Nanoscale Res Lett (2008) 3:355–360
DOI 10.1007/s11671-008-9165-x

NANO EXPRESS

Nanoporous Silicified Phospholipids and Application to Controlled Glycolic Acid Release

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Received: 23 June 2008 / Accepted: 25 August 2008 / Published online: 9 September 2008

Abstract This work demonstrates the synthesis and characterization of novel nanoporous silicified phospholipid bilayers assembled inorganic powders. The materials are obtained by silicification process with silica precursor at the hydrophilic region of phospholipid bilayers. This process involves the co-assembly of a chemically active phospholipids bilayer within the ordered porosity of a silica matrix and holds promise as a novel application for controlled drug release or drug containers with a high level of specificity and throughput. The controlled release application of the synthesized materials was achieved to glycolic acid, and obtained a zero-order release pattern due to the nanoporosity.

Keywords Nanoporous · Phospholipids · Silicification · Sol–gel · Controlled release · Glycolic acid

Introduction

Liposomes, or phospholipid vesicles, are good models for biological membranes, which are found in all species from bacteria to mammals [1–3]. Phospholipids are anionic, cationic, or zwitterionic, and the polar head groups, such as choline, ethanolamine, and serine, differ from each other in size and type of functional groups. The net charge of phospholipid vesicles can be adjusted through the use of different phospholipids. The binding of molecules to the phospholipid membranes also varies in relation to the net charge of phospholipid vesicles. Besides affecting the composition of the phospholipids, the preparation methods affect the structure and characteristics of the liposomes [4, 5]. Lecithin is an important component, especially with respect to membrane penetration. It has been widely used as a biosurfactant in the cosmetic industry for a long time. In addition, the chemical and physical properties of lecithin are largely dependent on its composition of different kinds of phospholipids. The commercial use of lecithin is increasing in the fields of biological membranes, skin-care formulations, and drug delivery. In modern cosmetic products, liposomes can encapsulate the active ingredients required by the skin; as a result, they can be applied directly to the skin cells. Most cosmetic and pharmaceutical liposomes are composed of various types of lecithins of natural, semi-synthetic, and synthetic origins; the major component is usually phosphatidylcholine (PC). The stability of the liposome is closely related to the composition of unsaturated fatty acids and the PC content.

Silica-based surfaces of phospholipids on inorganic surfaces have been formed by the spreading or physicochemical adsorbing of vesicles from solution such as supported lipid bilayers (SLBs). A detailed image of the structural intermediates during the formation of SLBs is emerging from both experimental and theoretical studies [6–10]. Even though many issues regarding the formation of SLBs have been reported recently, such as the role of calcium and the influence of vesicle size on rupture, the role of the inorganic solid support during vesicle formation remains poorly understood [11–13].

Metal cations have a greater influence on the membranes of anionic lipids than on neutral or zwitterionic membranes because of the stronger attractive Coulombic forces. In particular, divalent cations such as Mg^{2+}, Ca^{2+}, and Ba^{2+} affect the stability and structure of phospholipid bilayers

