Surface Modification of Vesicles with Methylol Urea

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ABSTRACT: Surface-modified vesicles were prepared using *N*-[3-(dimethylamino)propyl]-octadecanamide and stearic acid as bilayer-forming lipids, and *N*-methylol urea–dodecylamine conjugates (MU–DOA) as a surface modifier. The conjugation of MU to DOA was confirmed by FTIR spectra. MU–DOA was incorporated into the vesicles by co-homogenization of the lipids and MU–DOA, and the incorporated MU–DOA was then reacted with MU in aqueous bulk phase through a self-condensation reaction between the methylols under an acidic condition at 70°C. On a scanning electron microscope, the vesicles were spherical and multilamellar, and they exhibited thin polymer films on their surfaces. The incorporation of MU–DOA into the bilayer and the surface coating of the vesicles did not significantly influence the transition temperature of the vesicles. The absolute values of zeta potentials of the surface-modified vesicles were smaller than those of the unmodified vesicles, and the point of zero charge was shifted from *ca.* pH 9.5 to *ca.* pH 6.5 by the surface modification.

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KEY WORDS: *N*-Methylol urea, self-condensation, surface-modification, vesicles.

Encapsulation of food ingredients, pharmaceuticals, cosmetics, and agrochemicals has been of much interest among scientists involved in the area of vesicles, microcapsules, and microspheres. Microencapsulation techniques are used for protecting unstable substances from oxidation and atmospheric moisture, converting volatile materials into a nonvolatile form, releasing the active ingredients on demand, and targeting pharmaceuticals to the site of action (1,2). Various methods, such as emulsification–solvent evaporation (3,4), spray-drying (5,6), coacervation (7,8), interfacial polycondensation (9), *in situ* polymerization (10,11), and complexation (12) have been used to prepare microcapsules and microspheres.

Our method of preparing microcapsules by forming surface-modified vesicles involves a self-condensation reaction, a key technology of *in situ* polymerization where *N*-methylol urea or *N*-methylol melamine is used as a precondensate for the wall material of microcapsules; they are polymerized in the continuous phase of oil-in-water (O/W) emulsions through the self-condensation of the *N*-methylol groups. In this study, *N*-methylol urea–dodecylamine conjugate (MU–DOA) was used as a surface modifier of vesicles. MU–DOA was incorporated into the vesicles by co-homogenization with bilayer-forming lipids, *N*-[3-(dimethylamino)-propyl]-octadecanamide (DMAPODA), and stearic acid (SA). DMAPODA and FA are reported to form a building block for vesicle formation by creating a salt bridge between NH_3^+ of DMAPODA and COO^- of FA (13,14). The incorporated MU–DOA was reacted with MU in the aqueous phase through a self-condensation reaction between the methylols under acidic conditions at 70°C. The complete process is represented in Scheme 1. Observation of the physical properties of the surface-modified vesicles using thermal phase transition and microelectrophoresis is included in this report.

MATERIALS AND METHODS

Materials. DMAPODA (M.W. 369) was a gift from Inolex Chemical Co. (Philadelphia, PA). SA (99%), urea (99.5%), formaldehyde (37% solution), and dodecylamine were purchased from Sigma (St. Louis, MO). DMAPODA and SA were used together as bilayer-forming materials for vesicle formation. Urea, formaldehyde, and dodecylamine were used for modification of the vesicular surface.

Preparation of the MU precondensate. Urea (80 g, 1.33 mol) was dissolved in 400 g of an aqueous solution of formaldehyde (30 wt%, 3.99 mol). After pH was adjusted to 9.0 with 1 N NaOH, the solution was heated to 70°C and stirred at constant temperature. After a 1-h reaction, the reacted solution was cooled to room temperature and kept for further use. This step is shown in step 1 in Scheme 1.

