Influence of Lipid Composition and Ionic Strength on the Physical Stability of Liposomes

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Received February 3, 1983, from the *Department of Pharmaceutics, Pharmaceutical Laboratory, University of Utrecht, 3511 GH Utrecht, The Netherlands.* Accepted for publication November 22, 1983.

Abstract □ The effect of including charge-inducing agents (stearylamine or phosphatidylserine) on the zeta potential of phosphatidylcholine-cholesterol-containing liposomes in aqueous media with varying ionic strength (sodium chloride) was investigated. In 150 mM ionic strength solutions, the experimentally obtained zeta potentials were in good agreement with predictions made with the Gouy-Chapman equation, after proper correction for bulk ionic interaction. However, at low ionic strength, substantial deviations occurred which were ascribed to incomplete dissociation of the phosphatidylserine molecules or protonation of the stearylamine molecules due to the large diffuse double layer (Debye length). Predictions were made on the physical stability of the liposomes based on the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and current knowledge of hydration effects on interparticle interaction. For negatively charged liposomes, both at low and high ionic strength, no increase in particle size was found after storage. For low ionic strength solutions, this agreed with the calculations; for the high ionic strength solutions, aggregate formation in a secondary minimum was expected. For positively charged liposomes, the physical stability predicted from theoretical considerations did not correlate with the experimentally observed stability.

Keyphrases □ Liposomes—phosphatidylcholine-cholesterol, ionic strength, lipid composition physical stability □ Stearylamine—liposomes with phosphatidylcholine and cholesterol, physical stability □ Phosphatidylserine—liposomes with phosphatidylcholine and cholesterol, physical stability

The surface potential is an important parameter influencing liposomal behavior. *In vivo*, the surface charge density has been found to influence the distribution of liposomes (1, 2) and *in vitro*, a high potential might contribute to their physical stability by reducing the rate of aggregation and fusion. Frøkjaer *et al.* (3) reported an increase in physical stability of liposomes (against aggregation or fusion) by decreasing the ionic strength and increasing the surface charge density of liposomes consisting of phosphatidylcholine (I) and phosphatidylserine (II). However, if there is no electrostatic repulsion between two liposomes this will not necessarily result in aggregate formation or fusion of the colliding particles, as many uncharged liposomes are fully stabilized by hydration energies (4, 5).

The influence of the surface charge density and ionic strength on the zeta potential of I and cholesterol-containing liposomes with stearylamine (III) or II was investigated by microelectrophoresis. The zeta potentials obtained experimentally were compared with surface potentials (Ψ_0) predicted by the Gouy-Chapman equation.

Using the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (6) and information on hydration energies between phospholipid vesicles (4, 5), calculations were made on the physical stability of the liposomes against aggregation or fusion. These predictions were verified by determining the actual size by dynamic light scattering. Because the surface potential cannot be measured by electrophoresis, the zeta potential, instead of Ψ_0, was used for the evaluation of the experimental results.

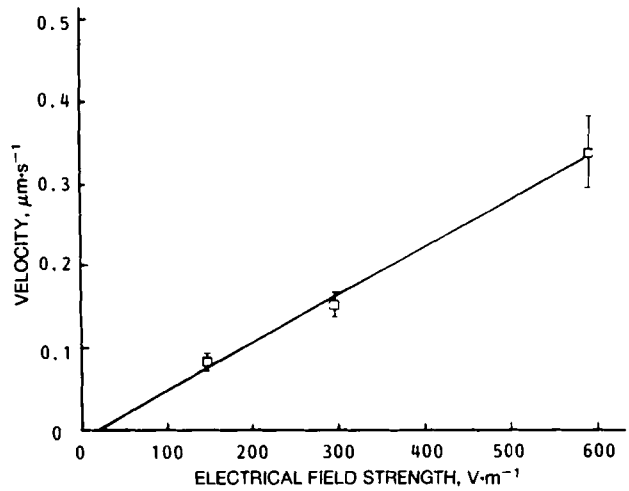

Figure 1—*Dependence of velocity on electrical field strength for liposomes composed of phosphatidylcholine-cholesterol-stearylamine (10:4:2); ionic strength, 2.3 mM. The line represents least-squares fit and the vertical bars are* SD.

