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Vesicle Size and Stability of Biomimetic Liposomes from 3'-Sulfo-Lewis a (SuLe^a)-Containing Glycolipids

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Abstract

We report on the use of a natural Lewis type saccharide ligand, 3'-sulfo-Lewis a (SuLe^a) for glyocalyx-mimetic surface modification of liposomes. Two SuLe^a-containing glycolipids, monovalent SuLe^a-lipid and trivalent SuLe^a (TSuLe^a)-lipid, were synthesized, and used with 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol to prepare unilaminar vesicles (ULVs) by a freeze-thaw and extrusion method. The effects of the glycolipid concentrations and the pore sizes of extrusion membranes on vesicle size and stability were investigated by photon correlation spectroscopy (PCS). Glycoliposomes, with 5% SuLe^a- or TSuLe^a-lipids obtained by 50 nm extrusion, had 25-30% more vesicles less than 100 nm in diameter compared with the 100 nm extrusion. TSuLe^a-liposomes always produced larger vesicle size than SuLe^a-liposomes, which we attribute to the larger TSuLe^a headgroup. Both SuLe^a- and TSuLe^a-liposomes increased their vesicle size with increasing glycolipid concentration from 5% to 15%, and demonstrated good stability at room temperature for over one month. Further increasing the glycolipid concentration to 20% resulted in large vesicle aggregation after 5 days for TSuLe^a-liposomes, while the SuLe^a-liposomes remained stable for 10 days. SuLe^a- and TSuLe^a-liposomes with 15% glycolipids demonstrated better stability due to the electrostatic effect from the negatively charged SuLe^a and TSuLe^a headgroups. The results indicate that the biomimetic liposomes with SuLe^a- and TSuLe^a-lipids with 5 to 15% incorporation are sufficiently stable for the potential applications in targeted drug delivery.

Keywords

Liposomes; saccharides; 3'-sulfo-Lewis a; glycolipids; vesicle size

1. Introduction

Liposomes have been widely used as drug carriers due to their structural versatility in terms of size, composition, surface charge, bilayer fluidity, and the facility to incorporate various hydrophilic and hydrophobic drugs [1-5]. The level of interest increased dramatically in the 1990s, with the discovery that the circulation lifetimes of liposomes can be prolonged from minutes to about a day, when vesicle surfaces are modified with poly(ethylene glycol) (PEG) [5-9]. This is achieved through the PEG properties of minimum interfacial free energy with water, large excluded volume and steric stabilization effect [10]. However, PEG lipids for

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liposome surface modification still has limitations, such as micelle formation [9,11], clustering induced collapse [12], and weak protein complex formation [13,14].

The glycocalyx of erythrocytes (red blood cells, RBCs) is a membrane-bound saccharide-rich layer, consisting of various proteoglycans and glycolipids, including glycoporphin A (GPA) with 10^6 copies per RBC [15-18]. This abundant GPA-containing glycocalyx on the RBC surface appears to play a critical role in the cell's extended circulation, which can be as long as 120 days. The glycocalyx prevents protein adsorption, non-specific interactions with other cells, and aggregation between RBCs. Therefore, the RBC glycocalyx is an attractive model for biomimetic surface modification of liposomes, with the objective of prolonged circulation time. A glycocalyx-mimetic saccharide layer on the liposome surface is expected to act as a hydrophilic barrier that will suppress nonspecific protein adsorption [18-20]. As an example, monosialoganglioside (G_{M1}) enhances circulation lifetimes of liposomes in mice [21,22]. G_{M1} suppresses nonspecific protein adsorption and helps to reduce recognition and clearance by the reticuloendothelial system (RES) in a manner comparable to that observed for PEG [23]. Further study showed that the function of G_{M1} in prolonging liposome circulation time is animal species dependent [24].

Saccharides also provide appropriate specificity when designed to target desired sites. Numerous saccharide ligands are known to exist on cell surfaces and have been used for targeted drug delivery [25-28]. One of the most important is Lewis antigens, including 3'-sialyl Lewis x (SLe^x) and 3'-sialyl Lewis a (SLe^a) [29-32]. They bind E-, P- and L-selectin receptors present on activated endothelial cells, platelets and leukocytes during the inflammation process [33-36]. Therefore, much effort has been focused on the development of new therapeutic strategies for inflammatory diseases, based on using $SLe^{x/a}$ ligands and their analogs [37-40]. To explore this approach, we have synthesized a natural Lewis type saccharide ligand, 3'-sulfo-Lewis a ($SuLe^a$), as well as its glycolipids, and incorporated them into liposomes for targeting P-selectin expressed on activated platelets [41-42].

