Preparation of pH-sensitive Anionic Liposomes Designed for Drug Delivery System (DDS) Application
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Abstract: We prepared pH-sensitive anionic liposomes composed solely of anionic bilayer membrane components that were designed to promote efficient release of entrapped agents in response to acidic pH. The pH-sensitive anionic liposomes showed high dispersion stability at neutral pH, but the fluidity of the bilayer membrane was enhanced in an acidic environment. These liposomes were rather simple and were composed of dimyristoylphosphatidylcholine (DMPC), an anionic bilayer membrane component, and polyoxyethylene sorbitan monostearate (Tween 80). In particular, the present pH-sensitive anionic liposomes showed higher temporal stability than those of conventional DMPC/DPPC liposomes. We found that pH-sensitive properties strongly depended on the molecular structure, \( pK_a \) value, and amount of an incorporated anionic bilayer membrane component, such as sodium oleate (SO), dimyristoylphosphatidylserine (DMPS), or sodium \( \beta \)-sitosterol sulfate (SS). These results provide an opportunity to manipulate liposomal stability in a pH-dependent manner, which could lead to the formulation of a high performance drug delivery system (DDS).

Key words: pH-sensitive anionic liposome, anionic bilayer membrane–component, acidic pH–sensitivity, drug delivery system (DDS)

1 INTRODUCTION
Phospholipid vesicles, or liposomes, have been extensively studied as drug carriers or drug delivery systems (DDSs) since the formation of a vesicular structure was discovered in a suspension of egg-yolk lecithin in 1964.¹ Currently, liposomes are prepared from a variety of phospholipids, and incorporation of anionic and/or cationic lipids into the bilayer membrane provides an opportunity to manipulate drug release and intracellular targeting in DDS applications.²³⁶ For practical use of liposomes as DDSs, there are two conflicting requirements that must be simultaneously fulfilled: high stability and high pH sensitivity of liposomal structures before and after administration. More specifically, a pH-sensitive, liposome-designed DDS must be able to retain a drug at a physiological pH of approximately 7.4 and is taken up by endocytosis in the endolysosomal pathway at pH 5.0. Pronounced pharmacological effects can thus be expected because of sustained release of a drug embedded in the liposome before its decomposition in a lysosome. In this research, we aimed to produce a series of functional liposomes equipped with such characteristics for use as drug carriers.²⁵

Recently, Hafez et al. reported preparation of pH-sensitive liposomes composed of both cationic and anionic lipids.⁷ Cationic/anionic liposomes formed stable carriers at neutral pH. However, in the endosome, the liposome was positively charged because of protonation of the anionic lipid, and the consequent electrostatic interactions lead to liposomal fusion with the endosomal membrane. Shi et al. also formulated pH-sensitive liposomes composed of both cationic and anionic lipids.⁸ Most research on pH-sensitive liposomes has been limited to a mixture of dioleoylphosphatidylcholine (DOPE) and an amphiphilic molecule involving a carboxyl group.⁹–¹³ DOPE gives rise to the destabilization of liposomal structure at acidic pH. Because the major driving force for the destabilization and subsequent fusion or destruction of DOPE-based liposomes essentially comes from an inherent tendency of DOPE to
form inverse hexagonal structure, such systems are accompanied by liposomal instability, even at natural pH.

This also holds true for liposomal systems involving non-bilayer forming lipids with a small polar head group, such as phosphatidylethanolamine (PE), diacylglycerol (DG), and cholesterol. There are few reports on pH-sensitive liposomes mainly composed of phosphatidylcholine (PC), because a PC-based liposome generally preserves its conformation against pH variation. Then, attempts have been made to equip a PC-based liposome with pH sensitivity by hybridization with another anionic lipid.