EXPERIMENTAL SECTION

Positively and negatively charged multilamellar liposomes were prepared by the "film" method (7). Positive liposomes consisted of phosphatidylcholine (I)[1], cholesterol[2], and stearylamine (III)[3] in the molar ratio 10:4:1, 2, or 3, corresponding to maximum (positive) surface charge densities of 0.019, 0.037, or 0.054 C·m^{-2}, respectively. For negative liposomes, phosphatidylserine (II)[4] was used instead of III. With II, maximum (negative) charge densities of ~0.018, 0.034, or 0.047 C·m^{-2}, respectively, can be reached.

After film formation in pear-shaped flasks in a rotary evaporator at 40–45°C, the film was left under reduced pressure for ≥2 h in a desiccator. It was hydrated with NaCl solution (ionic strength, 2–150 mM). The water was

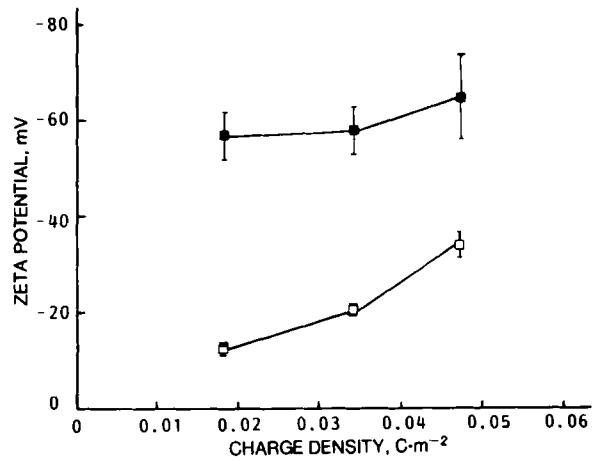

Figure 2—*Zeta potential of negative liposomes as a function of surface charge density for 6.6 mM (■) and 152 mM (□) ionic strengths. Liposome composition: phosphatidylcholine-cholesterol-phosphatidylserine (10:4:1, 2, or 3) with a charge density of 0.018, 0.034, or 0.047 C·m^{-2}, respectively. Vertical bars represent* SD.

[1] Egg yolk, L-α-phosphatidylcholine type V-E; Sigma Chemical Co., St. Louis, Mo.
[2] Sigma Chemical Co.
[3] ICN Pharmaceuticals, Plainview, N.Y.
[4] Bovine brain; Sigma Chemical Co.

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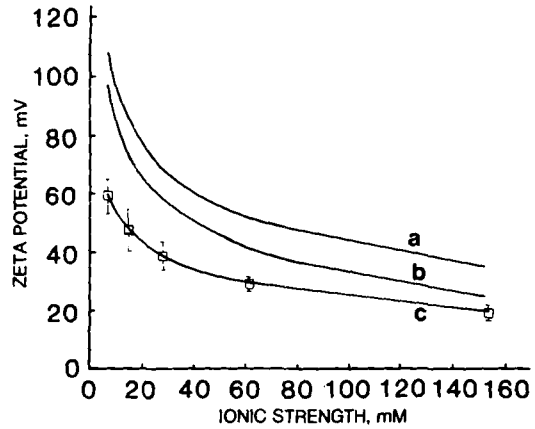

Figure 3—*Effect of ionic strength on the zeta potential for negative liposomes composed of phosphatidylcholine-cholesterol-phosphatidylserine (10:4:2). Key: (a) surface potential calculated according to Eq. 1; (b) surface potential calculated according to Eq. 2 (K = 0.0008 $m^3 \cdot mol^{-1}$), and Eq. 1; (c) experimental zeta potential. Vertical bars represent* SD.

freshly distilled in glass, and nitrogen was passed through the solution for 10 min. The final phospholipid concentration was ~7.5 μmol/mL. Negative liposome dispersions contained 1 mM EDTA. The pH was adjusted to 5.9 ± 0.1 with dilute HCl or NaOH. After pH adjustment, the concentration of NaCl in the dispersion was determined conductometrically[5]. If necessary, corrections for EDTA were applied. After complete dispersion of the film by shaking and vortexing at 40-45°C, the liposomes were stored overnight at 4-6°C and extruded the next day through membrane filters[6] with 400-nm pores (8).

Mobilities of the liposomes were measured in a cylindrical-cell microelectrophoresis apparatus[7] at 25°C. Heat effects were minimized by using currents of <2 mA and short current passage times. The liposome dispersions were diluted with the hydration medium to avoid an overcrowded field of view. To minimize electrode polarization, successive measurements were made in both current directions. At least 20 particles were measured in each direction at the lower and upper stationary levels. Data obtained for both directions of liposome movement did not differ significantly (Student's t test; $p = 0.95$) and were averaged.