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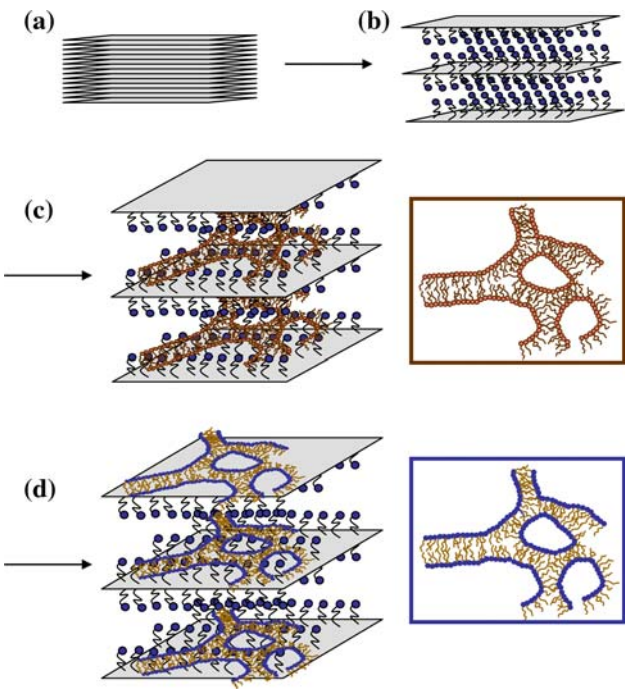
[14–16]. Magnesium is an essential mineral in vertebrates and is the fourth most abundant cation in the body within the cell second only to potassium. Furthermore, a large number of enzymes, especially those involving phosphate compounds such as ATPases, kinases, and phosphatases, have long been known to require the Mg^{2+} cation for activation. The magnesium cation is involved in several physiological and biochemical processes including the synthesis of RNA, DNA, or protein and the stabilization of membranes [17]. Recent research has confirmed that the Mg^{2+} cation plays an important role in the regulation of membrane channels as well as excitation–concentration coupling in skeletal muscle.

In this work, we demonstrated the synthesis and characterization of nanoporous silicified phospholipid bilayers assembled inorganic powders as shown in the Scheme 1. The nanoporous silicified phospholipids are obtained by a silicification process with a silica precursor of tetraethoxy orthosilicate on the hydrophilic domains of phospholipids. The characterization was achieved by combining a sequential measurement of electron microscopy such as transmission electron microscopy (TEM), field-emission scanning electron microscopy (FE-SEM), and electron spectroscopy for chemical analysis (ESCA). In addition, the controlled release application was demonstrated with glycolic acid on both nanoporous silicified phospholipids and non-silicified phospholipids. This approach involves the co-assembly of a chemically active phospholipids layer within the ordered porosity of a silica matrix and holds promise as a novel application for drug release systems or drug containers with a high level of specificity and throughput.



Scheme 1 The routes for the preparation of nanoporous silicified phospholipids-coated inorganic powders. (**a**) Inorganic powders only, (**b**) Mg-chelator bound inorganic powders, (**c**) liquid phospholipids-coated inorganic powders, and (**d**) nanoporous silicified phospholipids-coated powders

Experimental

The phospholipid vesicles consisted of 2 wt% of hydrogenated soybean lecithin from 70% PC. The hydrogenated lecithin (2 g) was added to the deionized (DI) water (98 g) and homogenized at 80 °C. The hot phospholipid vesicle solutions were passed through high-pressure homogenizer three times. The nanoemulsion of the phospholipid vesicle was dropped to the magnesium-grafted talc surface; the magnesium-grafted talc contained enough deionized water and the various concentrations of magnesium sulfate were as follows: 0.35, 1.75, 2.5, 3.5, 7, and 10 mM. The mixtures were aged for 24 h, and the powder was filtered, and then dried in air. Using a Quantachrome Nova e-4000 system with a sample pretreated at 100 °C overnight in a vacuum line, the Brauner–Emmet–Teller (BET) results were obtained by the Barrett–Joyner–Halenda (BJH) method from nitrogen adsorption and desorption isotherms. The distribution of the pore size was calculated from analysis of the adsorption branch of the isotherm. The pore volume was taken at the five points of P/P_0. The electron microscopic measurements such as TEM and SEM were achieved by a JEOL JEM-4010 TEM (400 kV) and a JEOL JSM 6700F, respectively. Infra-red (IR) and ultraviolet-visible (UV–Vis) spectra were obtained from a JASCO V-460 and JASCO V-550 including an attenuated total reflectance Fourier transform IR (ATR-FTIR) technique and diffused reflectance UV–Vis spectroscopy, respectively.