In the application of saccharides to achieve extended circulation lifetimes combined with a strong binding affinity for a target, an important consideration is to maximize the saccharide concentration at the liposome surface while preserving the structural integrity of the liposome. Previous research has demonstrated that liposome size and stability affects vesicle distribution and clearance in vivo after systemic administration [43-45]. This knowledge is essential for developing strategies for biomimetic liposomes as drug carriers. In this study, we report on vesicle size, distribution, and stability of $SuLe^a$ -modified liposomes. $SuLe^a$ -containing glycolipids, including monovalent $SuLe^a$ -lipid and trivalent $SuLe^a$ ($TSuLe^a$)-lipid, were used with 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol to prepare unilamellar vesicles (ULVs) by a freeze-thaw and extrusion method. The effects of glycolipid concentration and the pore size of extrusion membranes on vesicle size and stability were investigated by photon correlation spectroscopy (PCS).

2. Experimental Section

2.1. Materials

1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol (Chol), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG) were purchased from Avanti Polar Lipids (Birmingham, AL). Lactose-lipid with two octadecanoyl hydrophobic tails, were synthesized according to the previously reported method [21]. Monovalent $SuLe^a$ -lipid (**1a**) and trivalent $SuLe^a$ ($TSuLe^a$)-lipid (**1b**) were synthesized by the conjugation of protected $SuLe^a$ amino-derivative **2** with lipids **3a** (with a monovalent carboxylic group) and **3b** (with triple carboxylic groups), respectively, according to the method previously reported (Scheme 1) [41,42].

2.2. Preparation of liposomes

Liposomes were prepared by the freeze-thaw and extrusion method. Briefly, a desired volume of lipid stock solutions (20 mg/ml), including DSPC, DSPE-PEG or glycolipids in chloroform and methanol (3:1,v/v) (total lipids, 6.0 μ mol), was mixed with a cholesterol stock solution (10 mg/ml) in chloroform (0.154 ml, 4.0 μ mol) in a 10 ml round-bottom flask. The total volume was made up to 1 ml by chloroform. The mixtures were deposited in a thin film on the interior of the flask by rotary evaporation under reduced pressure for 30 min, followed by drying in a vacuum oven at room temperature overnight. Following the addition of phosphate-buffered saline (PBS) (1 ml), lipid thin films were hydrated in 70°C water bath for 30 min to obtain multilamellar vesicles (MLVs). The MLV suspension was subjected to 3-5 freeze-thaw cycles, involving quenching in liquid nitrogen, followed by immersion in a 70°C water bath. Unilamellar vesicles (ULVs) were produced by extrusion through Nucleopore track-etch polycarbonate membranes (Avanti Polar Lipids Inc.) with pore size of 100 nm for 10 times on an Avanti Mini-Extruder (Avanti Polar Lipids Inc.) operated at 75°C. Half of the 100 nm extruded liposomes were then extruded through 50 nm membranes for 10 more times according to the reported sequential extrusion method [46]. The extruded liposomes were stored at room temperature and their stability over time was followed by measuring the vesicle size and distribution.

2.3. Photon Correlation Spectroscopy

Photon correlation spectroscopy (PCS) was used to measure the mean vesicle size and distribution and to characterize vesicle stability over time periods extending out to one month. The analysis was performed on a BIC 90Plus Particle Size Analyzer (Brookhaven Instruments Corp.) with a laser at 656 nm. Analysis was performed at an angle of 90° and a temperature of 25°C. Suspensions of the original liposomes were diluted by PBS. Dilute particle concentration was maintained to ensure that multiple scattering and particle-particle interactions were negligible in this system. Five measurements were performed for each sample at each time point.

2.4. Zeta (ζ)-potential measurements

ζ -potential was measured on a Brookhaven Zeta Plus Analyzer (Brookhaven Instruments Corp.) at 25°C by Laser Doppler Anemometry (LDA) using electrophoretic light scattering. The analysis is based on the scattering of light from particles that move in a liquid under influence of an applied electric field. Liposomes were diluted in a 10 mM KCl solution for the measurement of ζ -potential. Five measurements were performed for each sample.