Mean diameters of the liposomes were measured with a dynamic light-scattering technique[8] 1 d after extrusion and after 7 and 14 d. A polydispersity index was also obtained[8]. This index ranges from 0 to 9; 0 refers to a monodispersed system, 9 to an extremely polydispersed system. A gold suspension approaching a normal size distribution with a mean diameter of ~50 nm and a CV of 15% has a polydispersity index of 3[9]. The dispersions were stored at

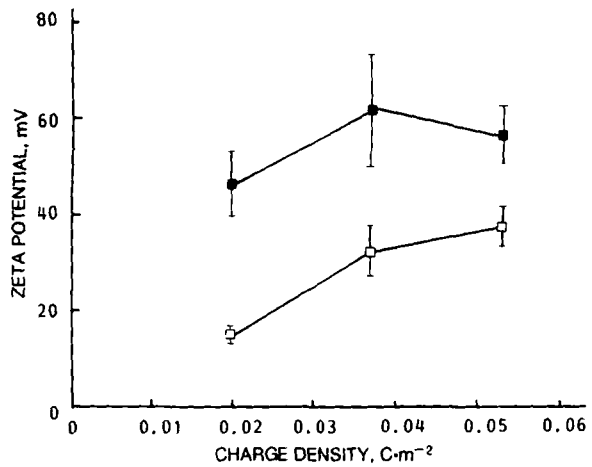

Figure 4—*Zeta potential of positive liposomes as a function of surface charge density for 2.3 (■) and 153 mM (□) ionic strengths. Liposome composition: phosphatidylcholine-cholesterol-stearylamine (10:4:1, 2, or 3) with a charge density of 0.019, 0.037, or 0.054 $C \cdot m^{-2}$, respectively.*

[5] PW 9505; Philips Cambridge, U.K.
[6] Unipore filters; Biorad, Richmond, Calif.
[7] Mark II; Rank Brothers, Bottisham, U.K.
[8] Nanosizer; Coulter Electronics Ltd., Luton, U.K.
[9] J. Leuvering, personal communication.

Table I—Effect of Ionic Strength on the Zeta Potential of Positive Liposomes [a]

Ionic Strength, mM[b]	Zeta Potential, mV	CV, %	Ψ_0, mV[c]
2.3	62	13	133
15.3	57	13	85
150	34	15	38

[a] Composition: phosphatidylcholine-cholesterol-stearylamine (10:4:2); charge density, 0.037 ($C \cdot m^{-2}$). [b] NaCl solutions. [c] Calculated according to the Gouy-Chapman equation (Eq. 1).

4-6°C, protected from light. Before measuring the mean diameter, the dispersions were equilibrated for at least 3 h at room temperature (20-25°C). Appropriate corrections were made for temperature-dependent parameters (*e.g.*, medium viscosity). Before taking the readings, the dispersions were diluted 10-100 times with solutions of the same ionic strength. For these dilute dispersions, the influence of the ionic strength on the particle size measurement is negligible (9).

The influence of dilution on the calculated diameters and polydispersity indices was investigated. In the concentration range covered by the dynamic light-scattering technique, the average size of primary or fused vesicles and the aggregate dimensions did not depend on the phospholipid concentration. The absorption (at 700 nm) per μmol/mL of phospholipid per cm path length was constant after dilution. These findings justify the conversion of the data obtained with the dilute dispersions to the original dispersions.

The purity of I and II was assessed as described by Frøkjaer *et al.* (3). Lysophosphatidylcholine and lysophosphatidylserine were separated from intact phospholipids by TLC [silica gel[10], chloroform-methanol-water (65:35:5)] visualized with iodine vapor. The spots were scraped off, and the compounds were extracted with methanol. The lysophosphatidylcholine and lysophosphatidylserine content was determined by phosphate analysis (10). Before the liposome preparation, <2 and 5% of these compounds were detectable in I- and II-containing vials, respectively. In a comparable system, Kitagawa *et al.* (11) found that the presence of these compounds in these concentrations had a negligible effect on particle size. One week after liposome preparation, no detectable lysophosphatidylcholine and lysophosphatidylserine increase was observed.

RESULTS AND DISCUSSION

A linear relationship between the velocity of liposomes and the electrical field strength is predicted by theory. In our experiment, results agreed with theory (Fig. 1). There was no difference between mobilities at the lower and the upper stationary levels for the same dispersion. All calculated zeta potentials were taken from upper stationary level data. The Helmholtz-Smoluchowski equation was used to calculate the zeta potential from mobility data. In cases of low ionic strength, particles with an estimated diameter >1 μm were selected for mobility measurements to avoid corrections for relaxation (12, 13).

