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Formation of Drug-Bearing Vesicles in Mixed Colloids of Bile Salts and Phosphatidylcholine

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Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). We used small-angle neutron scattering to study drug interactions with mixed colloids of bile salt and phosphatidylcholine. Because the mixed colloids form liposomes spontaneously, this system is a model for drug-bile interactions that are important in understanding the efficacy of oral drug formulations and in advanced applications for liposome drug delivery systems. We studied particle formation on incorporation of enzymatic products formed in the gut and the effects of cholesteric drugs and taxol on vesicle formation. Our studies show that particle morphology is not affected by inclusion of most cholesteric drugs and taxol, and is not affected by incorporation of the products of enzymatic action. Our findings suggest that particle form is important for the physiological function of bile and we are beginning to show which drugs affect liposome formation.

Background and Research Objectives

Mixed aqueous colloids of bile salts and swelling lipids (phosphatidylcholine or monoolein and oleate) have important and broad applications in pharmaceuticals, pharmacology, medicine and physiology, largely because these systems are models of bile. Bile is excreted by the liver into the bile duct, where bile acts as a carrier of lipophilic materials, such as cholesterol, then into the intestine where bile acts to emulsify dietary fats. Studies of the mixed colloids are the bases of understanding the physiology of these important processes. Our emphasis is in relating this physiological information to pharmaceuticals and medicine by considering the influence of drugs on particle self assembly and structure.

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The medical and pharmacological applications involve drug interactions with bile; thus bile models are of prime importance to the drug industry in drug delivery, drug uptake in the intestine, the development of therapies for gall stones and certain other gastroenterological disorders. The colloid science issues that need to be addressed in studying these problems are well matched to the capabilities of small-angle neutron scattering (SANS). Our work on the isotropic phase of aqueous mixed colloids of glycocholate and phosphatidylcholine [1-7] has shown that SANS is an appropriate and powerful tool for investigations of particle morphology in these types of systems.

Drug delivery by aqueous solutions of amphiphiles, liposomes, has proven successful in *intravenous* (IV) delivery in transporting drugs of limited solubility, in reducing toxicity, and in protecting the drug from biodegradation. These systems have additional potential in targeting cell types, bypassing healthy organs and systems and targeting those where the drug is needed. Liposomes are spherical objects made up of lipid bilayer walls of phosphatidylcholine. The drug is bound to the hydrophobic parts of the PC in the walls of the vesicle. Standard methods of liposome production by sonication produce metastable vesicles. Further the particles are large, making them susceptible to uptake by the immune system.

The bile salt-phosphatidylcholine systems show promise in dealing with some of these limitations to liposome therapy. These systems form liposomes spontaneously [1-7], even in blood [8]. This addresses the issue of mass production and stability, as the phospholipid-bile salt systems are easily reconstituted from dried mixtures by the addition of a physiological aqueous solvent. Our ultimate goal is to understand how to design formulations in an easily maintainable form such as solid mixtures, which the health professional could readily and accurately reconstitute, making the preparation of the formulation easy and well within the capabilities of clinicians. The potential benefits are increased availability and reduced cost of the technology. The small size and very monodispersed nature of the liposomes produced by this approach limit the uptake by the immune system and make this self assembling mixed system a natural candidate for insoluble anti tumor agents such as amacrine, bruceantin, melphalan, taxol, tenipside, echinomycin and didenmin B, to name just a few. Thus the approach is a complement to conventional methodology that produces very large, polydispersed liposomes for which the immune system is a natural target.

To use this system in drug delivery the conditions under which the vesicle are stable must be understood. Thus we must show that the liposomes can be produced under conditions that would actually be used in the clinical setting. Thus one objective of this

study is to understand the effect of drug loading on spontaneous mixed micelle and vesicle formation. Can we devise statements about the effects of certain classes of drugs that can be used by pharmaceutical formulators? In effect, we need to know the rules that formulators can use in engineering systems that are likely to be useful in this application. We hope to relate our results to recent theoretical work on the spontaneous formation of vesicles [9,10] and mixed micelles.

The effectiveness of oral formulations may well be the result of bile-drug interactions and the effect on intestinal uptake. This is an important issue to the pharmaceutical industry, as some orally administered drugs have poor bioavailability due to limited intestinal uptake. There are two goals: 1) to understand the effects of specific drugs on intestinal bile self-assembly and to correlate this with pharmaceutical studies; and 2) to study the effects of phospholipid-bile salt micelles as uptake enhancers. Since dietary triglycerides are cleaved by pancreatic lipase in the gut, yielding fatty acids and 2mono-glycerides [11], the effect of this on particle stability and the subsequent transport of drugs must also be investigated.

The other aspects of this system that are impacted indirectly by this work are also important. Every year tens of thousands of people undergo surgery for precipitation of bile in the gall bladder—gall stones. Considerable benefit would be derived from therapies that could dissolve the stones *in situ*, and some effort using the bile salt, ursodeoxycholate, has been expended by drug companies towards this end. A highly important factor in the development of drugs is the development of appropriate pharmaceutical models for the system, and this study provides the information needed to do this.