In this study, we aimed to produce a series of functional pH-sensitive liposomes that can be used as drug carriers, as illustrated in Fig. 1. We prepared pH-sensitive anionic liposomes that have high stability and sharp pH sensitivity at natural pH and acidic pH, respectively. These liposomes were based on a rather simple formulation of only three components: dimyristoylphosphatidylcholine (DMPC), an anionic bilayer membrane component, and polyoxyethylene sorbitan monostearate (Tween 80). These new systems take advantage of inherent high fluidity of the DMPC bilayer membrane. At the same time, incorporation of an additional anionic bilayer membrane component, including sodium oleate (SO), dimyristoylphosphatidylserine (DMPS), or sodium β-sitosterol sulfate (SS), modulates membrane stability at acidic pH. Membrane stability is a key to controlling pH sensitivity of the ternary anionic liposomes produced. Change of ionization state of anionic bilayer membrane components by lowering pH causes destabilization of the membrane, leading to an increase in membrane fluidity, liposomal fusion (or destruction), and particle size.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

DMPC and DMPS were purchased from NOF Co., Ltd. (Tokyo, Japan). SO, Tween 80, and pyrene as a fluorescence probe were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). SS was a gift from L.V.M.C. Co., Ltd. (Chiba, Japan). Dulbecco’s phosphate buffered saline (PBS) (pH 7.4) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other buffer solutions (pH 6.0, 5.0, 4.0, and 3.0) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Molecular formulae of these ionic materials are shown in Table 1.

2.2 Preparation of pH-sensitive liposomes

All liposomes were prepared by a probe-type sonication method. DMPC, an anionic bilayer membrane component (SS, SO, or DMPS) and Tween 80 were dissolved in methanol. These solutions were added to a vial to attain a molar ratio of DMPC and an anionic bilayer membrane component: Tween 80 = 200: 2. Then, DMPC-based anionic liposomes were prepared in 20 mM PBS solution under the same conditions mentioned above. By diluting the suspension with buffer solution, pH of the liposome dispersion was adjusted to an optimum value. The majority of organic solvent was evaporated at 60°C under vacuum and subsequently under the flow of N₂ gas. To remove the residual solvent, the resulting lipid film was dried in a vacuum for 16 hours in the dark. PBS solution (150 mM, pH 7.4) was added to the lipid film until the solvent reached a concentration of 20 mM. The solution was then warmed at 37°C for 30 minutes. The suspension was ultra-sonicated using a homogenizer (40 W, UD-200; TOMY SEIKO Co., Ltd., Tokyo, Japan) for 30 minutes.

2.3 Small angle x-ray scattering (SAXS)

We performed small angle X-ray scattering (SAXS) ex-

Fig. 1 Strategy of inferring pH sensitivity of the anionic liposomes at acidic pH.
Preparation of pH-sensitive anionic liposomes

Experiments on the pH-sensitive liposomes to examine their static structures using a SAXSess camera (Anton-Paar, Graz, Austria). A PW3830 sealed-tube anode X-ray generator (GE Inspection Technologies, Germany) was operated at 40 kV and 50 mA. A monochromatic, line-shaped primary X-ray beam of Cu-Kα radiation (λ = 0.1542 nm) was provided by focusing multilayer optics and a block collimator. The sample temperature was controlled with a thermostated sample holder unit (TCS 120, Anton-Paar). Two-dimensional (2D) scattering patterns were recorded by an imaging plate (IP) detector (Perkin Elmer, USA). By integrating the 2D profiles, one-dimensional (1D) scattering intensities were obtained as a function of the magnitude of the scattering vector \( q \), where \( q = 4\pi\lambda \sin(\theta/2) \), and \( \theta \) is the total scattering angle. A semi-transparent beam stop enabled us to monitor an attenuated primary beam at \( q = 0 \). All measured intensities were calibrated for transmission by normalizing a zero-\( q \) primary intensity to unity. The background scattering contributions from the capillary and solvent were corrected. Absolute intensity calibration was performed using water as a secondary standard.

2.4 Dynamic light scattering (DLS)

To determine liposome stability, we monitored the time-dependent particle size of the liposomes as a function of pH. Dynamic light scattering (DLS) experiments were carried out in dilute dispersions of the liposomes, in which a NICOMP 370 ZLS (Particle Sizing Systems Co., Santa Barbara, USA) was employed. The wavelength of the light source was 632.8 nm. Time-dependent scattering intensity was detected at a total scattering angle of 90°, which corresponds to the fixed scattering vector of 0.022 nm\(^{-1}\) in aqueous media (refractive index \( n = 1.33 \)). The intensity autocorrelation function, \( g_1(\tau) \), is connected to the (normalized) field correlation function, \( g_2(\tau) \), via \( g_2(\tau) = 1 + \beta |g_1(\tau)|^2 \), where \( \tau \) is the correlation time and \( \beta \) is the coherence factor. The diffusion constant, \( D_0 \), is related to the hydrodynamic radius, \( R_{\text{H}} \), of the particle via the Stokes–Einstein relation:

\[
D = \frac{k_B T}{6\pi\eta R_0}
\]

where \( k_B \) is the Boltzmann constant, \( T \) is temperature, and \( \eta \) is the solvent viscosity. Particle systems with a narrow size distribution are expected to show single-step relaxation behavior. We used the second-order cumulant technique\(^{18}\) to analyze the \( g_1(\tau) \) functions of dilute liposomal systems.