3. Results and Discussion

3.1. Biomimetic liposome modification

In this report, SuLe^a, one of the most potent natural selectin ligands, was used to create a saccharide coating as a glycocalyx-mimetic layer on the liposome surface, as shown in Figure 1. The strategy for the preparation of biomimetic liposomes is that monovalent SuLe^a-lipid (**1a**) and trivalent SuLe^a (TSuLe^a)-lipid (**1b**), as shown in Figure 2, are synthesized first, and are then used with 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol (Chol) to prepare unilaminar vesicles (ULVs) by a freeze-thaw and extrusion method. The purpose of incorporating TSuLe^a is to maximize the number of carbohydrate ligands linked to the liposome surface with minimal impact on liposome stability. Additionally, these liposomes are designed to exploit the multivalent effect for increasing the binding affinity to the target tissue or cells [39].

3.2. Synthesis of SuLe^a- and TSuLe^a-lipids

SuLe^a-lipid **1a** and TSuLe^a-lipid **1b** were synthesized by multiple-step reactions from a key intermediate, protected SuLe^a amino-derivative **2** in presence of 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), respectively, followed by deprotection of 4-methoxybenzyl (Bzl(OMe)) groups, 3'-sulfatation and deprotection of allyl (All) and benzyl (Bzl) groups [41,42], as shown in Scheme 1. In the natural form, Lewis antigens are attached through their reducing ends to lipids and/or proteins. If Lewis antigen conjugates have the lipids attached to the 2-amino group of the glucosamine residue with the reducing end capped by an unreactive group [38], it may affect the conformation of the antigen and their binding to selectins. Therefore, SuLe^a-containing glycolipids **1a** and **1b** with the lipid moiety at the saccharide reducing end have the advantage of stability under physiological conditions. This is attributed to the lower susceptibility of the glycosyl ether linkage to glycosidases, compared with natural *O*-glycosides with reducing ends.

3.3. SuLe^a- and TSuLe^a-modified liposomes

Glycolipids (5 mol%) were used with DSPC (55 mol%) and cholesterol (40 mol%) to prepare glycoliposomes by a freeze-thaw and extrusion method [45]. Multilamellar vesicles (MLVs) were first prepared by the freeze-thaw method, followed by extrusion through membranes with nanoscale pore size to produce unilamellar vesicles (ULVs). The ability to generate ULV liposomes with a well-defined pore size was examined using 100 and 50 nm extrusion membranes. The vesicle size and distribution was determined by PCS and the results are listed in Table 1. The mean vesicle sizes for SuLe^a-liposomes are 105.8 and 80.1 nm after extrusion through 100 and 50 nm membranes, respectively. This result is similar to the size range for DSPC- and PEG-liposomes, which were narrowly distributed with a polydispersity index (PDI) less than 0.2. The mean vesicle sizes for TSuLe^a-liposomes are 119.6 and 95.3 nm, after extrusion through 100 and 50 nm membranes, respectively. This is larger than the equivalent SuLe^a-liposomes, and is attributed to the larger headgroup of TSuLe^a.

The effect of the pore size of extrusion membranes on the vesicle size can be observed directly from the size distribution curves, as shown in Figure 3. The results show that the SuLe^a- and TSuLe^a-liposomes are narrowly dispersed with only one distribution peak, no matter what pore size is used for extrusion. However, the glycoliposomes have smaller sizes with considerable shifts to the left in the size distributions, when extruded through 50 nm membrane. This resulted in a decrease of about 25 nm in vesicle size, while the percentage of vesicles less than 100 nm in diameter, increased by 25-30% for SuLe^a- and TSuLe^a-liposomes (Table 1). The results suggest that both SuLe^a- and TSuLe^a-glycolipids with incorporation of 5 mol% can be used to form liposomal vesicles.

The stability of liposomes over time is an important concern in pharmaceutical applications, since liposomes must remain their vesicle integrity while in the circulation. Figure 4 shows the stability of the glycoliposomes with 5% of glycolipids incorporation in storage at room temperature. Both SuLe^a- and TSuLe^a-liposomes, extruded by either 100 or 50 nm membranes, maintained their vesicle size in the same range over a one month period, comparable with results for DSPC- and PEG-liposomes. This indicates that SuLe^a- and TSuLe^a-liposomes with 5 mol% glycolipid incorporation form stable vesicles with DSPC and cholesterol.

