피부미백의 최적 방출기능을 지닌 하이브리드 소재 연구

김명훈*, 김선남[1], 송윤구[2]

*캠브리지대학교 화학과, [1]나드리화장품㈜ 기술연구소, [2]연세대학교 지구시스템과학과
(mhk22@cam.ac.uk, myunghunkim@ymail.com*)

The study on hybrid material with optimal released function for skin whiteness

Myung Hun Kim*, Seon Nam Kim[1], Yungoo Song[2]
*Department of Chemisty, University of Cambridge, Cambridge, CB2 1EW, UK
[1]Technology Institute, The Nadree Co. Ltd., Pyongtaik-Si, 451-863, Korea
[2]Department of Earth System Sciences, Yonsei University, Seoul, 120-749, Korea
(mhk22@cam.ac.uk, myunghunkim@ymail.com*)

Arbutin is a new compatible and effective natural whitening substance without skin stimulation and allergy [1] as much more effective than its isomer, arbutin; and the whitening effect of arbutin was more than 10 times higher than β-arbutin [2-3]. In many parts of the world, arbutin is wildly used for cosmetic whitening agent. The demand for arbutin has increased significantly in many regions throughout the world in recent years. Arbutin is used as a skin lightening agent in cosmetics. The substance can be cleaved via metabolic pathways in the skin into D-glucose and hydroquinone. Hydroquinone is a sus-pected carcinogen which is banned in Europe in cosmetic products such as skin lightening agents. The EU Commission's scientific committee for consumer safety (SCCS) is currently re-assessing the active substance.

Montmorillonite (MMT) clay is one of the smectite group, composed of silica tetrahedral sheets layered between an alumina octahedral sheets. The imperfection of the crystal lattice and the isomorphous substitution induce a net negative charge that leads to the adsorption of alkaline earthmetal ions in the interlayer space. Such imperfection is responsible for the activity and exchange reactions with organic compounds. MMT also contains dangling hydroxyl end-groups on the surfaces [4]. MMT has large specific surface area; exhibits good adsorb ability, cation exchange capacity, standout adhesive ability, and drug-carrying capability. Thus, MMT is a common ingredient as both the excipient and active substance in pharmaceutical products [5]. The intercalation of organic species into layered inorganic solids provides a useful and convenient route to prepare organic–inorganic hybrids that contain properties of both the inorganic host and organic guest in a single material [6]. In recent years, smectite clays intercalated by drug molecules have attracted great interest from researchers since they exhibit novel physical and chemical properties. Some researchers have investigated the intercalation of ibuprofen into MMT as a sustained release drug carrier and studied the intercalation using MMT as drug carrier [7-9].

The aim of present study was to develop a highly effective synthesis processes for enhancing arbutin accumulation. To accomplish this goal, arbutin intercaltaed hybrid-materials using

montmorillonite were screened and the releasing conditions were also optimized.

Experimental

The experiments were carried out to determine the optimum pH value for intercalation of arbutin into the interlayer of MMT from 5wt% to 50wt%. These studies were performed by treating arbutin and MMT mixture at different pH and constant temperature, time and concentration. The arbutin intercalated montmorillonite separated from 9 ml suspension was placed into test tubes with 9 ml of 0.1 M HCl (pH 1.2) or 0.1 M sodium phosphate buffer containing 0.85% sodium chloride (PBS) of pH 7.4, and incubated on a constant temperature shaking bed at 37 ◦C and 100 rpm. After specific intervals, samples were ultra-centrifuged and then 3 ml aliquots of the supernatant were withdrawn and immediately replaced with the same amount of fresh medium. The release amounts of arbutin were quantified spectrophotometrically at 280 nm. All the experiments were done in triplicate and mean values were reported.

Results and Discussion

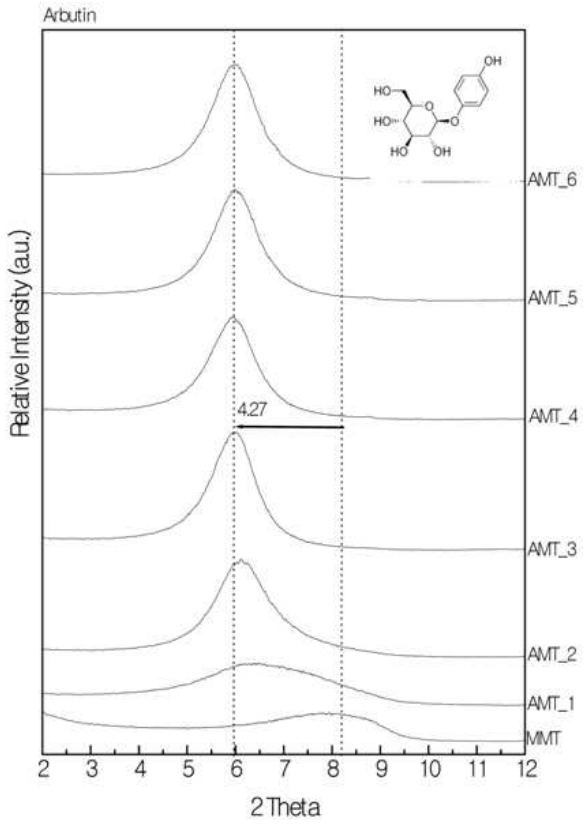
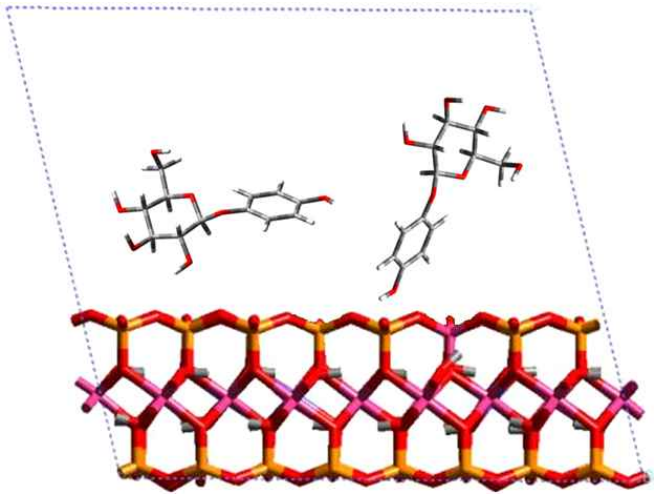