2.5 Membrane fluidity measurements

A membrane fusion assay was carried out according to a previously reported procedure\(^{19, 20}\). Since the excimer formation frequency of pyrene depends on its diffusion rate, which is affected by the microscopic environment in the membrane, the fluorescence spectrum of pyrene gives us information about membrane fluidity. Using a RF-5300PC spectrofluorometer (Shimadzu Co., Kyoto, Japan), pyrene fluorescence intensity was measured at an excitation wavelength of 330 nm, and emission was detected at 475

Table 1  Schematic representation of molecular structure of lipids used as constituents of the investigated liposomes.
Pyrene-labeled liposomes (total lipid concentration of 20 mM, incorporating 40 mM pyrene) were prepared by a polycarbonate membrane extrusion technique. The liposomes were dispersed in PBS solution and succinic acid buffer solution to attain pH 7.4 and pH 5.0, respectively. All measurements were performed at 37°C every 10 minutes for 1 hour, and the ratio of the fluorescence intensity at pH 7.4 to that at pH 5.0, \( I_{7.4}/I_{5.0} \), was calculated as a function of incubation time.

2.6 Effects of anionic lipids on pH–sensitivity of liposome ionization state

The electrophoretic mobility of liposomes (lipid concentration: 5 mM) were determined at 37°C with the help of laser doppler velocimetry (Zetasizer Nanoseries, Malvern Instruments, Malvern, Worcestershire, UK), where dielectric constant 74.4 and viscosity 0.6864 cP were used for dilute dispersions. Sample pH was determined using a pH meter with a pH electrode (D–51AC, HORIBA, Ltd., Kyoto, Japan) at 37°C. The zeta (\( \xi \)) potential was determined by measuring electrophoretic mobility, \( U_E \). The Smoluchowski equation was employed with Henry’s coefficient, \( f(\kappa R) = 1.5^{11} \), in the calculation of the \( \xi \) potential:

\[
U_k = \frac{\varepsilon \varepsilon_0 \zeta}{\eta} \cdot f(\kappa R) \quad (2),
\]

where \( \varepsilon \) is the dielectric constant, \( \eta \) is the solvent viscosity, \( \kappa \) is the Debye–Hückel parameter \( (\kappa^{-1} \text{ representing the double layer thickness}) \), and \( R \) is the radius of the particle. Measurements were repeated 5 times to confirm reproducibility.

3 RESULTS and DISCUSSION

3.1 Static structure of pH-sensitive anionic liposomes as obtained by SAXS

In Fig. 2, we show collimation-corrected SAXS intensities, \( I(q) \), of the pH-sensitive anionic liposomes incorporating SO, DMPS, and SS on an absolute scale. Generally, static structure factor, \( S(q) \), of a multilamellar stack such as a multilamellar vesicle or lamellar liquid crystal produces pronounced equidistant peaks. In the experiments, \( I(q) \) of the pH-sensitive anionic liposomes exhibited no such interference peaks, which were independent of the incorporated anionic bilayer membrane components. This finding indicates \( S(q) = 1 \), and that the vesicular structures were mostly unilamellar.

Since the whole size of the liposomes was beyond the resolution of our SAXS experiments \( (q_{min} = 0.08 \text{ nm}^{-1}) \), the present SAXS data mainly provided us information regarding local structures of the bilayer membrane. As shown in Fig. 2, lowering pH from 7.4 to 5.0 resulted in a reduction of the scattering intensities and somewhat smeared shape of the \( I(q) \) curves. These observations indicate that liposomal structures cannot be kept stable in acidic conditions, implying partial decomposition of the bilayers. At the same time, the overall features of the \( I(q) \) curves were found to be rather similar at natural and acidic pH, which suggests that the local membrane structures, such as thickness and internal electron density distributions, were partly preserved, even at pH 5.0.