3.4. Effect of glycolipid concentration on vesicle size

The results in Table 2 shows that vesicle size, extruded through either 100 or 50 nm membranes, increased with the increasing concentration of glycolipids. All liposomes had a narrow size distribution with a PDI from 0.1 to 0.3. For SuLe^a-liposomes with the concentration increased from 10 to 20%, the vesicle size increased from 131.3 to 189.1 nm (after 100 nm extrusion), and 103.7 to 123.0 nm (after 50 nm extrusion), while the percentage of vesicles within 100 nm

decreased from 35.6% to 3.9% for 100 nm extrusion, and 56.8% to 35.9% for 50 nm extrusion. In contrast, the vesicle size for the TSuLe^a-liposomes increased from 147.2 to 205.5 nm (100 nm extrusion), and 136.4 to 166.7 nm (50 nm extrusion), while the percentage of vesicles less than 100 nm in diameter decreased from 19.2 to 2.5% for 100 nm extrusion, and 23.4 to 9.0% for 50 nm extrusion. The TSuLe^a-liposomes always had a larger vesicle size compared with the SuLe^a-liposomes due to the larger TSuLe^a headgroups.

The concentration effect on vesicle size is shown in Figure 5. DSPE-PEG, as shown in Figure 2 (1c), has a very large and flexible poly(ethylene glycol) headgroup, and tends to form micelles spontaneously, even before extrusion. The high curvature of micelles reduces steric repulsions between the PEG head groups. Consequently, at a concentration of greater than 10%, the vesicle size of PEG-liposomes decreased with increasing concentration, due to the formation of micelles (Table 2) [9,11]. In contrast, lactose-lipids (1d, Figure 2) tend to form bilayer structures, and the vesicle size of its liposomes changes little until the concentration exceeds 15% (Figure 5). The vesicle size increases dramatically with incorporation of 20%, due to the strong repulsive force between the lactose headgroups after forced extrusion [21].

The results in Figure 5 indicate that both SuLe^a- and TSuLe^a-liposomes exhibit increased vesicle size with increasing glycolipids. The results suggest that they tend to form bilayer structures before and after extrusion, with no micelle formation at higher concentrations, as observed for lactose-lipid [21]. However, they demonstrated smaller vesicle size change than lactose-liposome, maybe due to the stabilization from the negatively charged headgroups.

3.5. Effect of headgroups on liposome extrusion

The polycarbonate membranes used for extrusion have roughly cylindrical pores formed by chemical etching along ion tracks. Vesicle extrusion involves pushing a solution of large MLVs through the pore of polycarbonate membranes several times. Flow of the vesicle through the pore results in shear-induced tension in the bilayer, which could lead to instability inside the pore or at the pore exit that would result in vesicle formation, as shown schematically in Figure 6. Early studies showed that multiple passes are required to reduce the size and the lamella number of extruded vesicles, and that their size depends on the pore size, extrusion pressure and lipid properties. A blowing bubble model has been proposed to predict pressure, lysis tension, and pore size dependencies for vesicles extruded through pore sizes ranging from 50 to 100 nm [47,48]:

$$\frac{D}{D_p} = a\sqrt{\frac{\gamma}{PD_p}} + b \quad (1)$$

where D is the diameter of the final vesicle, D_p is the pore diameter of the extrusion membrane, P is the applied extrusion pressure, γ is the lysis or rupture tension, a and b are the fitting parameters ($a = 0.64$, $b = 1.08$). From the asymptotic value of Equation 1, the extruded vesicle size is usually ~10% larger than the pore size of the extrusion membranes.

As shown in Table 1, the size of DSPC liposomes, as well as 5% PEG liposomes, is about 10% larger than the pore sizes by either 50 or 100 nm extrusion, consistent with the estimation from Equation 1. However, from Tables 1 and 2, the SuLe^a- and TSuLe^a-modified liposomes, except 5% SuLe^a-liposomes by 100 nm extrusion, are much larger than the extrusion pore size as estimated by Equation 1. For the 50-nm extrusion with more than 10% incorporation, the liposome size was at least double the extrusion pore size. This effect can be attributed to the larger and less flexible saccharide headgroups, compared with DSPC and PEG liposomes. In equilibrium, the surface tension of a vesicle is zero (Figure 6a), which means there is no repulsive force between the headgroups in the bilayer ($F_e=0$). During extrusion, large vesicles block the entrance to the pores of the polycarbonate membrane. Surface tension is induced in