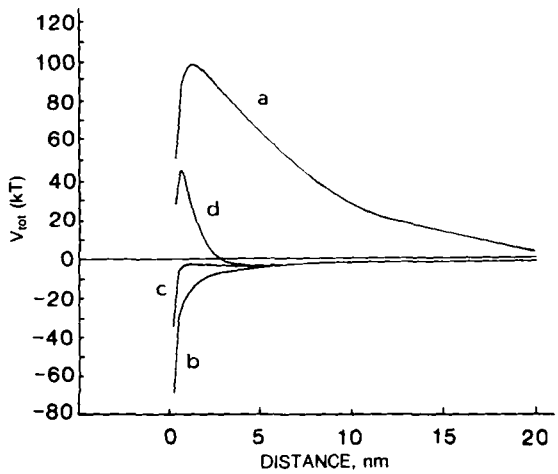

Figure 5—*Calculation of interaction energies (V_{tot}) between two liposomes (radii 200 nm) according to the Derjaguin-Landau-Verwey-Overbeek theory as a function of interparticle distance. The Hamaker constant is 5 × 10^{-21} J, based on data reported by Rand (5). Key [zeta potential (mV)/ionic strength (mM)]: (a) 30/3; (b) 10/150; (c) 20/150; (d) 30/150.*

[10] Precoated silica gel 60 F_{254}; E. Merck, Darmstadt, F.R.G.

Table II—Effect of Changing the Ionic Strength of the Outer Aqueous Medium of Liposome Dispersions on the Zeta Potential [a]

Charge	Initial: Ionic Strength, mM [b]	Initial: Zeta Potential mV [c]	After Changing: Ionic Strength, mM [b]	After Changing: Zeta Potential, mV [c]
+[d]	2.3	62	150	27
+	150	34	3.3	62
−[e]	6.6	59	150	20
−	150	20	—[f]	--

[a] Mobilities were measured at least 2 h after changing the medium. [b] NaCl solutions, for negative liposomes in combination with 1 mM EDTA. [c] Zeta potentials are the mean of duplicate experiments. [d] Composition of positive liposomes: phosphatidylcholine-cholesterol-stearylamine (10:4:2). [e] Negative liposomes: phosphatidylcholine-cholesterol-phosphatidylserine (10:4:2). [f] Not available.

Figure 2 is a plot of the zeta potential *versus* the surface charge density (σ). This parameter was calculated assuming a surface area of 0.70 nm^2 per I or II molecule. Values from 0.67 nm^2 (14) to 0.72 nm^2 (15, 16) were reported for I and 0.70 nm^2 for II (17, 18). The value used for the area covered by one cholesterol molecule was 0.28 nm^2 (7), and for one molecule of III the value used was 0.25 nm^2. For 1:1 bilayer combinations consisting of I and cholesterol, an area of 0.96 nm^2 was found for a unit consisting of one molecule of I and one molecule of cholesterol (5). This indicated that interactions between the different molecular species in the bilayer do not substantially influence the area covered by the individual molecules. The surface charge density could be calculated (19) from these data. It was assumed that the phospholipids and additives spread evenly and laterally over the bilayers (20). For I- and II-containing vesicles with relatively large radii of curvature (like those used in this study), the distribution over the two halves of the bilayer is expected to be fairly symmetrical (20, 21). At the experimental pH, both the deprotonation of II and the protonation of III were almost complete (22, 23). For negative liposomes, zeta potentials were obtained at two ionic strength levels as a function of II content. For an ionic strength of 152 mM, a nearly linear increase of the zeta potential with surface charge density was observed. For low ionic strength data (6.6 mM), this increase was less pronounced.

A comparison was made between the experimentally obtained zeta potentials and surface potentials calculated from the surface charge density. The effect of ionic strength on the zeta potential for the negative liposomes with an intermediate II content (charge density 0.034 $C \cdot m^{-2}$) is shown in Fig. 3. Line a represents the effect on the surface potential as predicted by the Gouy-Chapman equation for a single symmetrical electrolyte (Eq. 1):

$$\sigma = (8 \cdot N \cdot \epsilon_r \cdot \epsilon_0 \cdot kT)^{1/2} \cdot C^{1/2} \cdot \sinh (0.5 \cdot e \cdot \Psi_0/kT) \quad \text{(Eq. 1)}$$

