SYNTHESIS OF ULTRAFINE, MULTICOMPONENT PARTICLES USING PHOSPHOLIPID VESICLES

H. LIU, G. L. GRAFF, M. HYDE, M. SARIKAYA, and I. A. AKSAY

Department of Materials Science and Engineering, and Advanced Materials Technology Center, Washington Technology Center, University of Washington, Seattle, WA 98195

Because of their unique properties of self-assembly and selective ion permeability across the lipid bilayers, phospholipid vesicles were used as reaction vessels for the synthesis of ultrafine, multicomponent ceramic particles containing Y, Ba, Cu, and Ag. Chemical inhomogeneities in the system were limited to the individual particle size (< 50 nm), which was a considerable improvement over particles prepared using bulk precipitation routes. The consistent barium deficiency was a serious problem that arose when attempting to control the stoichiometry of the multicomponent system. Our experimental evidence suggests that chemical interactions between the barium cations and the vesicle-forming phospholipid may inhibit the precipitation of barium salts. In a parallel study, we performed consolidation studies on vesicle-precipitated Ag_2O *particles before and after the removal of the phospholipid molecules. Particle packing was greatly improved in the surfactant coated particles. This demonstrates the multifunctionality of this biomimetic system in which the vesicle membrane simultaneously acts as: (i) a reaction cell for particle precipitation, (ii) an ion selective membrane that affects precipitation kinetics, (iii) a barrier to prevent spontaneous agglomeration of the ultrafine particles, and (iv) a lubricant/dispersant that facilitates particle rearrangement during consolidation.*

INTRODUCTION

Recent trends in powder processing have placed emphasis on the use of ever-smaller particles as starting materials. With the use of gas-phase precipitation routes such as CVD and the gas-condensation method, remarkable properties such as ductility in ceramic materials, and significant increases in strength and hardness have been observed in nanophase materials.[1-4] Particle size reduction into the nanometer range can also result in substantially altered chemical, electrical, optical, and magnetic properties.[5]

Although the improved properties of these nanophase systems are enticing, the synthesis, dispersion, and consolidation of these ultrafine particles is extremely challenging.[6] Gas phase particle synthesis typically requires sophisticated equipment and high vacuum. Once formed, nanometer-sized particles are susceptible to rapid agglomeration due to van der Waals attractions and can fuse at contact points due to the high surface energies of the particles, thus making dispersion into primary particles impossible.

One possible solution is to utilize colloidal techniques to produce ultrafine particles and regulate particle interactions. Surfactants or polymeric additives are commonly added to colloidal dispersions to control interparticle forces and stabilize the individual particles. Another level of sophistication is to form particles within surfactant-containing structures such as micelles, vesicles, or lamellae thereby precipitating and coating the particles against agglomeration simultaneously.

Intravesicular precipitation of inorganic, crystalline particles is common in nature. Nanometer-sized magnetite particles, for example, are fabricated in intracellular vesicles by certain types of bacteria with precise control over particle morphology and orientation.[7] In addition, Mann et al.[8,9] have demonstrated that single component particles can be precipitated within synthetic vesicles as a model system for the study of biomineralization. To date, we are unaware of the use of intravesicular precipitation techniques with complex, multicomponent powders. Therefore, we investigated the possible application of this particle-forming technique to multicomponent systems. Accordingly, the goals of this study were to: (i) produce unagglomerated, nanometer-sized multicomponent particles using colloidal techniques, (ii) to examine the chemical homogeneity of the particles, and (iii) to observe the consolidation behavior of vesicle-coated versus noncoated particles. In this paper, we present some preliminary results towards achieving these goals.

EXPERIMENTAL

Dried films of egg yolk phosphatidylcholine (Sigma Chemical Co., St. Louis, Mo.) were swollen in the presence of an aqueous salt solution containing 0.15 M $Cu(NO_3)_2 \cdot 3H_2O$, 0.1 M $Ba(NO_3)_2$, 0.05 M $Y(NO_3)_3 \cdot 6H_2O$, and 0.0125 M $AgNO_3$ (3:2:1:0.25) to form a 0.67 wt% lipid solution. This solution was then sonicated using a probe-type sonicator at 60W output at room temperature until the solution was no longer turbid, which indicated the formation of small, unilamellar vesicles. The dispersion was then centrifuged for 5 minutes to eliminate any particulate contamination from the probe tip. For the consolidation studies, single component Ag_2O particles were synthesized from 0.1M $AgNO_3$ solutions by the same procedure.