Preparation of the MU–DOA conjugate. Dodecylamine (10 g, 53.9 mmol) was added to 120 mL of distilled water contained in a 250-mL round-bottomed flask, and the mixture was heated to 80°C. After dissolving 0.73 g of borax in the mixture, 23.3 g of MU solution (64.7 mmol of urea), prepared as in the previous section, was added and the reaction mixture was stirred at 80°C for 1.5 h. This step is shown in step 2 in Scheme 1. Upon standing, the reaction was separated into the upper layer of oil (MU–DOA) and the lower layer of aqueous phase. The upper layer was isolated from the lower layer using a separation funnel.

IR spectroscopy. FTIR spectra were recorded on a Nicolet 560 spectrometer (Madison, WI) purged with N_2. Aliquots of samples (MU precondensate, DOA, and MU–DOA) were applied onto a discoid (32 mm × 3 mm) internal reflection element (KRS-5; Nicolet) for data collection. Bulk water in the MU precondensate was removed using a dry N_2 stream.

Preparations of vesicles. DMAPODA (2.82 g, 7.64 mmol) and 2.18 g of SA (7.66 mmol) were added to a 250-mL

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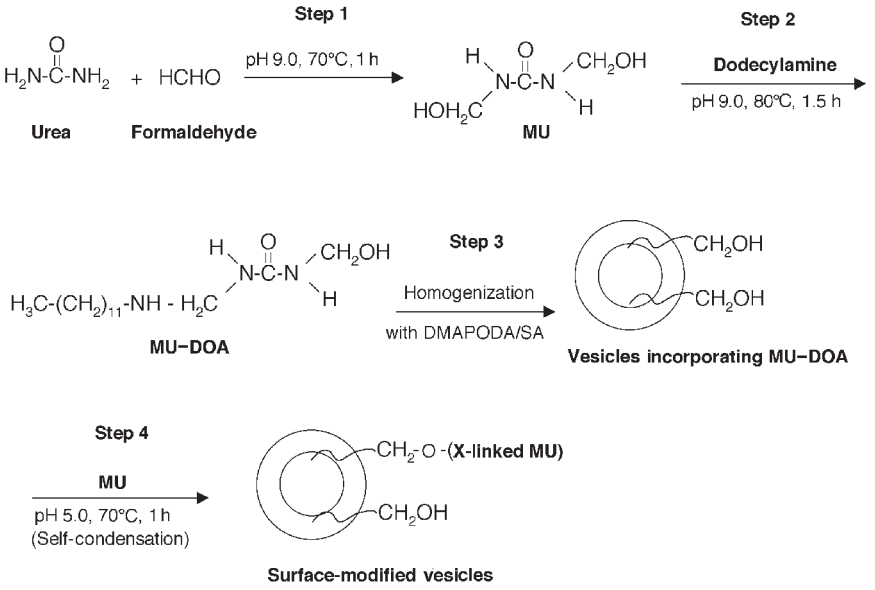

SCHEME 1

beaker. The mixture was then heated in a water bath at 70°C until it melted and became clear. Distilled water, preheated to 70°C, was added to the melt so that the total mass was 200 g (2.5 wt% lipids). The dispersion was stirred at 500 rpm until it had cooled to room temperature. The procedure for the preparation of DMAPODA/SA vesicles incorporating MU–DOA was the same as that for preparation of the DMAPODA/SA vesicles except that 0.54 g of MU–DOA was added to the mixture of DMAPODA and SA in the melting step. This step is shown in step 3 in Scheme 1. The DMAPODA/SA vesicles coated with polymerized MU were prepared as follows. MU solution (1.26 g, 3.5 mmol of urea) was added to a 100-g suspension (2.5 wt% lipids, 0.27 wt% MU–DOA) of the vesicles incorporating MU–DOA. After the pH was adjusted to 4.8 with acetic acid (10 wt%), it was stirred at approximately 70°C for 1 h. This step (i.e., surface modification by the self-condensation reaction between MU–DOA on vesicle bilayers and MU in the bulk aqueous phase) is shown in step 4 in Scheme 1. Finally, the pH was adjusted to 8.0 and then the suspension was cooled to room temperature.