Line b gives the surface potential calculated (with Eq. 1) after correction of the surface-charge density for the sodium ion interaction with II (Eq. 2). This interaction reduces the effective surface charge density (24):

$$\sigma_{corr} = \frac{\sigma}{1 + K \cdot C \cdot \exp\left(\frac{-e \cdot \Psi_0}{kT}\right)} \quad \text{(Eq. 2)}$$

where σ_{corr} and σ are the corrected and uncorrected surface charge density ($C \cdot m^{-2}$), respectively; e is the charge of a proton (C); k is the Boltzmann constant ($J \cdot K^{-1}$); T is absolute temperature (K); K is the intrinsic binding constant (m^3/mol); C is the concentration (mol/m^3); N is Avogadro constant (mol^{-1}); ϵ_0 is the permittivity ($C \cdot V^{-1} \cdot m^{-1}$); and ϵ_r is the relative permittivity (no units).

A value of 0.8×10^{-3} $m^3 \cdot mol^{-1}$ was used (25) for the instrinsic binding constant of the sodium ion to II, and the value for the relative permittivity of water was 78.3 (26). The zeta potential data comply reasonably well with the corrected surface potentials for the dispersions in 61 and 152 mM NaCl. In principle, the surface potential always has to exceed the zeta potential as the hydrodynamic plane of shear does not coincide with the surface, but is positioned at a finite distance from it (27). For the lower NaCl concentrations, the deviation between experimental and theoretical data increased. The influence of the divalence of the $EDTA^{2-}$ ion was neglected in the calculations, as the inclusion of a divalent ion complicated them considerably. This approach was justified because it was found experimentally that for the negative liposomes, under conditions where the influence of the $EDTA^{2-}$ ion is expected to be most pronounced (low II content, low ionic strength), the presence of $EDTA^{2-}$ ion in the aqueous medium did not change the zeta potential significantly [Student's t test, $p = 0.95$]: with EDTA, 58 mV (SD 8 mV, $n = 42$), without EDTA, 55 mV (SD 7.5 mV, $n = 35$).

The correction proposed by Nir and Bentz (24) takes into account interactions of the bilayer II molecules with cations from the bulk. The large deviation from the theoretical predictions at low ionic strength might be caused by a limitation in the ionization of II. In a solution of NaCl with an ionic strength of 5 mM, the Debye length ($1/\kappa$) is 4.4 nm and exceeds the distance between the molecules (2-3 nm, assuming the II molecules to be spread evenly over the bilayer). Therefore, mutual interference cannot be excluded. For a solution of 150 mM ionic strength, the Debye length is only 0.8 nm and no interaction is expected. Hauser *et al.* (20) suggested an analogous mechanism for the incomplete ionization of fatty acids clustering in bilayers.

Table III—Physical Stability of Positive and Negative Liposomes: Particle Size of Liposome Dispersions 1 and 7 d After Extrusion [a]

Ionic Strength, mM	Zeta Potential, mV	Diameter, nm [b]: Day 1	Diameter, nm [b]: Day 7	Stability [c]: Observed	Stability [c]: Predicted [d] ($V_{rep}^{el} + V_{att}$)
Phosphatidylcholine-Cholesterol-Stearylamine (10:4:*N*)					
2.3					
$N = 1$	46	271[e]	270[e]	+	+
$N = 2$	61	522[f]	—[g]	−	+
$N = 3$	57	—[g]	—[g]	−	+
150					
$N = 1$	17	—[g]	—[g]	−	−
$N = 2$	34	571[f]	—[g]	−	+/−
$N = 3$	39	376[e]	300[e]	+	+/−
Phosphatidylcholine-Cholesterol-Phosphatidylserine (10:4:*N*) [h]					
6.6					
$N = 1$	−57	300	309	+	+
$N = 2$	−58	276	267	+	+
$N = 3$	−65	320	311	+	+
150					
$N = 1$	−12	301	285	+	−
$N = 2$	−20	350	346	+	−
$N = 3$	−34	238	234	+	+/−

[a] Protected from light at 4-6°C. [b] Mean of duplicate experiments. CV of sequential readings of the mean diameters of the same dispersion, 3%. [c] (+) stable; (−) unstable; (+/−) secondary minimum over −3 kT at an interparticle distance >2.5 nm. [d] (V_{rep}^{el}) electrostatic repulsive energy; (V_{att}) van der Waals attractive energy (effective Hamaker constant, 5×10^{-21} J). Predictions made using the Derjaguin-Landau-Verwey-Overbeek theory. [e] Polydispersity index 2-3. [f] Polydispersity index 7. [g] Strongly heterodisperse with mean diameters >1000 nm. [h] Polydispersity index 2-3 for all negative liposomes.

