Reaction of either methyl *cis*-3-pentenylsulfide (**9**) or methyl *trans*-3-pentenylsulfide (**10**) with complex **3** yielded the trans 2,3,5-trisubstituted cyclopentenone **11** as the major product (Scheme III). We have shown that epimerization can take place after formation of the cyclopentenone.[17] Warming a toluene solution of a 4:1 mixture of **11**:**12** for 12 h in the presence of **3** gave rise to an 11:1 ratio of **11**:**12** in 95% yield. Reaction of either the *cis*- or *trans*-3-pentenyldimethylamine with cobalt complex **3** led to formation of the desired cyclopentenone; however, isomerization of the trisubstituted olefin to the tetrasubstituted isomer (entry 9 in Table I) was competitive.

In summary, the use of a directing ligand on the olefinic partner in the cobalt catalyzed olefin–acetylene cyclization provides a very high degree of regiocontrol in the intermolecular reaction. In addition, compared to previous intermolecular cycloadditions, the use of a directing ligand also contributes to a significant improvement in the overall yield. Applications to the synthesis of natural products will be reported in due course.

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(16) Dicobalt octacarbonyl obtained from Strem Chemical Company was of higher purity than that which was obtained from other sources. We thank Dr. Peter Wuts (Upjohn Co.) for this suggestion.

(17) We cannot, however, rule out the possibility of olefin isomerization prior to cycloaddition.

A New Method for the Instant Preparation of Large Unilamellar Vesicles

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In aqueous solutions phospholipid (PL) molecules form self closed spherical structures where one or several PL bilayers entrap part of the solvent in its/their interior.[1] In the case of multilamellar structures they are called liposomes or multilamellar vesicles (MLV), while terms such as small and large unilamellar vesicles (SUV and LUV, respectively) are used when a single bilayer separates internal and external solutions.[2]

SUV's and LUV's are very important in the studies of membranes, membrane proteins, and as delivery vehicles for drugs and genetic material into cells, and many different methods for their preparation exist.[3,4] However, most of them are time consuming and require relative demanding laboratory equipment. These methods involve the exposure of PL's and material to be encapsulated to physical stresses (sonication, high hydrostatic pressures) and/or a nonmild chemical environment (organic solvents, detergents, low/high pH) which may harm these sensitive substances. A simple, quick, and harmless method for vesicle preparation is still being searched for.

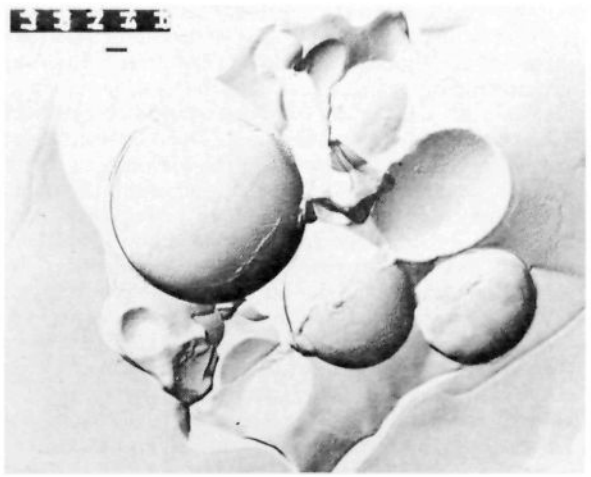

Figure 1. Freeze fracture EM micrograph of vesicle preparation. Vesicles were formed instantly by swelling PL film deposited on a silicon wafer in excess water. Bar indicates 100 nm.

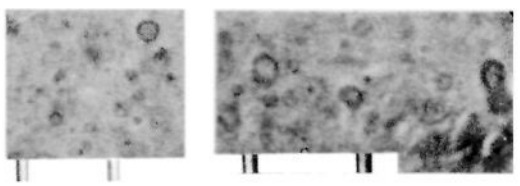

Figure 2. Phase contrast optical micrographs of instantly formed vesicles at two different magnifications. The distance between bars is 10 μm.

MLV's are formed spontaneously when dry PL films are hydrated and swollen in excess water by gentle shaking.[1] In contrast to the finite swelling behavior of uncharged films, charged PL films exhibit infinite swelling in excess water, and the spontaneous formation of heterogeneous populations of vesicles has already been reported.[5,6] By similar procedure, homogeneous preparations of SUV's have also been prepared.[7] However SUV's are not very suitable for the encapsulation because of their small internal volume.

In this communication we report on the "instant", spontaneous formation of rather homogeneous preparations of LUV's by an extremely simple technique. This procedure is completely analogous to the spontaneous formation of MLV's in that here LUV's are formed only by adding water to the dry PL film. The formation of multilamellar structures is prevented by inducing a surface charge on the bilayers while the size of the vesicles is controlled by the topography of the support surface on which PL film was deposited.

We deposited 0.5–1 mg of egg yolk lecithin (EYL) doped with 1.5–5 wt % of cationic detergent cetyltrimethylammonium bromide (CTAB) in 3 mL of $CHCl_3/CH_3OH$ on a specially etched 2-in. silicon wafer (silicon dioxide–silicon, heavy P^+ (boron) doped, vertical topography 0.5-μm steps, horizontal topography 6–10-μm random area).[8] This wafer was put in place of the original bottom

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(8) Wafer was prepared by standard procedures of microelectronic industry: cleaning of silicon wafer, oxidation in water vapor, dehydration bake, applying photoresist, prebake, covering with predesigned mask, exposure to UV light, development, rinsing, drying, final bake, etching in HF, stripping, and ion implantation. The wafer was cleaned in hot H_2SO_4/H_2O_2 mixture, dip etched in 10/1 HF, washed in deionized water, and dried. See, e.g.: Brodie, J.; Muray, J. J. *The Physics of Microfabrication*; Plenum Press: New York, 1982.