To remove unwanted ions surrounding the vesicles, the dispersions were passed through a desalting column prepared from Sephadex G-50 beads. A UV detector set at 200 nm was used to monitor the movement of vesicles and ions at the column outlet. Once the solution had been desalted, intravesicular precipitation of the particles was induced by introducing 0.1 N NaOH to the multicomponent (Y, Ba, Cu, Ag) system and 0.1N NH_4OH to the single component (Ag) system.

Transmission electron microscopy (TEM) was used to acquire information about morphology, crystal structure, composition, and consolidation behavior of the particles formed within the vesicles. TEM samples were prepared by loading a small drop of the dispersion containing particles onto a carbon-coated nickel grid, and allowing the sample to dry. Energy dispersive x-ray spectroscopy (EDS), with spot sizes of 20 and 50 nm, was used to determine elemental compositions of the ultrafine particles.

Inductively coupled plasma emission spectroscopy (ICP) was used to determine elemental concentrations of ions in solution both before and after intravesicular precipitation. Quasi-elastic light-scattering measurements were used to determine vesicle sizes in solution.

RESULTS AND DISCUSSION

Multicomponent Particle Formation

Particle precipitation within vesicles has several fundamental differences from bulk precipitation methods due to the unique properties of the lipid bilayer. In addition to forming a reaction cell which limits particle size, the bilayer serves as a semipermeable membrane to ion diffusion. Generally, phospholipid vesicles are nearly impermeable to cations, with typical permeability coefficients between 10^{-12} to 10^{-14} cm/s.(Ref.10-12) Diffusion rates of anions, on the other hand, are usually two to three orders of magnitude higher than those of cations,[13] but are still quite low (10^{-10} cm/s for Cl^-). This characteristic imposes a kinetic restraint on precipitation due to the diffusion restrictions across the bilayer,[14] and produces a system in which cations are essentially "trapped" within the phospholipid cage until precipitation can occur. This could potentially enhance chemical homogeneity within the system and facilitate the aqueous precipitation of water soluble phases (such as $Ba(OH)_2$).

Figure 1(a) shows a typical TEM micrograph of the vesicle-formed particles using the Y, Ba, Cu, and Ag nitrate precursors. The lipid membrane was not stained in our experiments, and therefore, is not visible. The particles are roughly spherical, crystalline, and well dispersed. A mean particle diameter of 34.8 nm with a standard deviation of 13.2 nm was determined from TEM micrographs of 470 different particles. It is important to compare the particle sizes with the starting sizes of the original vesicles. Theoretically, the particle sizes should be smaller than the vesicles from which they were formed. Light-scattering experiments showed the vesicle size in deionized water to be approximately 59 nm. Since the presence of ions in solution can have pronounced effects on the morphology and phase transitions of vesicles,[15] we also measured sizes in the presence of the nitrate salts. The vesicle size increased to 99 nm under the high ion concentrations. In both cases, the results confirm that the particle size is smaller than the corresponding vesicle size.

The EDS spectra collected on a single particle in Figure 1(b) positively confirms the presence of all four starting components. This suggests that chemical inhomogeneity has been confined to the physical dimensions of each particle (< 50 nm), which is a significant improvement over bulk precipitation. The large nickel ($Ni_{K\alpha}$) peak is due to the TEM grid and the phosphorus ($P_{K\alpha}$) peak from the phospholipid molecules. The particles are also multidomain, which suggests that compositional differences may exist between different regions within a single particle. Preliminary EDS work with a 5 nm probe tends to support this hypothesis.

Electron diffraction patterns were also taken on the particles shown in Figure 1(a). By comparing calculated d-spacings with standard x-ray data files, the possible constituents of the particles include Ag_2O, Y_2O_3, CuO, $Y(NO_3)_3 \cdot H_2O$, $Cu(NO_3)_2 \cdot 6H_2O$, $AgNO_3$, and $Ba(NO_3)_2$. However, this system is very complex, and the identification of these phases is by no means certain unless electron microdiffraction is performed on individual particles.