Scanning electron microscopy (SEM). To verify whether the surfaces of vesicles were modified with polymerized MU, a structural analysis was carried out using a cold-stage scanning electron microscope (JEOL JSM-840A). An aliquot of the vesicle suspensions was placed on a metal stub and then immersed in liquid nitrogen. The specimen was transferred to a prechamber (Bio-Rad E7450) located outside the scanning electron microscope and then etched at –60°C, 10^{-5} mbar, for 2 h. The specimen was then sputtered with gold at –130°C to a thickness of 100–200 Å and viewed in the scanning electron microscope using an accelerating voltage of 10 kV at a stage temperature of –180°C.

DSC. Suspensions of bare vesicles, vesicles incorporating MU–DOA, and surface-modified vesicles were thermally scanned on a differential scanning calorimeter (TA Instruments DSC 2010). For each sample, 10–20 mg was weighed into an aluminum DSC pan and scanned from 10–70°C at a heating rate of 3°C/min.

Microelectrophoresis. Zeta potentials of vesicle suspensions of bare vesicles, vesicles incorporating MU–DOA, and surface-modified vesicles were measured using ZetaPlus (Brookhaven Instrument Co., Holtsville, NY). The concentrations of the vesicles in the suspensions were adjusted to approximately 0.06%. The ionic strengths of the suspensions were fixed to 1 mM with NaCl. The pH of the suspensions were varied from 3.0 to 11.0 with 1 mM NaOH and 1 mM HCl solutions.

RESULTS AND DISCUSSION

Conjugation of MU and DOA. Figure 1 shows FTIR spectra of the MU precondensate, DOA, and MU–DOA. A peak around 3320 cm^{-1} of spectrum A, characteristic of the OH alcohol stretching, indicates the presence of *N*-methylol groups in the MU precondensate (step 1 in Scheme 1). The peak around 3350 cm^{-1} of spectrum B, the vibration characteristic of amine groups of DOA, disappeared and a small and broad band of 3250–3550 cm^{-1}, corresponding to the OH stretching of *N*-methylol groups of MU–DOA, was observed following the reaction between DOA and the MU precondensate (step 2 in Scheme 1), indicating that DOA was successfully conjugated to the MU precondensate (see spectrum C). The relatively weak OH signal of MU–DOA, compared with the signal of the MU precondensate, was caused by a portion of the OH groups of the MU precondensate reacting with the amine groups of DOA.

Structures of vesicles. Figure 2 shows electron micrographs of the surface-modified vesicles. The particles were spherical and less than 20 μm in size. Wrinkled coats show-