The glycolic acid release experiments were achieved from the overnight immersion of nanoporous silicified phospholipid-coated talc powder in a saturated drug solution, the mixture of which was dried over a period of 3 days at room temperature. The dried mixture was then immersed in 10 mL of a phosphate buffer (PBS, pH 7.4, 10 mM) and kept in a temperature-controlled shaker for stepwise temperature changes. The drug released concentration of the solution was measured with a UV–Vis spectrophotometer at different time intervals. After each measurement, 10 mL of the PBS buffer was replaced.

Results and Discussion

The formation of the nanoporous silicified phospholipids was achieved by a sol–gel reaction on the hydrophilic head

domain in the phospholipids with the silica precursor tetraethoxy orthosilicate. Figure 1 shows a conceptual diagram and the molecular simulation and indicates the multipurpose application of various kinds of phospholipids. The nanoporous silicified phospholipid vesicle was formed via self-assembly of amphiphilic surfactant in the formation of nanoporous inorganic materials, and the phase consists of a three dimensional random packing of a multiply connected bilayers of the surfactant and co-surfactant dividing the space into two sub-spaces filled with solvent based on the liposome structure. The pore size and distance between silicate layers of porous structure were controlled by changing the hydrophilic domain such as water-amounts. Note also that the thickness of the silica can be controlled by the concentration of silica precursors. The initial silica domain on the nanoporous silicified phospholipids caused to control the release of molecules such as drug or chemicals due to the nanoporosity, and to the strong binding between the liquid phase phospholipids and the inorganic powders.

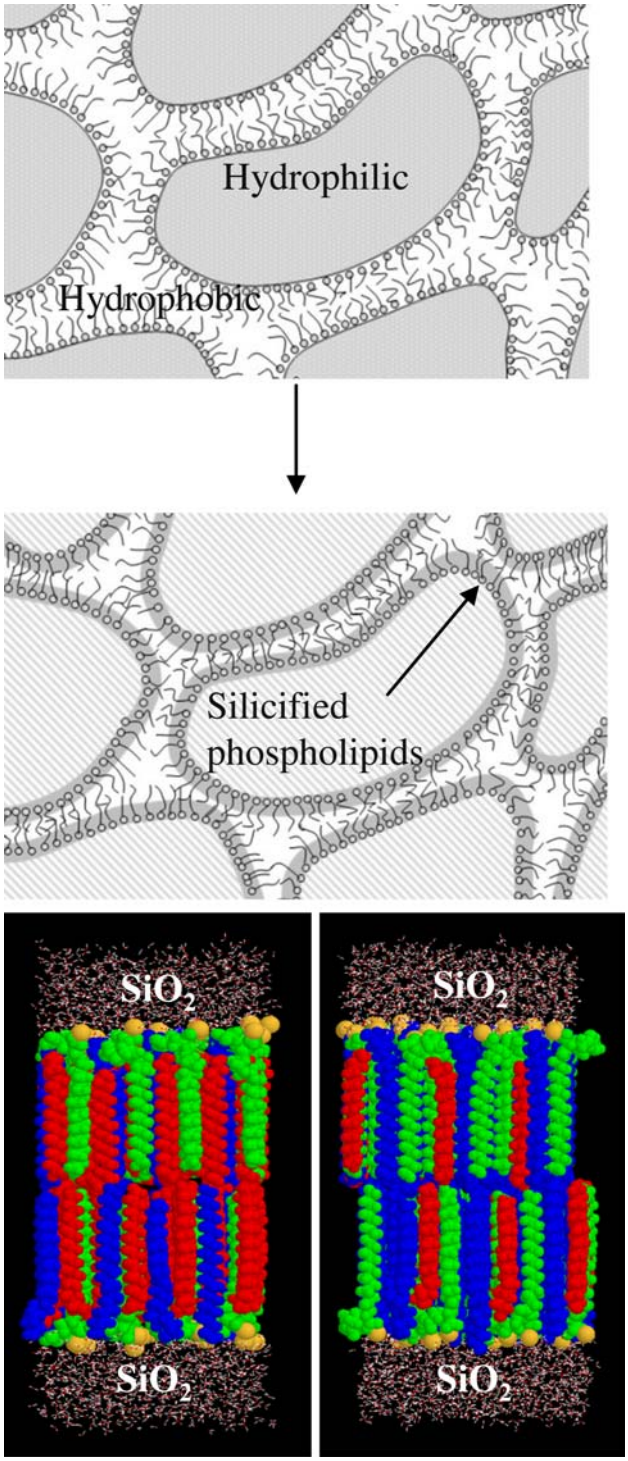


Fig. 1 Schematics for the preparation of silicification process on hydrophilic region of phospholipids, and molecular simulation images