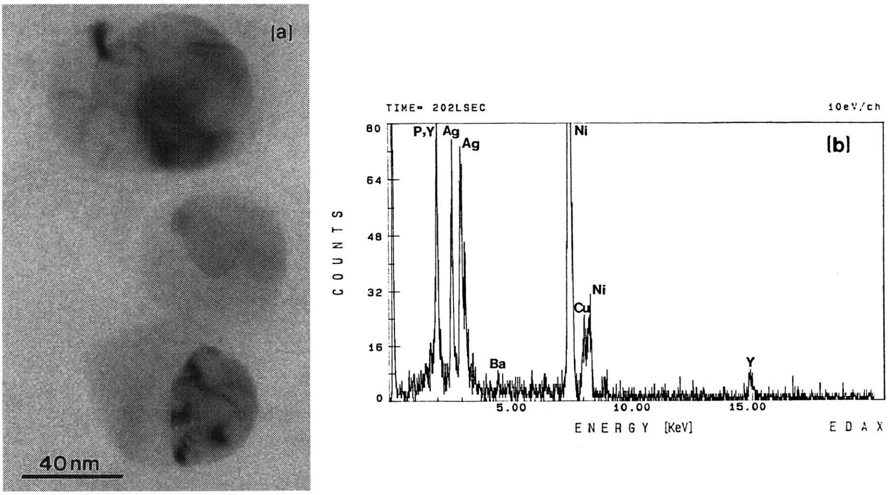

Figure 1. (a) TEM micrograph of multicomponent particle formed within vesicle, and (b) EDS spectra of single particle.

Stoichiometric Considerations

Although the vesicle-mediated particle formation system exhibits some exciting advantages over more conventional methods of powder production, there are also some distinct problems with the system. By far the most serious is the observation that the ratio between elements in the formed particles was not consistent with the beginning solution ratios. Most pronounced was the barium to silver ratio. Though the starting solution contained eight times more barium than silver, the final particles were always rich in silver as shown in Figure 1(b).

There are several possible explanations for the observed barium deficiency. The first possibility is that the barium (II) ions may leak out of the vesicle bilayers. The second possibility is that the barium (II) ions are not as easily incorporated within the vesicles as the silver (I) ions, and third, specific interactions between the divalent barium cation and the phosphatidylcholine head group on the lipid molecules may inhibit the precipitation of barium salts. These possibilities will be considered individually in the following paragraphs.

To evaluate the possibility of barium leakage, a fundamental understanding of the retention time of barium (II) is required. The gel filtration (Sephadex) column is an ideal system for such measurements. If all ions are contained within the vesicles, the chromatographic trace of absorption as a function of time (Figure 2) will show a single peak. As ions leak through the bilayer, a second absorption peak will arise in the trace due to the presence of free ions. Based on this principle, a number of chromatographic traces were taken at various time intervals after barium (II) encapsulation within the vesicles. Figures 2(a-d) show that, at one hour, only the single vesicle peak exists. At three hours, the second apex began to rise and became more and more pronounced as time progressed. Since all our TEM samples were prepared within 2 to 2.5 hours, the barium ions should have remained within the vesicles.

The possibility also exists that barium ions may be chemically bound to the exterior of the vesicle and slough off while passing through the column during the initial desalting procedure. Baseline resolution was never acheived between the respective vesicle and ion peaks in our chromatographic separations on the barium system, whereas baseline resolution was common in the silver system. This could account for some of the barium loss. Experiments are currently underway to better understand this observed phenomena.

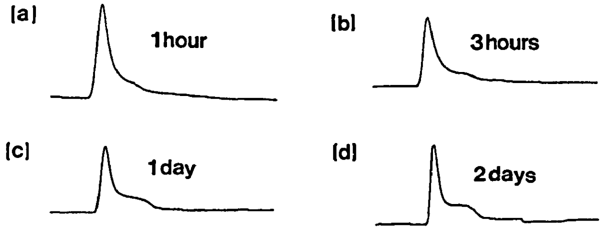

Figure 2. Gel filtration curves at various times for vesicles containing barium (II) ions.