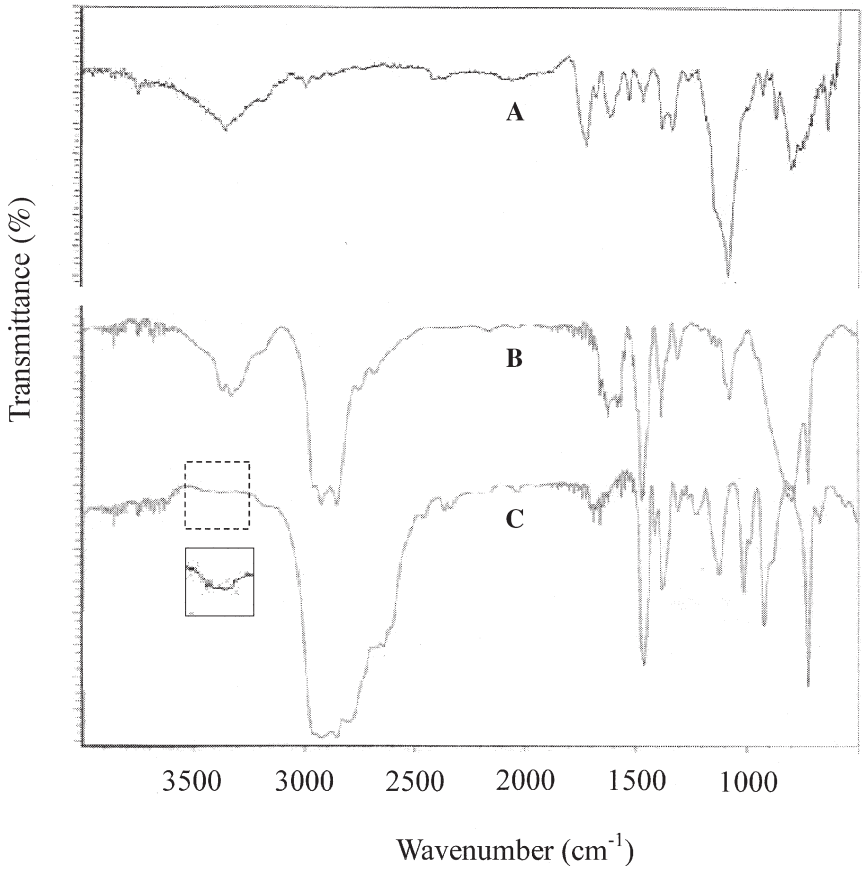

FIG. 1. FTIR spectra of *N*-methylol urea (MU) precondensates (A), dodecylamine (DOA) (B), and MU–DOA conjugates (C). The spectrum in the solid box was obtained by intensifying the spectrum in the dotted box.

ing a polymeric membrane of MUwere observed on the surface. In the process of microcapsule preparation using *in situ* polymerization, MU in the aqueous phase of the O/W emulsion was deposited onto the surface of oil droplets by a self-condensation reaction, leading to the formation of microcapsules (10,11). It was postulated that the MU precondensate became hydrophobic during the condensation reaction and that the hydrophobicity could be a driving force in its deposition. In our systems, MU–DOA intercalated into vesicle bilayers would play a major role in depositing the MU precondensate onto the surfaces of vesicles because the vesicular MU–DOA could react with the MU precondensate in the bulk aqueous phase through their reactive methylol groups. On the other hand, the cross-section micrographs revealed that the vesicles had a mutilayered structure, indicating that the surface modification did not affect the integrity of the vesicles. DMAPODA and FA are reported to form a building block for vesicle formation by creating a salt bridge between the NH_3^+ of DMAPODA and the COO^- of FA (13,14).

Thermal phase transition. Figure 3 shows thermograms of DOA, MU–DOA, and vesicles. The transition temperature of MU–DOA (21.1°C) was lower than that of DOA (30.3°C). This is probably because the intermolecular interaction of MU–DOA would be hindered by its bulky MU group. On the other hand, the transition temperatures of bare vesicles, vesicles incorporating MU–DOA, and the surface-modified vesicles were 52.7, 51.5, and 52.7°C, respectively (there was no significant difference in the transition temperatures). The thermal transition of MU–DOA disappeared when it was in the vesicle suspension. This indicates that hydrocarbon chains of MU–DOA were incorporated into the lipid bilayer of vesicles. The incorporation of MU–DOA did not significantly influence the transition temperatures of vesicles but led to broader peaks.

Microelectrophoretic behavior. Figure 4 shows the pH-dependent zeta potentials of bare vesicles, vesicles incorporating MU–DOA, and the surface-modified vesicles. The zeta potentials of bare vesicles decreased with increasing pH, and the isoelectric point was observed around 9.5. FFA carboxyl groups have a pK of approximately 5, so they will be deprotonated above a pH of 5, increasing the negative charge on the surface of the vesicles. Similarly, the number of protonated tertiary amine groups of DMAPODA will decrease with increasing pH, decreasing the positive charge on the surface. Therefore, the deprotonations of the carboxyl groups and the protonated tertiary amine groups would be responsible for the reduction of the zeta potential with increasing pH. The incorporation of MU–DOA into the vesicles did not significantly affect the zeta potentials. The surface-modified vesicles, however, exhibited quite a different curve. The absolute values of zeta potentials were smaller than those of the others and had