the SuLe^a- and TSuLe^a-lipid bilayer and leads to a critical repulsive force (F_c) between the headgroups when MLVs pass through the entrance of the pore (Figure 6b). Consequently, the applied pressure induces a surface tension that exceeds the lysis or rupture tension of the bilayer, and the big MLVs break up to form small ULVs. When more SuLe^a- and TSuLe^a-lipids are incorporated, higher surface tension is induced in the bilayers, which results in a greater F_c . That means the headgroups are more compressed. After extrusion, the bilayer of the vesicle reduces this surface tension, in order to achieve a relaxation state with the repulsive force (F_r) between the headgroups close to zero (Figure 6c). The mechanism for reducing the surface tension of the vesicles is to enlarge its size, analogous to a compressed spring by extending its length once the compressing force is removed. If the headgroups are large and rigid, this size-increasing effect is more apparent as the glycolipid concentration increases.

3.6. Effect of glycolipid concentration on vesicle stability

The stability of liposomes over time is an important concern in pharmaceutical applications. For specific drug delivery, liposomes must remain vesicle stability while in the circulation. When the incorporation of SuLe^a- and TSuLe^a-lipids was increased from 5 to 15%, the vesicle size was maintained in the same range for at least one month, similar to DSPC and PEG liposomes extruded by either 100 or 50 nm membranes (Data not shown). It indicates that glycoliposomes with both SuLe^a- and TSuLe^a-lipids form stable vesicles with DSPC and cholesterol. Figure 7 shows the stability of liposomes with 15% glycolipids in storage at room temperature. The pore sizes of extrusion membranes appear to have no influence on vesicle stability. Compared with the lactose-liposomes with 15% glycolipids [21], the presence of SuLe^a and TSuLe^a on the liposome surface results in better stability, due to the electrostatic effects from the negatively charged headgroups, which prevent liposome aggregation.

ζ -potential analysis was carried out to determine the surface charge, as listed in Table 3. Lactose- and PEG-liposomes with incorporation from 5 to 15%, as well as DSPC-liposomes, gave ζ -potential close to zero, which indicates these liposomes were almost charge neutral. The results indicate that SuLe^a- and TSuLe^a-liposomes were negatively charged by the presence of sulfate groups, with ζ -potential increasingly negative with increasing incorporation of glycolipids. However, the negative charge is shielded partially by the bulky hydrophilic saccharide groups, which play an important role in the prevention of vesicle aggregation and interaction with plasma proteins [23]. SuLe^a- and TSuLe^a-lipids can be incorporated more than 15% and the resulting vesicles have better stability than the equivalent lactose-liposomes. This is due to the electrostatic effects from the charged headgroups.

4. Conclusions

Two SuLe^a-containing glycolipids, monovalent SuLe^a-lipid and trivalent SuLe^a (TSuLe^a)-lipid, were synthesized, and used to make glycocalyx-mimetic liposomes by a freeze-thaw and extrusion method. The effects of the glycolipid concentrations and the pore sizes of extrusion membranes on vesicle size and stability were investigated by photon correlation spectroscopy (PCS). Glycoliposomes had larger portion under 100 nm by 50 nm extrusion than by 100 nm extrusion, and TSuLe^a-liposomes always had bigger vesicle size than SuLe^a-liposomes due to the larger TSuLe^a headgroup. Both SuLe^a- and TSuLe^a-liposomes increased their vesicle size with increasing glycolipids from 5% to 15%, and demonstrated good stability at room temperature for over one month. Compared with lactose-liposomes, SuLe^a- and TSuLe^a-liposomes with 15% glycolipids demonstrated better stability due to the electrostatic effect from the SuLe^a and TSuLe^a headgroups. The results indicate that these SuLe^a-modified biomimetic liposomes with 5 to 15% incorporation of glycolipids are sufficiently stable for the potential applications for targeted drug delivery.

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Figure 1. Model of glycocalyx-mimetic liposomes from SuLe^a-containing glycolipids. Cholesterol is not included in the vesicle structure in order to simplify the liposome model

Figure 2.
Structures of monovalent SuLe^a-lipid (**1a**), trivalent SuLe^a (TSuLe^a)-lipid (**1b**), DSPE-PEG (**1c**) and lactose-lipid (**1d**).

Figure 3.

Effect of extrusion pore sizes on vesicle size and distribution of SuLe^a antigens-modified liposomes. (a) SuLe^a-liposome (DSPE/Chol/**1a**, 55:40:5); (b) TSuLe^a-liposome (DSPE/Chol/**1b**, 55:40:5).

