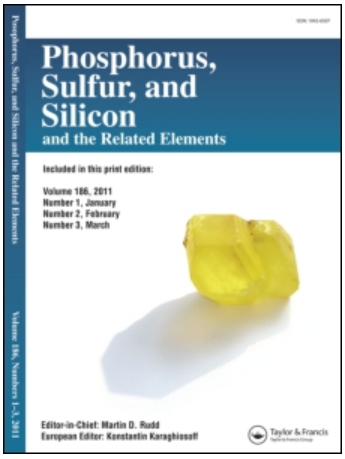

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ENHANCED PROCLIVITY TO SELF-AGGREGATION OF PHOSPHORUS-BASED AMPHIPHILES WHEN PERFLUOROALKYLATED : THE (PERFLUOROALKYL)ALKYL DIMORPHOLINOPHOSPHATES

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Abstract Dimorpholinophosphates **1**, with a single perfluoroalkyl chain, self-aggregate when dispersed in water to give tubular structures which reversibly transform into giant vesicles when heated. Compounds **1** also allow the preparation of stable reverse water-in-fluorocarbon emulsions destined to pulmonary drug delivery. None of this could be achieved with non-fluorinated analogs.

Key words : Fluorinated phosphorylated amphiphile, fluorinated surfactant, liposome, tubules, water-in-fluorocarbon emulsion, reverse emulsion, drug delivery, fluorocarbon.

INTRODUCTION

A variety of well-defined phosphorus-based amphiphiles fitted with one or two perfluoroalkylated chains have recently been synthesized in our laboratory, and their self-aggregation and emulsion-stabilizing properties were investigated. These amphiphiles include single-chain phosphocholine and double-chain phosphatidylcholine derivatives [1], single- and double-chain glycophospholipids [2] and mono- and dimorpholinophosphates [1a,3]. Earlier work on the colloidal behavior of fluorinated phosphorus-based amphiphiles includes that of Kunitake [4], Ringsdorf et al. [5] and Hui et al. [6].

The impact of the fluorinated chain(s) on the physicochemical and biological properties of amphiphiles has been analyzed in relation to their potential as components of drug carrier and delivery systems [7]. The intense hydrophobic effect developed by these chains results in the formation, not only of vesicles, but also of tubules and other fibers, even from single-chain amphiphiles [8]. When fluorinated vesicles form, they usually withstand heat sterilization [8,9]. Fluorinated liposomes were also obtained from combinations of standard phospholipids with (perfluoroalkyl)alkanes [10]. Perfluoroalkylated phosphocholines were shown to form long flexible fibers without recourse to hydrogen bonding, chiral effect or polymerization [11]. Bilayers made from

fluorinated phospholipids are characterized by the presence of a fluorinated film within the bilayer membrane, which results in enhanced stability and lower permeability to both hydrophilic and lipophilic drugs and other materials [7].

Perfluoroalkylated dimorpholinophosphates $C_nF_{2n+1}(CH_2)_mOP(O)[N(CH_2CH_2)_2O]_2$ **1**, were readily obtained in 65-80 % yields in two steps [3]. They constitute poorly hydrophilic, highly fluorophilic surfactants. Preliminary acute toxicity tests indicate LD_{50} values superior to 1g per kg body weight intravenously in mice for **1** (n = 8, m = 11); no hemolytic activity was found (n = 6 or 8, m = 2), even at a concentration of 5 g/L; and an inverse emulsion (see below) based on 1% of **1** (n = 8, m = 11), when injected intraperitonealy at a dose of 25 mL/kg in mice, was well tolerated. Actually, these truly miraculous dimorpholinophosphates were developed by pure chance and malice, to keep an unruly, though amiable, she-student busy.

TUBULAR SUPRAMOLECULAR AGGREGATES

When the dimorpholinophosphate with a hydrocarbon chain, $C_mH_{2m+1}OP(O)[N(CH_2CH_2)O]_2$, with m = 10, was dispersed in water (up to 20% w/v), no aggregates larger than micelles were observed to form, in line with the usual behavior of single chain amphiphiles. Dispersions of the longer analog (m = 15) underwent rapid phase separation. On the contrary, when fluorinated compounds **1** (n = 8 or 10, m = 2 or 5) were dispersed in water at a 1 to 6% w/v concentration, tubular assemblies were readily obtained. Within hours the tubules of **1** (n = 8, m = 2) typically reached 10 to 50 μm in length and 0.1 to 0.5 μm in diameter. For certain compounds, for example **1** (n = 8, m = 5), the tubules can reach several hundreds of nm (fig. 1a). These tubules appear to consist of rolled up bilayers (fig. 1b). Upon heating, one observes their reversible conversion into giant vesicles. The transformation temperature increases with the length of the fluorinated chain (40°C for n = 8, m = 2; 65°C for n = 10, m = 2). Optical polarization microscopy of the giant vesicles showed maltese crosses revealing a lamellar arrangement of the surfactant. These characteristic defects were no longer present in the tubules, indicating a more ordered, more crystalline structure. The formation of such highly organized structures from short single-chain amphiphiles, without the help of a chiral center, rigid rod(s) or hydrogen bonds usually required [12,13], while no such constructs were obtained from non-fluorinated analogs, illustrates the powerful driving force towards self-aggregation exerted by the strongly hydrophobic and bulkier perfluoroalkyl chains. Tubular self-assemblies were also obtained in non aqueous organic solvents such as dimethylformamide [14].