Importance to LANL's Science and Technology Base and National R&D Needs

This work directly relates to the bioscience and biotechnology thrust areas of biomedical research and technology, biomedical structure and functional analysis and in the use of neutron scattering for biomedical-biotechnological research. Our work done at Los Alamos (and using facilities of other national centers, as well), has shown that SANS techniques are appropriate, and essential to understanding these problems. The problem is one of understanding self-assembling systems; in particular in understanding the formation of vesicles. Thus this work provides a direct linkage between a biotechnological issue and basic colloid physics, physical chemistry and the physics of complex fluids. The issues

and length scales demanded by this problem are most appropriately probed by neutron scattering, as we have demonstrated in our published accounts. [1-7]

This work complements the Laboratory's growing effort in structural biology and biophysics. The developments in small-angle neutron scattering methodology in structural biology that are being carried out under this project will benefit the efforts to determine structural information using neutron scattering in other Laboratory programs.

Scientific Approach and Accomplishments

SANS probes the most appropriate length scales for this system, *ca* 10-1000 Å. It also provides much better contrast than with x-rays in these systems, particularly when D₂O is used as the solvent, as has been adequately demonstrated in our other work [1-4]. This gives considerable latitude in studying the concentration-dependence of the system that is not available to x-rays, an important consideration, as the particles structures are highly dependent on concentration. Finally, deuteration can be used to label specific components of the system to determine their distribution with SANS [4].

Early work demonstrated the existence of isotropic phases in the water rich parts of the bile salt-phosphatidylcholine-water phase map. Subsequently we used SANS to study particle structure in these phases [1-4]. The particle forms were studied as a function of type of bile salt, total lipid concentration and phosphatidylcholine to bile salt molar ratio. The picture of isotropic phase particle structure that has emerged from the SANS work may be summarized as follows: Globular mixed micelles are formed at the highest total lipid concentrations in the isotropic parts of the phase map. The micelles elongate when the solutions are diluted and form long rods with sufficient dilution. We have shown [4] that the phosphatidylcholine is arranged in the rods with the long molecular axis radial to the rod axis. The bile salts have the long molecular axes parallel to the rod axis. If the sample is diluted further there is a transition to vesicles with a region where both rod-like mixed micelles and vesicles coexist. The vesicles persist but become smaller as the system is diluted further past the micelle to vesicle transition.

We have completed work on understanding how enzymatic degradation of dietary triglycerides, which results in a combination of monoolein, oleate and oleic acid, affects the self-assembly and morphology in bile salt/phosphatidylcholine particles in the isotropic phases. We studied the particle morphology in mixtures of cholesterylglycine or

chenodeoxycholyglycine with monoolein, oleic acid, or deuterated monoolein and oleic acid.

We have found that the particle morphology in the isotropic part of the bile salt-swelling lipid-water phase map is qualitatively the same regardless of the type of swelling lipid. Further, the molecular arrangement in the rods is the same. These results are important, as they indicate that the particle morphology is conserved in the presence of lipolytic products, suggesting that there is a functional form of bile particle morphology that is retained. These observations suggest further that the bile salt is the most important determinant of the functional form of the bile particles, as monoglyceride has a single tail and is non-ionic, oleate also has a single tail, but is negatively charged, as opposed to the two-tailed, zwitterionic phosphatidylcholine.

We have also examined the effects on particle morphology of the drugs, taxol, cholesterol, and the cholesteric drugs, progesterone, hydrocortisone and deoxycorticosterone. The effects of taxol and cholesterol were studied with the bile salt cholyglycine. The effects of the cholesteric drugs were studied with cholytaurine and the related deoxy-form, chenodeoxycholytaurine.

The compound taxol [12], derived from the stem bark of the western yew, *Taxus brevifolia*, has potent antileukemic and tumor inhibitory properties, but its extreme aqueous insolubility has limited its therapeutic use. Thus, we are interested in the use of the mixed colloid system to solubilize the drug. We wanted to determine the effects of taxol on the structural transitions which occur with dilution using taxol concentrations near those envisioned for therapeutic use. The behavior of the system with dilution is particularly important, as the envisioned formulation would involve reconstitution of taxol in a concentrated mixture of lecithin and bile salt. This mixture would then be diluted to form liposomes for *intravenous* injection in a manner similar to that done here. We found that the presence of small amounts of taxol may affect the structure and association of rod-like mixed micelles, but that it did not affect the formation of vesicles. However, more measurements will be needed in the vesicle transition and vesicle regions of the dilution series to confirm this.

Cholesterol and the cholesteric drugs have the common feature of a cholesterol-like core, and thus, have analogous structures to the bile salts. They differ, however in their polarity. In shape these molecules are wedge-like. We found that hydrocortisone and progesterone had no effect on the structure of bile salt-phosphatidylcholine particles. On the other hand the addition of a relatively small amount of deoxycorticosterone, the least polar of these compounds, completely disrupts the micelle structure.

Publications

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