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Some properties of N-acyl sarcosinate lipid vesicles

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INTRODUCTION

In recent years considerable effort has been directed toward the development of lipid bilayer vesicle delivery systems employing lipid amphiphiles other than phospholipids. Amphiphiles have been shown to form lipid vesicles of different types and stabilities and include fatty acids (1,2), dialkyldimethylammonium amphiphiles (3–7), dialkyl amphiphiles with ionic or zwitterionic head groups (8,9), polyglycerol alkyl ethers and polyethoxylated analogues (10,11), two-tailed sucrose fatty acid esters (12,13), and appropriate mixtures of single-tailed cationic and anionic surfactants (14).

Our own efforts have focused on the production of non-phospholipid paucilamellar vesicles that we call "novasomes" (15–20). These vesicles are 0.1–1.0 microns in diameter, with 2–5 bilayer shells surrounding an unstructured space that can be occupied by aqueous or water-immiscible materials. These vesicles are formed by injection of a liquid amphiphile mixture into the excess aqueous phase at high velocity, followed by cooling under conditions of turbulent mixing. The injection step causes the lipid to form minute droplets that are quickly converted into micelles. The cooling step causes the micelles to fuse into paucilamellar vesicles within milliseconds. The vesicles can be produced in amounts ranging from milliliter batches to continuous flow at costs equivalent to those of making simple emulsions.

This paper concerns novasomes made from N-acyl sarcosinates, amphiphiles widely used in shampoos, soaps, skin cleansers, and a variety of other cosmetic and personal care applications. N-acyl sarcosinates have the following general structure:

$$R-\underset{\underset{O}{\|}}{C}-\underset{\underset{CH_3}{|}}{N}-CH_2-COOH \leftrightarrow R-\underset{\underset{O}{\|}}{C}-\underset{\underset{CH_3}{|}}{N}-CH_2-CH_2-COO^- + H^+$$

where R is a single fatty acyl chain, usually lauroyl or oleoyl or a mixture (e.g., coconut oil fatty acids).

The state of the N-acyl sarcosinates in the presence of water depends markedly on the ionization of the carboxyl group (pK near 4.5). At low pHs, where this group is fully protonated, N-acyl sarcosinates are water-insoluble and can form lipid bilayers. As the carboxyl group dissociates, N-acyl sarcosinates become water-soluble. Lipid vesicles made of N-acyl sarcosinates thus constitute simple carrier systems allowing pH-triggered delivery of active materials.

EXPERIMENTAL

N-lauroyl sarcosinate, N-cocoyl sarcosinate, and N-oleoyl sarcosinate (94% purity) were obtained from W. R. Grace (Lexington, MA) and R. T. Vanderbilt (Norwalk, CT), and cholesterol was obtained from RITA (Woodstock, IL). Lipid vesicles were formed essentially as in (17). Two ml of un-ionized sarcosinate or sarcosinate/cholesterol mixture, plus or minus oil cargo, were heated to 65–70°C and transferred to a 10-ml syringe. This syringe was connected via a stopcock to a second syringe containing 10 ml of deionized water at 60–65°C. The lipid mixture was immediately injected into the aqueous phase, and the resulting mixture was injected back into the first syringe. This process was repeated 20 times in 30 sec. The resulting cooled vesicle suspension was examined by dark-field and polarization microscopy, and sized using a Coulter NS particle counter. In separate experiments the above procedure was used to hydrate un-ionized sarcosinates at high proportions (0.4–0.75, v/v).

To measure vesicle water volume, 10 ml of the vesicle suspension was mixed with 10 ml of 20% dextran (M. Wt. 100,000–200,000; Sigma Chemical Co., St. Louis, MO) in deionized water. After centrifugation for 15 min at 3,500 rpm (Jouan bench top centrifuge), the vesicles had separated into a sharp layer floating atop a clear infranatant. The volumes of the layers were determined and the vesicle volume, in ml/g and ml/mMol sarcosinate, calculated, using average molecular weights of 270, 280, and 349 for N-lauroyl, N-cocoyl, and N-oleoyl sarcosinate, respectively. Vesicle diameters were obtained with a Coulter NS4DS submicron analyzer.