To elucidate the effect of silica precursor on the formation of silica coated phospholipids, we demonstrated the quantification of Si element in the nanoporous silicified phospholipids by the inductively coupled plasma-optical emission spectrometer (ICP-OES) with axial viewing configuration. This elemental analysis was achieved by the various concentration of silica precursor such as 0.5, 1.0, and 1.5 M. The results on Si element showed the 30.5, 58.5, and 89.3 wt% corresponding to 0.5, 1.0, and 1.5 M of silica precursor concentration, respectively. These results provided that the use of higher concentration of silica precursor was caused to strong cross-linking within the silica matrix to enhance the silica density as shown in molecular simulation images. However, the structural changes of phospholipids do not occur because this cross-linking interaction is just motivated by the hydrophilic interaction.

Figure 2 shows the FE-SEM and TEM images of the nanoporous silicified phospholipids-coated talc powder, where the talc powder has undergone magnesium chelation, phospholipids coating, and a silicification process. The FE-SEM images showed the comparative results after the phospholipids and silicification coating processes: the image of the phospholipids-coated talc revealed the disappearance of the ordered lamellar structure in the talc powder as a result of the liquid phospholipids coating. However, the initial lamellar structure on the powder was formed by the silicification caused by the interaction between the hydrophilic heads of the phospholipids and silica precursor. The TEM images confirmed the existence and sizes of the silicified phospholipid vesicle droplets, which were distributed in a range of 50–90 nm. Smaller droplets and a narrow size distribution were generally favored for good emulsion stability. In spite of the formation of smaller droplets, conventional emulsion has a low time-stability as a result of creaming or sedimentation, but the nanoemulsion prepared by high-pressure homogenization has better stability.