The results obtained with positive liposomes at two ionic strength levels are plotted in Fig. 4. For the 150 mM ionic strength series, the zeta potential increase leveled off at charge densities >0.037 $C \cdot m^{-2}$. Little increase of the potential with the surface charge density was found for solutions of 2.3 mM ionic strength.

Table I gives the zeta potentials of the positive liposomes with an intermediate III content over an ionic strength range from 2.3 to 150 mM. The experimental results were compared with the calculated surface potentials (Gouy-Chapman equation) listed in the last column. Here, as with the negative liposomes, for the high ionic strength dispersions a good agreement with theory was found; for low ionic strength, the experimental values for the zeta potentials were too low.

The proposed explanation for the deviation of the experimental zeta potentials from theory implies that changing the outer aqueous medium of the liposome dispersions from low to high ionic strength and *vice versa*, has in principle a reversible impact on the zeta potential. The effect of changes in the ionic strength in the aqueous phase outside the bilayer on the zeta potential was investigated. The positive and negative liposomes with intermediate III and II content, respectively, prepared under low ionic strength conditions, were mixed with at least 100-fold excess of an NaCl solution of high ionic strength (150 mM). After 2 h of equilibration, the mobilities were determined. For the positive liposomes, the reverse was done: dispersions in the high ionic strength medium were diluted with the low ionic strength solution. The results are listed in Table II. Changing the medium resulted in zeta potentials comparable with those existing after preparation of the liposomes at that particular ionic strength. This is an indication that preparing these types of liposomes at low or high ionic strength does not result in structures that differ irreversibly from each other.

The physical stability, on storage at 4°C, was investigated for various types of positively and negatively charged liposomes. Results of particle size measurements 1 d and 7 d after extrusion through 400-nm membrane filters are shown in Table III. Deviations of the reported particle diameter from the pore diameter (400 nm) were ascribed to differences in filtered liposome dispersion volumes. The initial filtrate generally contained smaller-sized liposomes. There was a distinct difference between the behavior of stable and unstable dispersions. For unstable dispersions, the mean particle diameter was at least double the diameter of the membrane pore with a polydispersity index >7 after 1

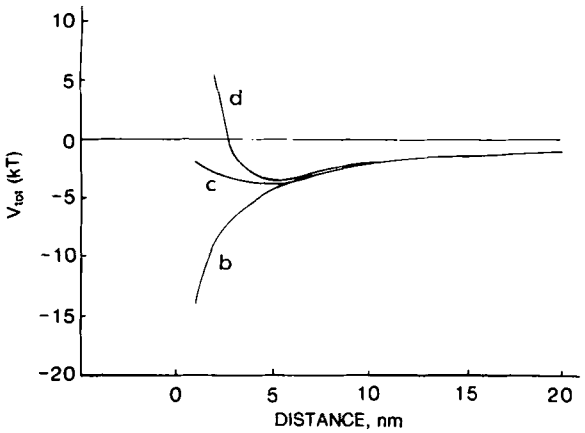

Figure 6—*Magnification of part of Fig. 5 to show occurrence of secondary minima.*

week. Stable dispersions had diameters <400 nm and polydispersity indices of 2–3 both at the day of extrusion and after 1 week. In all cases, changes in particle size occurred independently of phospholipid concentration within the first week of storage. No difference was found in particle diameters of the negative liposomes measured after 7 or 14 d of storage. This was also true for the two stable positive liposome dispersions.

Frøkjaer *et al.* (3) found, for I- and II-containing liposomes, an enhanced stability with decreasing ionic strength and increasing II content. Under the experimental conditions in this study, negative liposomes were stable independent of ionic strength and surface charge density. Positive liposomes had no correlation between the zeta potential and particle size stability; those with low surface charge density were stable at an ionic strength of 2.3 mM. Liposomes with high surface charge density, on the other hand, were stable at an ionic strength of 150 mM. A high positive zeta potential (61 or 57 mV), as was observed with the low ionic strength medium, did not therefore, guarantee a stable dispersion. Whether particle size growth was caused by aggregation or fusion of the particles was not studied.