To measure vesicle oil uptake, increasing proportions of mineral oil (Drakeol 19, Penreco, Butler, PA) were dissolved in the lipid phase prior to hydration, microscopy, and centrifugation over dextran. The limit to oil uptake is marked by the appearance of microscopically discernible oil droplets and, after centrifugation, separation of a layer of free oil atop the vesicle layer. Oil uptake is calculated in ml/g and ml/mMol sarcosinate.

Freeze fracture of samples frozen in liquid propane was carried out using a Balzers freeze fracture apparatus (BAF 400) followed by shadowing with platinum/carbon. Replicas were examined with a JEOL electron microscope (JEM 100 SX).

RESULTS

At proportions of 0.5 and less, using deionized water as aqueous phase, all of the N-acyl sarcosinates gave paucilamellar vesicles exhibiting no membrane birefringence upon polarization microscopy. Important features of these vesicles are given in Table I.

At a N-lauroyl sarcosinate proportion of 0.6 or a N-cocoyl sarcosinate proportion of

Table I
Aqueous Volume Capture and Mean Vesicle Diameters of Three Types of Sarcosinate Vesicles

Sarcosinate	pH	Mean diameter (microns)	Aqueous volume (ml/g)	Aqueous volume (ml/mMol)
N-lauroyl	3.67	0.600	4.16	1.12
N-cocoyl	3.24	0.466	4.08	1.14
N-oleoyl	3.54	0.399	2.55	0.89

Table II
Aqueous Volume Capture and Mean Vesicle Diameters of Three Types of Sarcosinate/Cholesterol Vesicles

Sarcosinate	pH	Mean diameter (microns)	Aqueous volume (ml/g)	Aqueous volume (ml/mMol)
N-lauroyl	3.67	0.209	4.41	1.19
N-cocoyl	3.24	0.339	4.41	1.23
N-oleoyl	3.54	0.255	2.19	0.76

Table III
Oil Capture and Mean Vesicle Diameters of Three Types of Sarcosinate and Sarcosinate/Cholesterol Vesicles

Sarcosinate	Oil uptake (ml/g)	Oil uptake (ml/mMol)
N-lauroyl	12.5	3.38
N-lauroyl/cholesterol	16.2	4.37
N-cocoyl	7.1	2.00
N-cocoyl/cholesterol	10.3	2.88
N-oleoyl	7.1	2.49
N-oleoyl/cholesterol	13.2	4.62

0.75, the vesicles are multilamellar with typical birefringence patterns. At a ratio of 0.75 the N-lauroyl sarcosinate gives a gel phase that gives rise to multilamellar vesicles upon mixing with water.

Important features of sarcosinate/cholesterol vesicles (3/1; M/M) formed using deionized water as aqueous phase are given in Table II. Oil uptake by sarcosinate and sarcosinate/cholesterol vesicles (3/1; M/M), formed using deionized water as aqueous phase, are given in Table III.

Lipid vesicles made of N-acyl sarcosinates as sole amphiphiles (or sarcosinate/cholesterol combinations) are stable at neutral and acidic pH values. At pH 8 or higher, they disintegrate as the amphiphiles go into solution. The cholesterol of sarcosinate/cholesterol vesicles precipitates under these circumstances. Vesicles made of mixtures of N-acyl sarcosinates and nonionic amphiphiles are less pH-sensitive than vesicles made only of N-acyl sarcosinates. Figure 1 shows the freeze fracture appearance of N-cocoyl