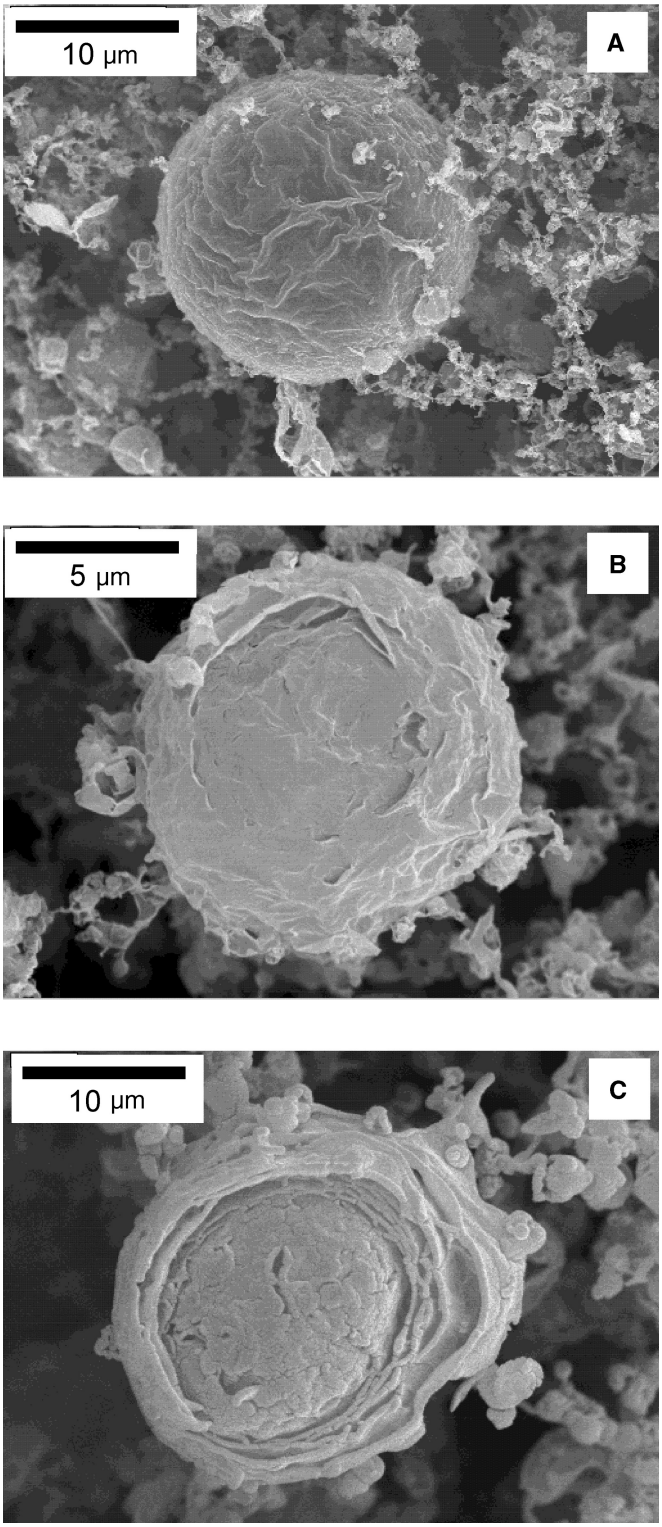

FIG. 2. Scanning electron micrographs of the surfaces (A,B) and a cross-section of surface-modified vesicles (C).

the point of zero charge around pH 6.5. The microelectrophoretic behavior of the surface-modified vesicles was dominated not by the surface of DMAPODA/SA but by the polymer coats formed on the surface of the vesicles. The deprotonations of the MU moieties in the polymer coats would