Figure 4.
Stability of SuLe^a- and TSuLe^a-liposomes with 5% glycolipids

Figure 5.
Effect of lipid concentrations on vesicle size of glycoliposomes by 100 nm extrusion (a) and 50 nm extrusion (b).

Figure 6. Schematic diagram of liposome preparation from MLVs to ULVs by membrane extrusion.

Figure 7. Stability of SuLe^a-modified liposomes extruded by 100 nm membranes (a) and 50 nm membranes (b) in storage at room temperature.

Scheme 1.**Synthesis of SuLe^a-lipid (1a) and TSuLe^a-lipid (1b)**

Reagents and conditions: (a) HOBt/EDC, DCM, RT, 36 h; (b) (NH₄)₂Ce(NO₃)₆, CH₃CN, CHCl₃/H₂O, RT, 2 h; (c) Pyridine, (CH₃)₃N·SO₃, RT, 48 h; (d) PdCl₂, NaOAc, HOAc/H₂O, RT, 48 h; (e) H₂, 10% Pd/C, CHCl₃/MeOH/H₂O (1:5:1), RT, 48 h.

Table 1

Effect of lipid concentrations on vesicle size and distribution of liposomes

Lipid ^a	Conc. (mol%)	100 nm extrusion		50 nm extrusion		
		D _v (nm ± SD) ^b	PDI ^c	D _v < 100 nm (%)	D _v (nm ± SD)	PDI
SuLe-lipid ^a	5	105.8 ± 1.5	0.19	54.6	80.1 ± 1.5	0.17
TSuLe-lipid ^a	5	119.6 ± 1.4	0.11	35.0	95.3 ± 1.3	0.10
DSPE-PEG	5	108.5 ± 1.2	0.12	34.6	66.4 ± 1.4	0.12
DSPC	60	109.5 ± 1.4	0.14	47.2	63.3 ± 1.4	0.13

^a All liposomes consisted of 60 mol% of total lipid (including DSPC and the functional lipid) and 40 mol% of cholesterol.

^b Volume-average diameter ± Standard deviation.

^c Polydispersity index.

Table 2

Effect of lipid concentrations on vesicle size and distribution of liposomes

Lipid	Conc. (mol%)	100 nm extrusion			50 nm extrusion		
		D _v (nm ± SD)	PDI	D _v < 100 nm (%)	D _v (nm ± SD)	PDI	D _v < 100 nm (%)
SuLe ^a -lipid	10	131.3 ± 1.6	0.22	35.6	103.7 ± 1.5	0.20	56.8
	15	154.2 ± 1.7	0.29	27.8	121.0 ± 1.6	0.23	43.3
	20	189.1 ± 1.4	0.13	3.89	123.0 ± 1.6	0.14	35.9
TSuLe ^a -lipid	10	147.2 ± 1.5	0.15	19.2	136.4 ± 1.4	0.13	23.4
	15	171.2 ± 1.5	0.11	5.53	162.7 ± 1.4	0.12	13.6
	20	205.5 ± 1.4	0.11	2.45	166.7 ± 1.5	0.17	9.04
DSPE-PEG	10	99.0 ± 1.4	0.06	57.6	68.0 ± 1.2	0.04	96.6
	15	72.1 ± 1.5	0.21	84.8	41.5 ± 1.5	0.18	99.6
	20	65.2 ± 1.5	0.17	91.2	38.1 ± 1.5	0.16	99.7

Table 3
 ζ -Potential values of various liposomes by 50 nm extrusion

Lipid	ζ -Potential (mV $\pm$ SD) of liposomes ^a		
	5 %	10 %	15 %
SuLe-lipid ^a	-8.46 $\pm$ 1.91	-15.94 $\pm$ 1.68	-22.80 $\pm$ 0.79
TSuLe-lipid ^a	-13.52 $\pm$ 1.06	-18.59 $\pm$ 1.02	-23.34 $\pm$ 0.68
Lactose-lipid	0.27 $\pm$ 1.26	1.30 $\pm$ 1.92	-1.97 $\pm$ 1.22
DSPE-PEG	0.41 $\pm$ 1.05	-0.42 $\pm$ 1.23	1.03 $\pm$ 0.68

^a ζ -Potential of DSPC-liposomes is -0.42 $\pm$ 1.21 mV.