Fig. 1. XRD patterns of MMT and MMT−arbutin hybrid materials.

XRD patterns from 5◦ to 45◦ in fig. 1 exhibit the crystal peaks of MMT and arbutin-MMT hybrid materials. One typical peak near 2θ = 8.2° as (001) plane can be observed for MMT,

but its diffraction intensities shifted drastically in the hybrid materials and the diffraction peak of (001) appears gradually as arbutin content increases. It is obvious that the crystallinities of arbutin-MMT were increased, which reveals that its intermolecular interaction was formed and the interaction between arbutin and MMT has taken place. Fig. 1 shows that the characteristic 2 peak of (001) plane for MMT and MMT−arbuyin hybrid is observed at 7.4° and 5.948°, with basal spacing of 1.18 and 1.47 nm, respectively. From the Bragg's law, the peak shifting fromhigher diffraction angle to lower diffraction angle is due to increase in the d-spacing which indicates that arbutin has been effectively intercalated into the interlayer of MMT (Scheme 1).



Scheme 1. Possible structural interaction of arbutin-MMT.

FT-IR spectra of MMT and arbutin-MMT hybrid-materials shows that the band at 3640 cm^{-1} is ascribed to the stretching vibration of the interlayer −OH group in MMT and the peak at 1485 cm^{-1} belongs to the characteristic absorption of the methyl groups of arbutin, but in the spectra of the hybrid-materials, the former peak nearly disappears while the latter one also weakens gradually. The result indicates that electrostatic interaction between arbutin and MMT has taken place, because the MMT surface is negatively charged and its OH- groups can interact strongly with the CH_3 groups in MMT. Besides, compared to arbutin, the spectra of the hybrid-materilas show a lower frequency of the peak at 3430 cm^{-1}, showing C−H bonded to O−H in arbutin, and the peaks between 3750 and 3000 cm^{-1} become wider, which may due to hydrogen bonding between the −OH group in MMT and the −CH− and −OH groups in arbutin. This fact also reveals the interaction between arbutin and MMT. The surface and cross-sectional morphology of arbutin-MMT hybrid-material is depicted in SEM images in fig. 2. Arbutin-MMT demonstrated a more rigid and flat surface as compared to MMT, whose surface was convoluted.

In the present study, we used human skin melanocytes as an in vitro model because of the need to measure cytotoxic effects. An MTT assay for cytotoxicity was employed before further in vitro testing in skin melanocytes was done to test tyrosinase inhibition and melanin content. Arbutin inhibitors are important constituents of cosmetics and skin-lightening agents. We used l-DOPA as the substrate to detect any arbutin inhibitory effect in HEMn cells (protein content per well, 40g). Among extracts, Pharbitisnil, Sophora japonica, Spatholobus suberectus and

Morus alba showed potent tyrosinase inhibitory effects.

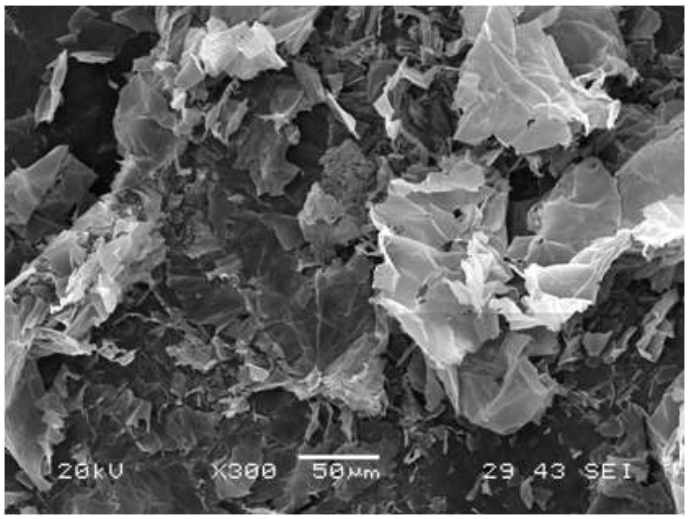


Fig. 2. SEM morphology of arbutin-MMT hybrid-materials.

Conclusion

In the present study, arbutin-MMT hybrid-materials than arbutin were investigated for potential effectiveness as skin-whitening agents and in maintaining skin health. arbutin-MMT hybrid-materials were shown to be potent tyrosinase and melanin synthesis inhibitors in human skin melanocyte cells.

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