A second possibility is a low incorporation efficiency of barium ions in the system. To test this hypothesis, standard solutions with known Ba:Ag ratios were prepared and the ion concentrations measured using ICP. Next, this same solution was used to swell the lipid films and form unilamellar vesicles. After desalting, the intravesicular ion concentration was again measured using ICP. The results were plotted as Ba:Ag starting ratio versus Ba:Ag intravesicular ratio in Figure 3. It is obvious that the silver ions are more readily entrapped and retained since a starting Ba:Ag ratio of 10:1 results in a intravesicular ratio of only 2:1. This implies that the barium ions are indeed more difficult to incorporate within the vesicles or they are lost during initial desalting. Even so, we found that upon precipitation of the 10:1 (Ba:Ag) sample, the final particles were still barium deficient. Therefore, the lower incorporation efficiency cannot account for all the observed particle precipitation behavior.

A final possibility is that interactions between barium ions and the phospholipid head groups may inhibit barium precipitation. At this time there is only indirect evidence for this phenomenon derived from ICP data taken after external ion removal (desalting). ICP analysis confirmed that the bulk Ba:Ag ratio was the same both before and after particle precipitation yet the EDS analysis on single particles showed a dominance of Ag in the crystallites. Since ICP confirms that the barium remains in the system, there is a possible interaction between the cations and phospholipid molecules which inhibit barium precipitation.

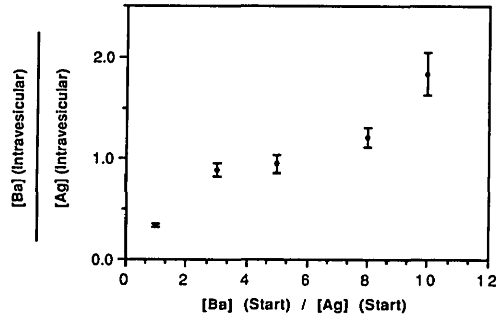

Figure 3. Comparison of incorporation efficiency within vesicles between barium (II) and silver (I) ions.

Consolidation Studies

Considering the surfactant nature of the phospholipid molecules, we were interested in determining whether the membrane could serve as an aid in the consolidation of ultrafine particles. To determine this, Ag_2O particles were first synthesized in vesicles. One drop of this suspension was allowed to consolidate on a TEM grid with the vesicles surrounding the particles. A second portion of the same sample was washed in chloroform with sonication, decanted, and rewashed a total of ten times in an attempt to remove the lipid membrane from the Ag_2O particles. A drop of this "washed" sample was also placed on a TEM grid and allowed to consolidate. The low magnification micrographs in Figures 4(a,c) show that the particles surrounded by membrane are well dispersed and consolidate as individual entities. In contrast, the washed particles are flocculated into large agglomerates. Careful examination of these micrographs reveals that each agglomerate is composed of many particles comparable in size to those in Figure 4(a). The high magnification micrographs, Figures 4(b,d) confirm that the vesicle-coated particles are separated from one another, while the noncoated particles are in direct contact. Further, the noncoated particles are fused at the contact points thus forming rigid agglomerates.

CONCLUSIONS

We have demonstrated that phospholipid vesicles can be used as reaction vessels for the synthesis of ultrafine, well-dispersed, multicomponent ceramic particles. The chemical inhomogeneity of the system can be restricted to the individual particle size of approximately 40 nm. We also found that precise control over chemical stoichiometry in multicomponent systems may be difficult to achieve due to differences in ion permeabilities, trapping efficiencies, and interactions between various cations and the vesicle membrane. Finally, we provided evidence that the vesicle membrane acts as a barrier against particle agglomeration and facilitates particle rearrangement during consolidation. In summary, this biomimetic system is truly multifunctional in that it simultaneously acts as: (i) a reaction cell for particle precipitation, (ii) an ion selective membrane that affects precipitation kinetics, (iii) a barrier to prevent spontaneous agglomeration of the ultrafine particles, and (iv) a lubricant/dispersant that facilitates particle rearrangement during particle consolidation.