We investigated whether the experimental results could be predicted by the existing theories on the physical stability of liposome dispersions. The interaction energy between two approaching liposomes was calculated according to the DLVO theory (6) using $V_{tot} = V_{att} + V^{el}_{rep}$, or:

$$V_{tot} = -\frac{aA}{12H} + 2\epsilon_r\epsilon_0\Psi_0^2 a \cdot \ln(1 + e^{-\kappa H}) \qquad \text{(Eq. 3)}$$

where V_{tot}, V^{el}_{rep}, and V_{att} are the total, electrostatic repulsive, and attractive energies, respectively; a is the radius of the liposome; H is the distance between the bilayer surfaces; and A is the effective Hamaker constant. In Eq. 3, V_{att} is calculated with a simplified equation for solid spheres and $H \ll a$. Results obtained with the complete equation for spherical shells, as given by Nir and Bentz (24), differed <5% for $H \leq 20$ nm.

The results suggest that, for low ionic strength (3 mM) dispersions with a zeta potential of >30 mV, a V_{tot} of at least 100 kT can be reached (Fig. 5). This implies that the rate of aggregation of these charged primary particles (compared with uncharged particles) is reduced by at least a factor of 10^9, and that dimer formation is limited to <10% of the primary particles over 2 weeks (6). Therefore, at low ionic strength the dispersions used should be stable (Figs. 2 and 4). Aggregation in the primary or secondary minimum was unlikely as the electrostatic repulsion exceeded the van der Waals attraction. These calculations are estimates because the effective Hamaker constants for the I, cholesterol, and II or III systems are not known precisely. Rand (5) reported a value of 9×10^{-21} J for pure I and 1.2×10^{-21} J for I-cholesterol (1:1) bilayers. We chose 5×10^{-21} J for our calculations. For low ionic strengths, all dispersions were predicted to be stable. However, for the positive liposomes, aggregation or fusion occurred with two of three dispersions.

For the 150 mM ionic strength dispersions, V^{el}_{rep} was not dominant. Secondary minima could be observed by using realistic Ψ_0 values (Fig. 6). The secondary minima are not affected by repulsive hydration energy, as the influence of this energy decreases steeply with increasing interparticle distance and can virtually be neglected for interparticle distances >2.5 nm (5). As far as electrical repulsion is concerned, the dispersions with low III and intermediate II content should behave similarly under 150 mM ionic strength conditions as they have similar zeta potentials (−17 and −20 mV, respectively). Experimentally, the negative liposomes were stable, and the positive liposomes were unstable. Apparently factors other than the electrical repulsion (*e.g.*, V_{att}) control stability. As stated above, the secondary minima shown in Fig. 6 are not affected by the hydration forces. In the case of negatively charged liposomes containing 100% II, aggregation in the secondary minimum is assumed to be a reversible process in the presence of sodium ions (28). In these dispersions, both aggregate formation and dissociation occur. In the dispersions under investigation, an analogous situation is likely. The mass average aggregate size at equilibrium was calculated for a −4-kT secondary minimum for negative liposomes of the compositions used in this study. Diameters of 200 and 400 nm were taken with phospholipid concentrations between 7.5×10^{-3} M (the concentration of the dispersions on storage) and 7.5×10^{-5} M (the minimum concentration used in dynamic light-scattering studies). The equations and boundary conditions for these calculations have been described by Bentz and Nir (28). Under the chosen conditions substantial aggregate formation was predicted, depending on the phospholipid concentration. This could not be confirmed experimentally, as no concentration-dependent mean diameter or polydispersity was observed in this study.

The negatively charged liposome dispersion (predicted to aggregate in the secondary minimum) was stable. If the physical model that was used to predict the stability is correct, the experimental results indicate that V_{att} is overrated for the negatively charged liposomes. This might be ascribed to an overestimation of the value for the Hamaker constant.

It is remarkable that two positively charged liposome dispersions (zeta potentials of 34 and 39 mV) that are both predicted to form aggregates in the secondary minimum (Table III) behave differently. The first one forms aggregates; the second is stable for at least 2 weeks.

CONCLUSIONS

Increasing the charge density from ~0.018 to 0.050 C·m^{-2} resulted in considerably larger zeta potentials for the dispersions with an ionic strength of 150 mM. Almost no increase was found at an ionic strength of ~5 mM. The zeta potentials were larger at low ionic strength, but not as large as predicted by the (corrected) Gouy-Chapman equation. Based on these data for low ionic strength, high electrostatic repulsive energies, and thus stable dispersions, were expected. However, two of the positive liposome dispersions were not stable. Apparently, in these systems particles can form aggregates because of a mechanism interfering with the "classical" DLVO theory (with consideration given to repulsive hydration forces).