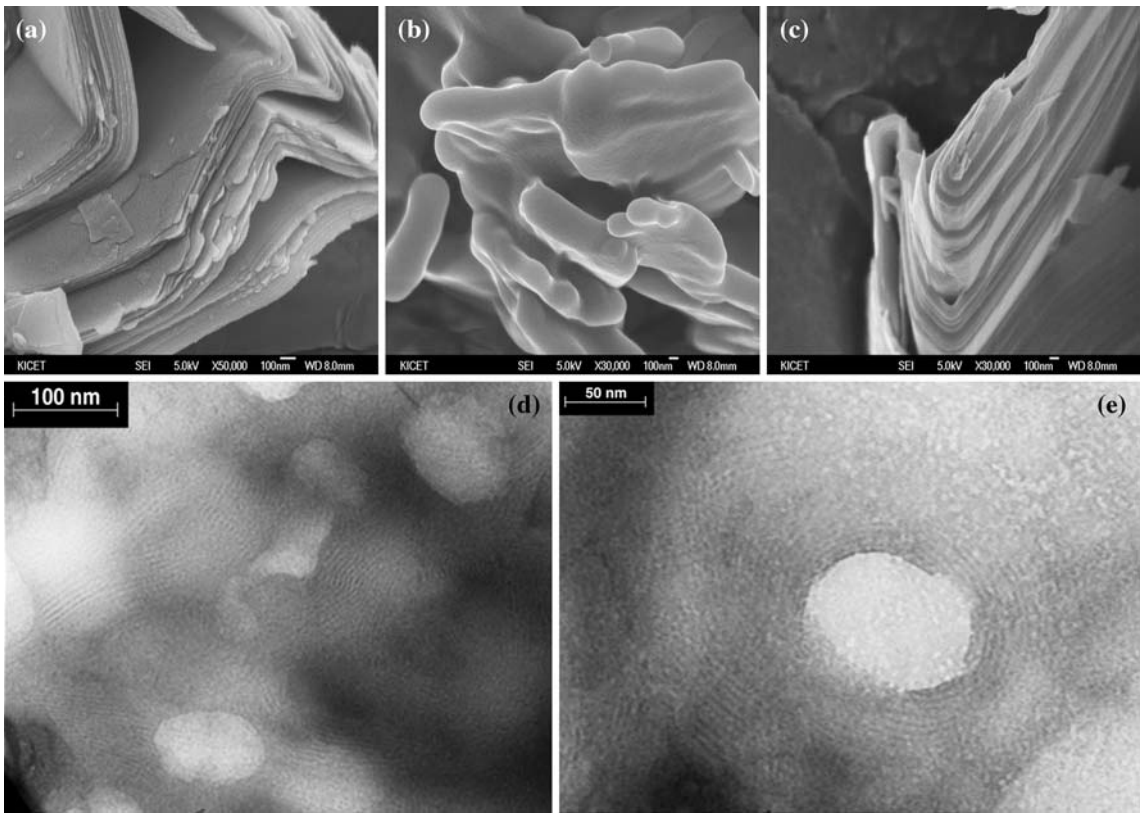


Fig. 2 SEM images of (**a**) talc powders, (**b**) phospholipids-coated talc powders, (**c**) nanoporous silicified phospholipids-coated talc powders, and TEM images of nanoporous silicified phospholipids-coated talc powders (**d–e**)

To demonstrate the surface activation and the fixation of phospholipids on the surface of talc powders, the various concentration of magnesium sulfate of 0.35, 1.75, 2.5, 3.5, 7, 10 mM as a chelator were added before the addition of phospholipids on the talc solution as shown in Fig. 3. After phospholipids addition, BET measurement with BJH method was achieved to measure the surface areas and pore-sizes of the materials. The BET surface areas of the materials were drastically decreased as a function of Mg^{2+} cation concentration: that is very low surface areas of 3–10 m^2/g due to the large attachment of nanoporous silicified phospholipid vesicles on Mg^{2+} grafted talc powders. However, the pore size of the materials was increased by the silicification process. In addition, the UV–Vis spectrum of the resident solution from the mixture of the Mg^{2+} bound talc powders and the phospholipids showed that the presence of magnesium has a much more pronounced influence on the phospholipid deposition process. The UV–Vis spectra of the phospholipids showed that as we increased the Mg^{2+} cation concentration, the intensity at 280 nm was diminished. This phenomenon explained how the decrease in resident phospholipids is caused by the attachment of the phospholipids to the Mg^{2+} substituted talc powders.

Figure 4 showed the wide-angle XRD data and an FT-IR spectrum taken with an attenuated reflectance method of nanoporous silicified phospholipids-coated talc powders. The XRD data were confirmed that the talc crystallinity was maintained after the phospholipids and silicification coating process; all the main peaks showed the *d* values of 9.34, 4.65, 3.11, 2.33, 1.87, 1.56, 1.39, 1.33, respectively. The lattice constants *a*, *b*, and *c* which were calculated in relation to the observed *d* values were 5.19, 9.29, and 18.89, respectively. From calculation of these values, the talc powder had a tri-layer monoclinic form of the mineral. After phospholipids coating process, some weak ripples from typical peaks of talc powder disappeared and shifted to form shoulders on the broad peaks at $2\theta = 22°$ and 60° because the phospholipids coating caused a stacking disorder in the talc powder. The silicification process on the phospholipids-coated talc powder showed that the peak position and intensity were similar to the talc powder: this outcome indicated that the disordered phospholipids domain was ordered by the silicification on the hydrophilic domains (heads) of the phospholipids. The proposed reactions produced the significant changes that were observed in the IR spectra of the surface modified talc powders. The spectrum of the talc powder showed the