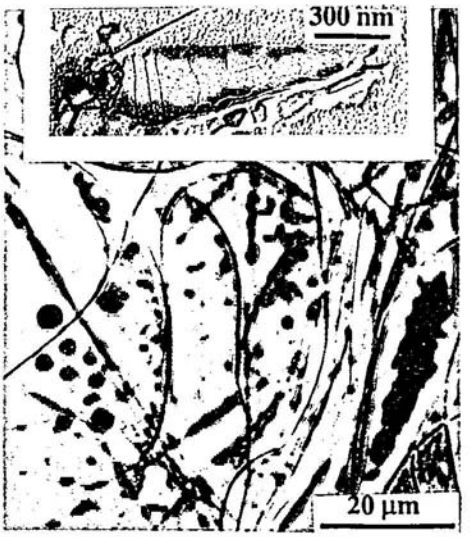

FIGURE 1: Optical and freeze fracture electron micrographs of a 6% aqueous dispersion of 1 (n = 8, m = 5) showing tubular structures .

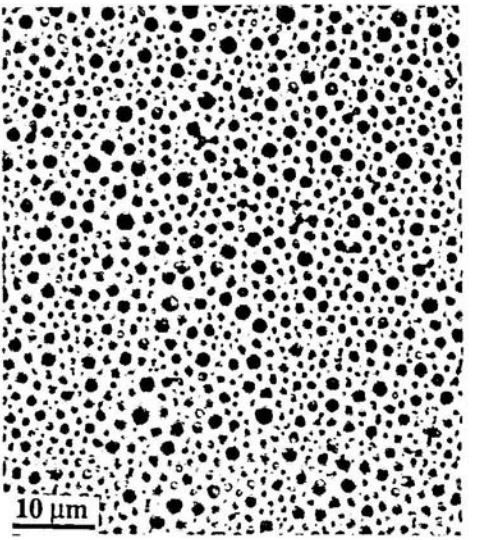

FIGURE 2: Optical micrograph (phase contrast) of a reverse water-in-perfluoroctyl bromide emulsion stabilized by 1 (n = 8, m = 11), obtained by simple hand-shaking.

STABLE WATER-IN-FLUOROCARBON REVERSE EMULSIONS

The dimorpholinophosphates 1 were also found to strongly stabilize reverse water-in-fluorocarbon emulsions [15]. Such emulsions show promise for pulmonary administration of drugs. They can be loaded with a variety of drugs, contrast agents, surfactants, immunoactive agents, genetic and other materials. Reverse emulsions also give access to multiple emulsions, including hydrocarbon-in-water-in-fluorocarbon emulsions and water-in-fluorocarbon-in-water emulsions. When an aqueous inner space is present, it can also be filled with vesicles, capsules, micelles, polymers, etc. Such systems could provide protection and controlled release for biologically active materials, as well as depot and other effects.

A series of water-in-fluorocarbon emulsions were obtained with a water content ranging from *ca* 1 to 30% v/v in a variety of linear and cyclic fluorocarbons. A typical emulsion with 5% v/v of water and 2% w/v of 1 (n = 8, m = 11) in perfluorooctyl bromide had an average particle size of *ca* 500 nm for 1 month at room temperature (fig. 2). This emulsion was stable enough to withstand heat sterilization (121°C, 15 min, 15 N m^{-2}). The reverse water-in-oil situation was verified by phase-contrast optical

microscopy after including a water soluble dye, and by the fact that these emulsions can easily be diluted in a fluorocarbon, but not in water. Smaller average particle sizes and further stabilization were achieved by addition of electrolytes such as NaCl, KI or $CaCl_2$. No stable emulsion could be obtained with dimorpholinophosphates that did not bear a fluorocarbon chain, while the fluorinated compounds **1** readily produced a stable film at the water/fluorocarbon interface.

Pulmonary drugs that were incorporated in such emulsions include acetyl cysteine (a mucolytic agent), pyrazinamide (a tuberculostatic agent), epinephrine (a bronchodilator) and prednisone.

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