On the other hand, for high ionic strength dispersions, DLVO calculations predicted an irreversible aggregation in a primary minimum or a reversible aggregation in the secondary minimum. However, no aggregation at all (negative liposomes and one positive liposome dispersion) or irreversible aggregation was found. This unexpected stability against aggregation in the primary minimum can be ascribed to the hydration forces. However, hydration forces do not extend far enough from the surface of the bilayer to affect the reversible aggregation in the secondary minimum.

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ACKNOWLEDGMENTS

The author acknowledges the helpful discussions with Dr. O. Patmamanoharan (Department of Physical and Colloid Chemistry of the University of Utrecht) and Dr. J. Leuvering (Organon B. V.), and the critical remarks by Dr. P. J. J. M. van Mil during preparation of the manuscript.

Hydrolysis of Some Poly(*ortho*-ester)s in Homogeneous Solutions

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Abstract □ The hydrolysis of poly(*ortho*-ester)s and a monomeric model compound, 3,9-dibenzyloxy-3,9-diethyl-2,4,8,10-tetraoxaspiro[5,5]undecane, was carried out in dioxane-d_8–dioxane and followed by ^{1}H-NMR and HPLC, respectively. Experimental results suggested that the polymer degradation proceeds to a large extent *via* random scission. The hydrolysis was catalyzed by the acid; the catalytic rate constant increased predictably with decreasing aqueous pK_a of the acid. The reaction is first order with respect to the catalyst concentration and the number of *ortho*-ester linkages present, and it is independent of water in the concentration range studied. Strain at the *ortho*-ester bond may be a factor influencing the hydrolysis rate.

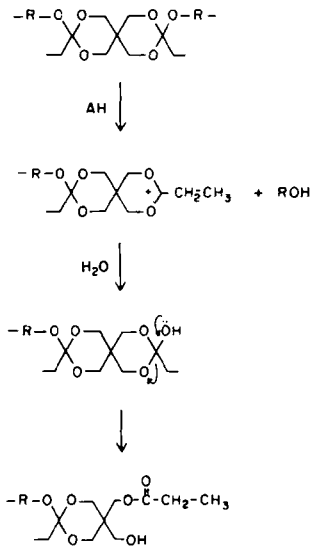
Keyphrases □ Hydrolysis—poly(*ortho*-ester)s, homogeneous solutions □ Polymers—hydrolysis of poly(*ortho*-ester)s, homogeneous solutions □ Degradation—polymers, hydrolysis of poly(*ortho*-ester)s, homogeneous solutions □ Poly(*ortho*-ester)s—hydrolysis, homogeneous solutions

Poly(*ortho*-ester)s are a novel class of polymers with potential utility in drug delivery systems. Their use as an erodible matrix for the delivery of a steroid has been demonstrated by Heller *et al.* (1). In these systems, water-soluble salts were incorporated into the polymer, and the release mechanism appeared to be similar in part to that described by Fedors (2). Osmotic imbibition of water induced the swelling of the matrix, which subsequently burst and released the drug.

Poly(*ortho*-ester)s, as with most *ortho*-esters, undergo acid-catalyzed hydrolysis quite readily and are relatively unreactive in neutral or basic media (3, 4). Thus, when exposed to an aqueous environment, the erosion of the polymeric matrix may be induced by the presence of an acidic catalyst. The acid may be external or may be generated *in situ* by an acid-producing agent such as acid anhydride incorporated in the device. Since acid anhydrides are neutral, and therefore noncatalytic, they add to the stability of the device during storage.

Following this rationale Shih *et al.* (5) have demonstrated the use of poly(*ortho*-ester)s delivery systems, achieving zero-order release of timolol maleate. The release rate was effectively controlled by the amount of acid anhydride incorporated into the device and the aqueous pK_a of the corresponding acid. It was proposed that the mechanism of drug release from such a system results from the contribution of several processes, namely, the permeation of water into the polymer matrix, the hydrolysis of the acid anhydride to the corresponding acid, the hydrolysis of the *ortho*-ester linkages, and the dissolution of the drug species into the medium (5).

The present studies were undertaken as part of an effort to understand the various physicochemical processes which govern the release of drugs from poly(*ortho*-ester)s delivery systems. In an attempt to separate the various contributing factors, we report the kinetics of the hydrolysis of poly(*ortho*-ester)s [poly(3,9-dialkyloxy-3,9-diethyl-2,4,8,10-



*Scheme I—Acid-catalyzed hydrolysis of poly(*ortho*-ester)s.*