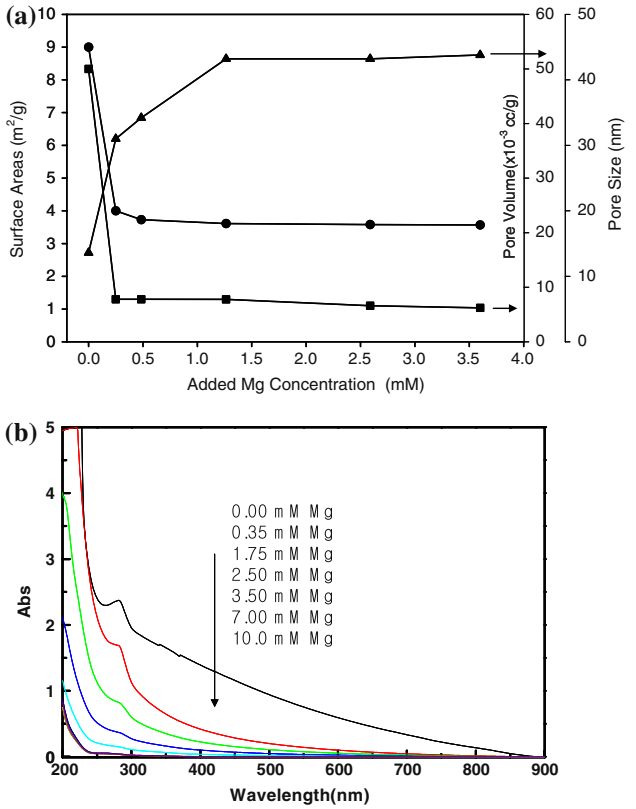


Fig. 3 Correlation plots for (**a**) surface areas (●), pore volume (■), and pore size (▲) from BET measurements of phospholipids-coated talc powders, and (**b**) UV-Visible spectra of a resident solution from the mixture of phospholipids-coated talc powders as a function of the Mg concentration

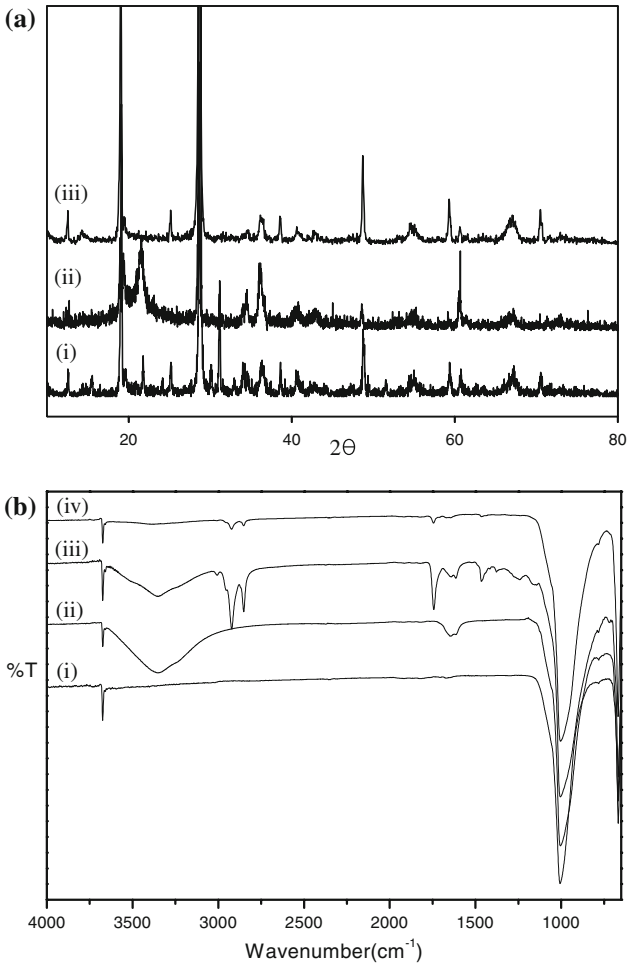


Fig. 4 (**a**) Wide-angle XRD data. ((i) Talc powders, (ii) phospholipids-coated talc, (iii) nanoporous silicified phospholipids-coated talc), and (**b**) FT-IR spectra of (i) talc, (ii) Mg-talc, (iii) phospholipids-coated talc, and (iv) nanoporous silicified phospholipids-coated talc powders