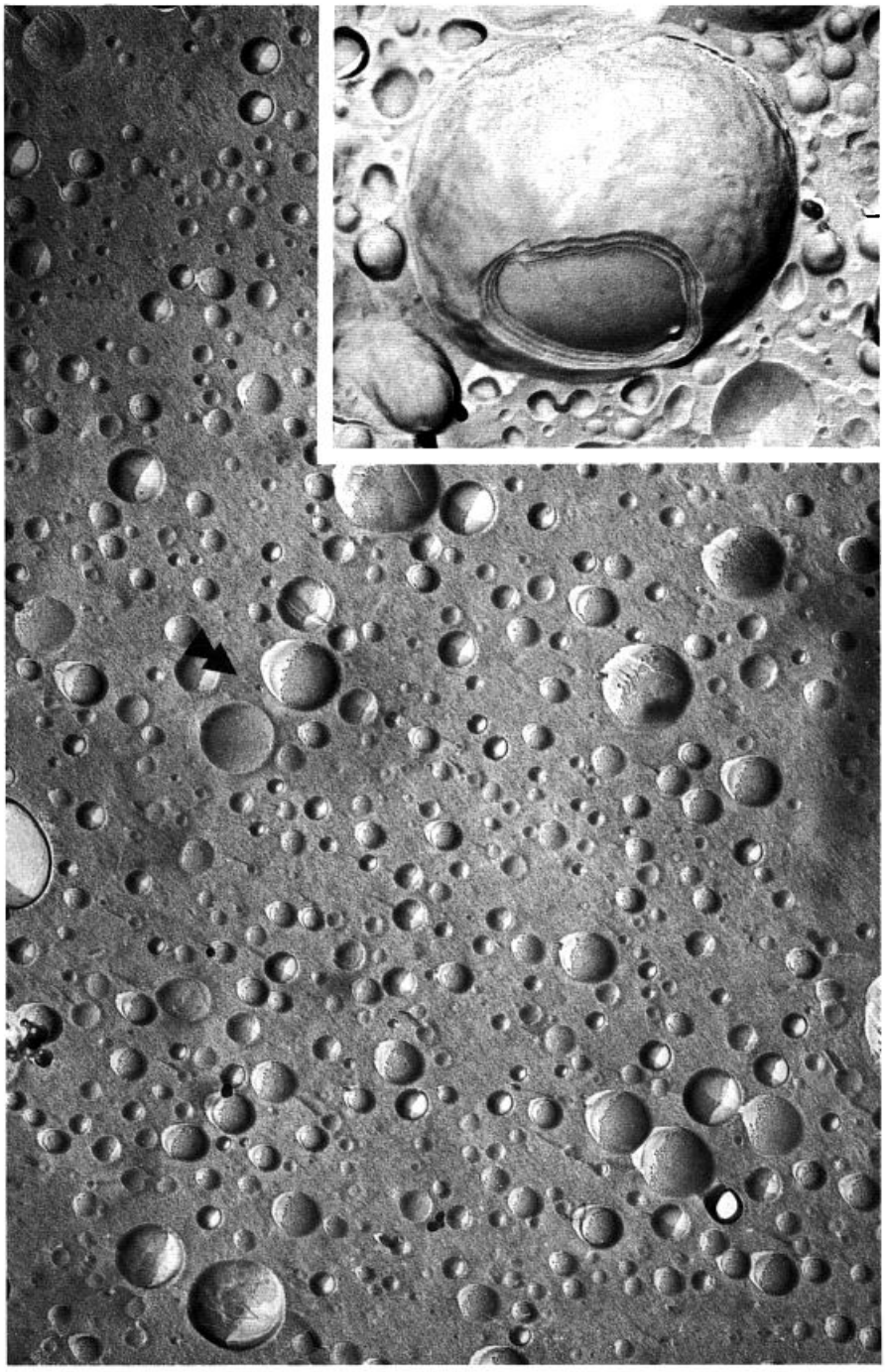

Figure 1. Freeze fracture electron micrograph of N-cocoyl sarcosinate vesicles laden with Dior fragrance 37 D. Most of the fields show 300–500-nm vesicles with two membranes. An occasional large multilamellar vesicle (inset) is observed, revealing greater structural detail. Magnification 45,000×.

sarcosinate vesicles laden with Dior fragrance 37 D (Parfums Christian Dior). Most of the fields show 300–500-nm vesicles with two membranes. Occasional large, multilamellar vesicles are also seen (Figure 1, insert).

DISCUSSION

N-acyl sarcosinates have a long and wide acceptance for skin and hair care. Because they are extremely mild and adsorb readily onto the skin at neutral or slightly acid pH, they are used as cleansers and moisturizers for oily skin.

Formulations containing N-acyl sarcosinates in vesicular form provide not only the desirable properties of the sarcosinates themselves, but also the benefits of delivering a variety of water-miscible and water-immiscible active ingredients in a programmable fashion. The high capacity for water-immiscible substances represents perhaps the major advantage in the cosmetic area, since it allows for the controlled delivery and sustained release of fragrant and emollient oils, conditioning silicone fluids, and active molecules such as the oil-soluble vitamins.

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