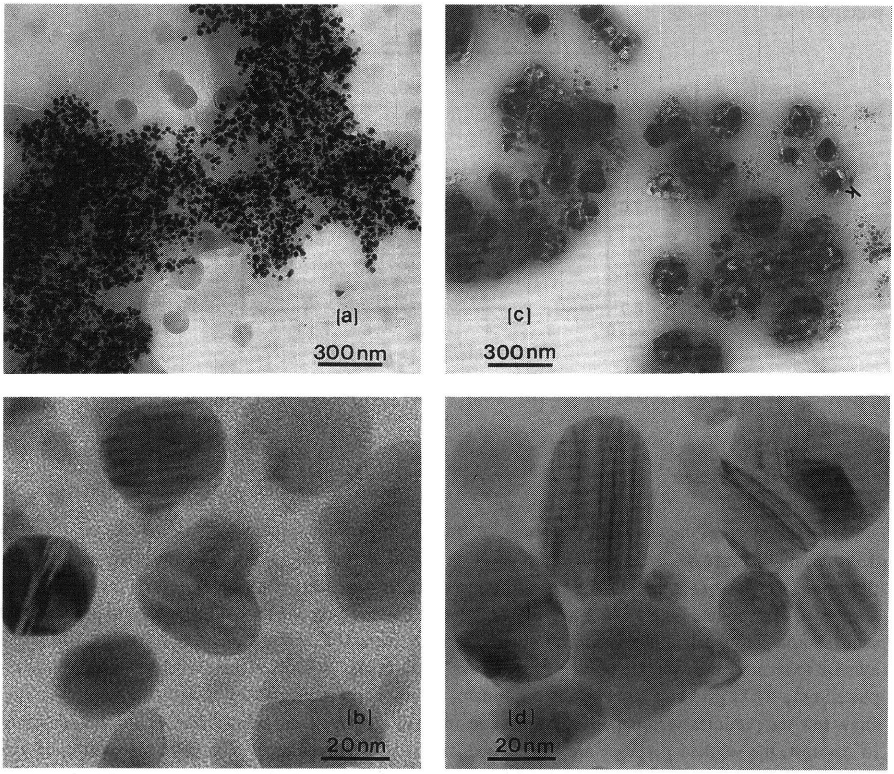

Figure 4. TEM micrographs of vesicle formed Ag_2O particles with (a, b) and without (c, d) vesicle membrane.

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REFERENCES

1. R. P. Andres et al., *J. Mater. Res.*, **4** [3] 704 (1989).
2. R. W. Seigel, S. Ramasamy, H. Hahn, Z. Li, T. Lu, and, R. Gronsky, *J. Mater. Res.*, **3** [6] 1370 (1988).
3. J. Karck, R. Birringer, and H. Gleiter, *Nature*, **330** [10] 556 (1987).
4. R. W. Seigel, S. Ramasamy, H. Hahn, Z. Li, T. Lu, and R. Gronsky, *J. Mater. Res.*, **3** [6] 1369 (1988).
5. J. H. Fendler, *Chem. Rev.*, **87**, 877 (1987).
6. B. J. Tarasevich, J. Liu, M. Sarikaya, and I. A. Aksay, ***in Better Ceramics Through Chemistry III, MRS Symp. Proc.***, Vol. 121, edited by C. J. Brinker, D. E. Clark, and D. R. Ulrich (Materials Research Society, Pittsburg, Pennsylvania, 1988), p. 225.
7. R. B. Frankel and R. P. Blakemore, *Phil. Trans. R. Soc. Lond.*, **B304**, 567 (1984).
8. S. Mann and R. J. P. Williams, *J. Chem. Soc. Dalton Trans.*, 311 (1983).
9. S. Mann and J. P. Hannington, *J. Colloid Interface Sci.*, **122** [2] 326 (1988).
10. S. M. Johnson and A. D. Bangham, *Biochim. Biophys. Acta.*, **193**, 82 (1969).
11. H. O. Hauser, M. C. Phillips, and M. Stubbs, *Nature*, **239**, 342 (1972).
12. D. Papahadjopoulos, *Biochim. Biophys. Acta.*, **241**, 254 (1971).
13. A. D. Bangham, M. M. Standish, and J. C. Watkins, *J. Molec. Biol.*, **13**, 238 (1965).
14. S. Mann, J. P. Hannington, and R. J. P. Williams, *Nature*, **324**, 565 (1986).
15. J. H. Fendler, ***Membrane Mimetic Chemistry***, (Wiley, New York, 1982), p. 113.