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of an Erlenmeyer flask. After having dried overnight at 10^{-2} Torr (~1 Pa), we resuspended the film by gentle shaking in 1-2 mL of water. Vesicles were formed instantly and were investigated by freeze fracture electron microscopy (EM), video enhanced phase contrast optical microscopy (PCM), gel chromatography, and ^{1}H NMR.

Figure 1 shows a freeze fracture EM micrograph of these vesicles while two PCM micrographs are shown in Figure 2. Most of the vesicles observed by freeze fracture EM (and fractured at their middle) have diameters between 0.5 and 1 μm. These data are supported by observations of completely unperturbed samples in the video enhanced phase contrast optical microscope where rather homogeneous populations of vesicles with diameters slightly below 1 μm were observed. The resolution of our system was ~0.4 μm. (Unfortunately, in reproducing the image on photographic film and paper much of the resolution was lost.) Occasionally vesicles were observed in clusters and a fracture through one of such clusters is shown in Figure 1. This indicates that in the fracture plane, only few vesicles were fractured through their center.

The contamination of vesicles with larger structures (MLV, giant vesicles, or other PL colloid particles) was ruled out by video enhanced PCM. This is an excellent technique for the observation of giant vesicles and liposomes.[9,10] Only a few larger structures, predominantly giant vesicles, occasionally with entrapped smaller vesicles, were observed in these samples.

To check the possible contamination of LUV's with SUV's we have used gel chromatography and ^{1}H NMR. In the gel chromatography experiment vesicles eluted in a symmetric peak in the void volume of Sephacryl S 1000 column (the recovery of PL was not measured) indicating that their minimal size is at least 30 nm.[11] Also, the absence of the ^{1}H NMR high resolution spectrum which is characteristic for SUV's (where the dipolar interaction and anisotropy of absorption lines due to the chemical shift tensor are averaged out due to fast vesicle tumbling and diffusion of PL molecules on the highly curved vesicle surface) indicates that the sample does not contain SUV's within the sensitivity of these two experiments. In addition, SUV's were not observed in the freeze fracture EM micrographs.

The losses of PL due to adsorption on the wafer were no larger than for the case where glass flasks are used as a support. The inconvenience of introducing detergent into the bilayer can be bypassed by doping EYL with ionic PL(s) instead of CTAB. The possible disadvantage of this vesicle preparation method is the relatively low concentration of vesicles obtainable (~1 mg/mL). However, they can be concentrated in a subsequent separate step. After its use, the wafer-bottomed Erlenmeyer flask was washed with $CHCl_3/CH_3OH$ (3:1), rinsed with distilled water, and dried. Several different preparations yielded, within the accuracy of our experimental methods, the same preparations of LUV's.[12]

This method of vesicle preparation was based on the theoretical model of vesicle formation which defines a bilayered PL flake (BPF) as an intermediate structure in the vesicle formation process.[13] Therefore the results of this study, which are in qualitative agreement with this model, also shed some light on the experimentally unproven model of vesicle formation.

We believe that vesicles are formed by the bending and sealing of BPF's which are thermodynamically unstable due to the exposure of hydrocarbon chains to water at their edges. In our experiment the size of BPF's is determined by the topography of the support surface. On swelling they peel off into solution where they are unstable. Each BPF continuously reduces the circumference of its exposed boundaries, i.e., the unfavorable contact interaction at its edges, by bending and finally eliminates it by closing upon itself.

The advantages of this method are its extreme simplicity, rapidity, and avoidance of all potentially harmful treatments. In addition, the LUV's which are formed are larger than vesicles prepared by most other techniques. This and their quick and harmless preparation make them extremely suitable for the encapsulation of drugs and genetic material.

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The Quinuclidine Dimer Cation Radical

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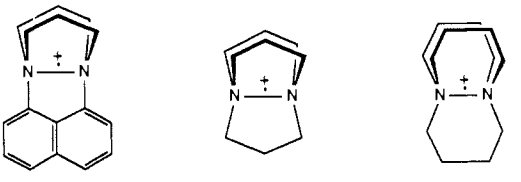
Homonuclear dimer cation radicals containing a three-electron σ bond between two group 15 elements have long been known for phosphines[1] and arsines.[1b,2] Curiously, amines do *not* form such dimer cation radicals. The first apparent exceptions to this generalization are the intramolecular "dimer" cation radicals **1-3**



1 **2** **3**

recently prepared by Alder.[3] Unfortunately, because of the uncertain constraints imposed by their carbocyclic frameworks, these examples shed little understanding on the more common failure of amines to form *inter*molecular dimer cation radicals.[4] We describe herein the preparation and characterization of the first intermolecular amine dimer cation radical and propose a simple rationale to account for its formation.

The general inability of amines to form dimer cation radicals might have two possible origins, termed here, orbital extension and structural reorganization. The first hypothesis recognizes that the nonbonding orbital on nitrogen is considerably contracted vis-a-vis those of the lower elements in group 15. Since the three-electron σ bonds in dimer cation radicals are considerably longer than the corresponding two-electron bonds,[3f,5] it seems plausible that the amine dimer cation radicals would suffer the poorest orbital interpenetration in the series.

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