The form factor, \( P(q) \), of a membrane can be written as the product of the thickness scattering function, \( P_t(q) \), and the Lorentz factor, \( 1/q^2 \), as

\[
P(q) = (2\pi A/q^2)P_t(q) \quad (3),
\]

![Fig. 2](image-url) The collimation-corrected SAXS intensities, \( I(q) \), of the pH-sensitive anionic liposomes on an absolute scale at pH 7.4 and pH 5.0 incorporating (a) SO, (b) DMPS, and (c) SS.
where $A$ is the area of the basal plane. $P_t(q)$ is connected with a thickness distance distribution function (thickness PDDF), $p_t(r)$, via the cosine transformation,

$$P_t(q) = 2\int_0^\infty p_t(r) \cos(qr) dr (4),$$

where $r$ is the distance between two arbitrarily chosen scattering centers, $p_t(r)$ is the convolution square of the electron density fluctuation, $\rho_t(r)$, in a perpendicular direction toward the midplane of the bilayers,

$$p_t(r) = 2\int_0^\infty \Delta \rho(r') \Delta \rho(r' + r) dr' (5).$$

When the thickness of the bilayer is sufficiently smaller than the remaining two other dimensions, a similar technique using the well-established indirect Fourier transformation (IFT) can be used to calculate $p_t(r)$ from the experimental $P_t(q)$.

In Fig. 3, we show $p_t(r)$ of the pH-sensitive anionic liposomes incorporating SO, DMPS, and SS. The bilayer thickness of the pH-sensitive anionic liposomes ($D_{\text{max}}$), as determined based on $p_t(r)$, ranged between 5.0–5.5 nm at pH 7.4 for all systems and seems to have been only slightly affected by the incorporated anionic bilayer membrane components. When going from $r = 0$ to longer distances, the polarity of $p_t(r)$ changed from positive to negative and then negative to positive, which is typical behavior of the lipid vesicles in aqueous media. Eq.5 shows negative and positive $\rho_t(r)$ in the hydrophobic tail and hydrophilic head groups within the bilayer, respectively.

### 3.2 Temporal stability of the liposomes at pH 7.4

As shown in Fig. 4, a conventional PC-based liposome consisting of DMPC and DPPC showed a rapid increase of the hydrodynamic diameter from ca. 95 nm observed soon after preparation to ca. 200 nm within 10 hours. Figure 5 displays the $\xi$ potential of the DMPC/DPPC hybrid liposome as a function of pH. The DMPC/DPPC liposome had a $\xi$ potential close to zero, independent of pH. This indicates that the insufficient dispersion stability of the DMPC/DPPC liposome was caused by charge neutralization and the resulting decrease of the electrostatic repulsion between the liposomes, leading to aggregation and fusion. Accounting for the enhanced permeation and retention (EPR) effect, an important factor of a drug carrier for passive targeting to cancer cells, the size (diameter) of the carrier needs to be in the range of 20–200 nm. Insufficient stability of the DMPC/DPPC liposome, whose size readily exceeded 200 nm after 12 hours, was found to be inappropriate as a drug carrier for cancer therapy.

On the other hand, we confirmed that all pH-sensitive anionic liposomes, which were composed of SO, DMPS, or SS, were sufficiently stable, preserving diameters smaller than 200 nm for at least two days, as shown in Fig. 4. The negative $\xi$ potential shown in Fig. 5 indicates that these anionic liposomes possessed negative surface charges, even in acidic pH. This finding indicates that electric repulsion between liposomes helped achieve high dispersion stability, preventing aggregation and fusion or disappearance of the liposome bilayer membrane. The particle size
of all anionic liposomes was in the range of 20–200 nm, which is suitable for a drug carrier for cancer therapy, even when taking into account the EPR effect.

We observed different time-dependent behavior of anionic liposome particle size depending on the molecular structure of the incorporated lipids. A probe-type sonication method often produces a liposome with a diameter smaller than 100 nm. The liposome incorporating SO preserved an almost constant diameter of ca. 70 nm for 48 hours. The DMPS–incorporated system showed an initial diameter increase for 3 hours after preparation, which remained less than 100 nm for 48 hours. The incorporation of SS led to the most pronounced increase of particle size among these three systems. The diameter increased linearly with time for 6 hours, reaching a plateau around 160 nm. It appears that the liposome containing SS preferred a smaller curvature because of an optimized configuration of the bulky, planar-like hydrophobic group of SS in the lipid bilayer.