presence of a sharp band at 3676 and 1000 cm^{-1} corresponding to *v*(Mg–OH) and *v*(Si–O–Si) vibrations, respectively. The spectrum of the Mg-chelator bound talc (Mg-Talc) showed an increase in the *v*(–OH) vibration at 3400 cm^{-1} due to the formation of magnesium hydroxide ($Mg–(OH)_2$). A spectrum of the phospholipids-coated Mg-talc powder revealed that the intensity of the magnesium hydroxide was reduced by the replacement of the lecithin, and that the initial bands of alkyl chains appeared in the range of 3000–2800 cm^{-1}. A spectrum of the silicified phospholipids-coated Mg-talc powder showed the *v*(Mg–OH), *v*(Si–O–Si), and *v*(C–H) vibration in the detection ranges confirming the presence of organic and inorganic groups on the matrices.

The controlled drug release application was achieved through the loading and releasing of glycolic acid, which is known as a skin-care drug for wrinkle, roughness, age spots, and other skin damage. For the nanoporous silicified phospholipids-coated Mg-talc materials, the pattern of drug loading and releasing were dependent on the pore confinement of the porous structure as a result of the weak hydrogen bonding between the surface hydroxy group(–OH) of SiO_2 or the hydrophobic alkyl chain of the lecithin and hydroxy group(–OH) of glycolic acid. Figure 5 shows the releasing profile of glycolic acid in both the phospholipids-coated Mg-talc (LC Mg-talc) and the nanoporous silicified phospholipids Mg-talc (SLC Mg-talc) powders by using the reflective method of a solid-state UV–Vis spectrophotometer. The overall delivery of the glycolic acid into the media is controlled by diffusion through the LC Mg-talc membrane and the SLC Mg-talc porous matrices. The glycolic acid diffusion in the porous matrices of the nanoporous silicified phospholipids was effected through a more controlled released than that of the only phospholipids-coated materials. This result was attributed to the steric hindrance of the silica layers because the porous matrices of the silicified phospholipids had a more complicated structure. Although the glycolic acid could be easily squeezed out of the LC Mg-talc powders, the silica layer of the Mg-talc porous matrices enables the release to be controlled. The release

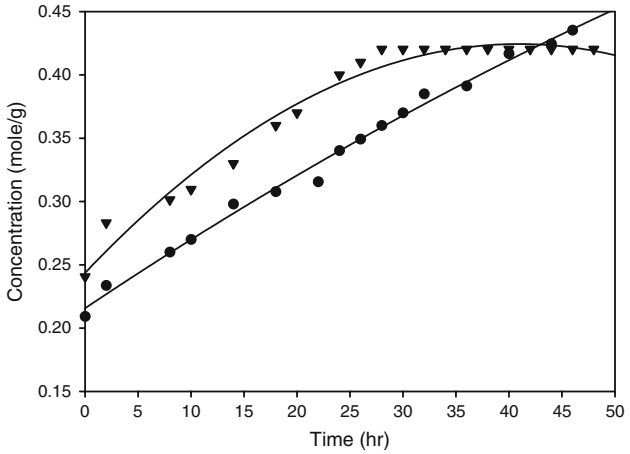


Fig. 5 Controlled release profile of glycolic acid as a function of the releasing time. (●) Phospholipids-coated talc powder and (▼) nanoporous silicified phospholipids-coated talc powder

profile of the SLC Mg-talc powders showed a first-order and zero-order release before and after 24 h, respectively. However, the first-order release pattern was only observed in the LC Mg-talc powders. From comparing the first-order release patterns, the release rate of glycolic acid for the initial 24 h was calculated to 6.2 and 3.7 mmol/(h g) for the SLC Mg-talc powder and LC Mg-talc powders, respectively.

