surface (25,000X) at pH 10.