3.3 pH-sensitive properties of the anionic liposomes

Figure 6 compares effects of pH and anionic lipid concentration on the fusion, aggregation, or disappearance of liposomes for liposomes containing different anionic bilayer membrane components (SO, DMPS, and SS) in terms of the mean diameters determined at 37°C after 60 minutes. The DMPC/SO/Tween 80 liposome showed virtually no size difference at pH 7.4 and pH 5.0 in the SO concentration range of 20–40 mol%. At 50 mol% of SO, however, decreasing pH from 7.4 to 5.0 led to a marked increase of particle size. At this composition, the ξ potential also sharply increased toward charge neutralization, indicating a pronounced decrease of the electrostatic repulsion between liposomes.

In contrast, the liposome containing DMPS showed weak dependence on lipid concentration and pH, which is linked to the ionization state of DMPS. The pKₐ values of an amino group and a carboxyl group in DMPS are known to be 8.7 and 3.3, respectively, which indicates that DMPS is in the zwitter–ionic form in the entire pH range of 7.4–5.0. This provides a plausible explanation of the high stability and weak pH sensitivity of the liposomal structure.

Alternatively, the incorporation of SS resulted in a significant increase of liposome size and was approximately proportional to SS concentration. These data suggest that the bulky steroid structure of SS embedded in the lipid membrane leaves a space between lipid alkyl chains. At 20 mol% of SS, the ξ potential slightly changed in the direction of charge neutralization when pH decreased from 7.4 to 5.0. The ξ potential of the SS-incorporated liposome showed a less pronounced change than that of the SO-incorporated liposome. The protonated SS resulted in decreased electrostatic repulsion.

Judging from the above-mentioned results, we yielded the liposome incorporating SO as the most favorable pH sensitivity relative to appropriate size of a drug carrier for cancer therapy. By lowering pH, the cation species of SO is substituted by hydrogen ion and protonated to produce oleic acid. A carboxyl group of the oleic acid formed a hy-
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Hydrogen bond with a phosphate group of the DMPC in the liposome. The structure of the complex (an association state) is similar to that of an inverse cone shape formed by DOPE in a liposome. DOPE is known to be a pH-sensitive, liposome-forming lipid. Consequently, the liposome structure cannot be kept stable in acidic conditions, leading to aggregation, fusion, or disappearance of the liposome bilayer membrane.

3.4 Membrane fluidity

We also evaluated pH sensitivity of the anionic liposome membrane fluidity by measuring the fluorescence intensity of pyrene-labeled liposomal samples. The labeled liposomes were dispersed in PBS and succinic acid buffer solutions to attain pH 7.4 and pH 5.0, respectively. Figure 7 shows the reduced fluorescence intensity, $I_{5.0}/I_{7.4}$ at 37°C as a function of time, where $I_{5.0}$ and $I_{7.4}$ are the fluorescence intensities at pH 7.4 and pH 5.0, respectively. As Figure 7a shows, $I_{5.0}/I_{7.4}$ of the liposome incorporating 20 mol% SO remained almost unchanged over time, exhibiting near unity, whereas $I_{5.0}/I_{7.4}$ for 50 mol% SO was clearly time-dependent, showing a successive increase. This means that the frequency of pyrene excimer formation was greater at pH 5.0 than at pH 7.4, which provides evidence for increased membrane fluidity by lowering pH. As for the DMPS-incorporated systems, which did not show pH dependence on liposome size, $I_{5.0}/I_{7.4}$ showed almost no time-dependence to yield a constant value of unity. This result demonstrates that neither lipid concentration nor pH variation affected membrane fluidity of the liposomes. Both 30 and 50 mol% DMPS concentrations yielded no change, indicating that neither liposome expressed membrane fluidity change in response to a change in pH.

$I_{5.0}/I_{7.4}$ of the liposome incorporating 10 mol% SS did not change over time, exhibiting a constant value of unity. This indicates that membrane fluidity did not increase, even in acidic conditions (pH 5.0), at this SS concentration. However, when the SS concentration increased to 20 mol%, $I_{5.0}/I_{7.4}$ showed a gradual increase as a function of time, indicating increased membrane fluidity at 20 mol% of SS.