Conclusion

In summary, the nanoporous silicified phospholipids-coated inorganic powders were systematically synthesized and characterized. The silicification process involves co-assembly of a chemically active phospholipid bilayer within the ordered porosity of a silica matrix. The concentration of the Mg-chelator was optimized to fix the phospholipids and activate the surface. The controlled release application of the synthesized materials was achieved to glycolic acid, and obtained a zero-order release pattern due to the nanoporosity. Consequently, the nanoporous silicification process on the phospholipids is very useful for bio-related fields such as cosmetics and drug delivery systems. We expect the basic results to lead to a general and simple approach for preparing a wide range of controlled releasing materials such as encapsulation with cosmetics or drugs.

Acknowledgments This work was supported by a grant from the Fundamental R&D Program for Core Technology of Materials funded by the Ministry of Knowledge Economy, Republic of Korea.

References

1. T.H. Watts, A.A. Brian, J.W. Kappler, P. Marreck, H.M. McConnell, Proc. Natl Acad. Sci. U.S.A. **81**, 7564 (1984). doi:10.1073/pnas.81.23.7564
2. T.S. Aurora, W. Li, H.Z. Cummins, T.H. Haines, Biochim. Biophys. Acta **820**, 250 (1985). doi:10.1016/0005-2736(85)90118-X
3. H. Hauser, N. Gaines, Proc. Natl Acad. Sci. U.S.A. **79**, 1683 (1982). doi:10.1073/pnas.79.6.1683
4. R. Tero, M. Takazawa, Y.J. Li, M. Yamazaki, T. Urisu, Appl. Surf. Sci. **238**, 218 (2004). doi:10.1016/j.apsusc.2004.05.203
5. R.P. Richter, A.R. Brisson, Biophys. J. **88**, 3422 (2005). doi:10.1529/biophysj.104.053728
6. C.A. Keller, K. Glasmaster, V.P. Zhdanov, B. Kasemo, Phys. Rev. Lett. **84**, 5443 (2000). doi:10.1103/PhysRevLett.84.5443
7. J. Radler, H. Strey, E. Sackmann, Langmuir **11**, 4539 (1995). doi:10.1021/la00011a058
8. V.P. Zhdanov, C.A. Keller, K. Glasmaster, B. Kasemo, J. Chem. Phys. **112**, 900 (2000). doi:10.1063/1.480617
9. R. Waninge, T. Nylander, M. Paulsson, B. Bergenstahl, Colloids Surf. B **31**, 257 (2003). doi:10.1016/S0927-7765(03)00145-0
10. J.H. Chang, C.H. Shim, B.J. Kim, Y. Shin, G.J. Exarhos, K.J. Kim, Adv. Mater. **17**, 634 (2005). doi:10.1002/adma.200400545
11. R.P. Richter, A. Brisson, Langmuir **20**, 4609 (2004). doi:10.1021/la049827n
12. T.E. Starr, N.L. Thomson, Langmuir **16**, 10301 (2000). doi:10.1021/la000992m
13. J. Penfold, E. Staples, I. Tucker, Langmuir **18**, 2967 (2002). doi:10.1021/la011575s
14. G. Puu, I. Gustafson, Biochim. Biophys. Acta **1327**, 149 (1997). doi:10.1016/S0005-2736(97)00052-7
15. I. Reviakine, A. Simon, A. Brisson, Langmuir **16**, 1473 (2000). doi:10.1021/la990806g
16. G.W. Feigenson, Biochemistry **28**, 1270 (1989). doi:10.1021/bi00429a048
17. L.W. Cynthia, D.L. Kunze, J. Physiol. **575**, 175 (2006). doi:10.1113/jphysiol.2006.113308