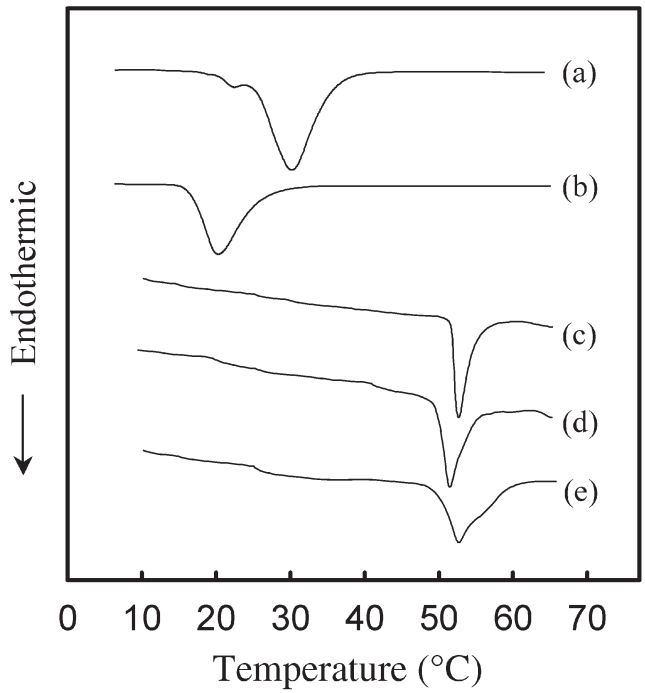

FIG. 3. Thermograms of DOA (a), MU–DOA (b), bare vesicles (c), vesicles incorporating MU–DOA (d), and surface-modified vesicles (e). For abbreviations see Figure 1.

be responsible for the decrease in zeta potential with increasing pH.

In conclusion, the surfaces of vesicles were coated by self-condensation reactions between MU–DOA in the vesicle bilayers and MU in the bulk aqueous phase. The coating changed the surface property of the vesicle, as shown by the

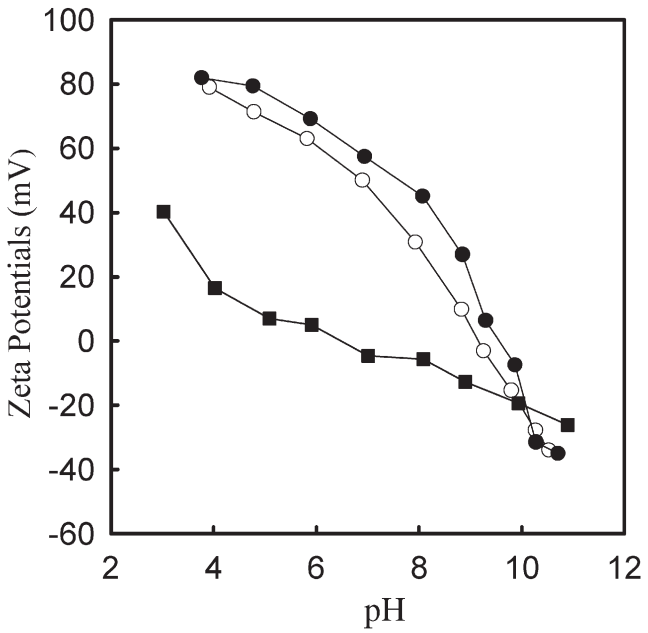

FIG. 4. pH-Dependent zeta potentials of bare vesicles (●), vesicles incorporating MU–DOA (○), and surface-modified vesicles (■). For abbreviation see Figure 1.

zeta potentials, but it did not influence the internal structure or the transition temperature of the vesicle. Therefore, the method presented in this study covers the surfaces of vesicles with thin polymer films without deteriorating their integrity. The polymer film is expected to act as a protective layer against the destabilization of the vesicles, e.g., their fusion, size reduction, and even solubilization.

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