To summarize these results, the behavior of $I_{5.0}/I_{7.4}$ does not simply depend on the type of anionic lipid, but also on the liposomes having a specific composition of DMPC and anionic lipids that resulted in pH-dependent size change, as inferred by $I_{5.0}/I_{7.4}$ values greater than unity, indicating enhanced membrane fluidity at an acidic pH. On the other hand, $I_{5.0}/I_{7.4}$ barely changed over time in systems that did not show pH-dependence of liposome size. These findings demonstrate that the pH-induced increase of liposome size.
was accompanied by an increase in membrane fluidity. The data also indicate that, as for the systems showing only weak or virtually no pH dependence of liposome size, lowering pH did not affect membrane fluidity. A cancer therapeutic drug carrier requires pH sensitivity to the switching property to pH, which provides high dispersion stability at neutral pH and enhanced membrane fluidity at acidic pH, resulting in aggregation, fusion, or disappearance of the membrane structures.

3.5 Membrane surface charge
To explore the state of the anionic liposome surface, ξ potentials were measured at various pH values. The results are displayed in Fig. 5. The DMPC/DPPC liposome has no apparent surface potential at any pH value. The ξ potential of the DMPC/SO/Tween 80 liposome or pH-sensitive liposome containing SO sharply changed toward a neutral direction with a decrease of pH value below pH 4, indicating that the carboxylate groups of SO are fully protonated. As for the DMPC/DMPS/Tween 80 liposome or pH-sensitive liposome with the DMPS, the ξ potential curve shifted in a positive direction at a pH less than 5.0.

When a liposome is used as a drug carrier, it must be stable in the pH range of intracellular environments (7.4–5.0). Pure DMPS yielded two pK_a values: 8.7 and 3.3\(^{27}\). However, they are biased by the presence of other lipids. The pH-sensitive liposome with DMPS prepared in this study increased the ξ potential only when the pH was less than 5.0. Therefore, this system is not appropriate for a drug carrier. On the other hand, the ξ potential of the pH-sensitive liposome incorporating SS slowly changed in a neutral direction when pH was lowered to 5.0, indicating that hydrophilic groups of SS form sulfate ions at pH 5. Although the ξ potential of the liposome incorporating SS is less sensitive to pH change than that of the pH-sensitive liposome containing SO, the SS-incorporated system seems to satisfy the requirement of a drug carrier because of the pH range in which the system exhibits surface charge variation. pH sensitivity was caused by protonation of the carboxylate group of SO and the sulfate group of SS as well as the resulting neutralization of the membrane surface charge, leading to the aggregation, fusion, or disappearance of liposomes by lowering pH.

4 CONCLUSION
In this study, we prepared liposomes composed solely of
anionic charged lipids that showed pH-sensitive properties, in particular high dispersion stability at neutral pH and enhanced membrane fluidity in acidic environments. The SAXS data confirmed that the unilamellar structure of the liposomes have bilayer thicknesses of ca. 5.0–5.5 nm at pH 7.4. The results of SAXS, DLS, and the membrane fluidity assay unambiguously demonstrated high stability of the liposomal structures at natural pH and their efficient destabilization in acidic conditions. Accounting for the EPR effect, the pH-sensitive liposomes are able to retain a drug at neutral pH because of their stable membrane structure, but will release the drug in response to the acidic environments of endosomes because of the induced instability. We expect that in actual medical applications, a drug is efficiently delivered into an endosome and released. In contrast to conventional anionic–cationic hybrid liposomes (DMPC/DPPC liposomes), the present anionic liposomes have the advantages of higher temporal stability (before administration) and pH–sensitive membrane stability. We revealed that pH sensitivity depends on the structure, \( pK_a \), and amount of anionic lipid added. In particular, the liposome incorporating SO has favorable pH sensitivity. By lowering pH, SO is protonated, and the protonated SO produces oleic acid. A carboxyl group of the oleic acid formed a hydrogen bond with a phosphate group of the DMPC in the liposome. pH sensitivity is recognized by the neutralization of the surface charge of the membrane. Consequently, the liposomal structure cannot be stably sustained in acidic conditions, and increased membrane fluidity leads to aggregation and fusion. Therefore, these systems are promising candidates for anticancer drug carriers, and these results offer an effective approach to producing high performance drug carriers.

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