



November 18, 2002

Dockets Management Branch (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

**Re: Docket No. 02D-0337  
Draft Guidance for Industry on Liposome Drug Products**

ALZA Corporation appreciates the opportunity to provide the following comments to the Food and Drug Administration ("FDA") regarding the Draft Guidance for Industry on Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation.

The comments follow the format of the draft guidance, and are correlated with specific line numbers. Items in quotations are taken directly from the draft guidance. This response is also being submitted electronically to the e-mail address provided in the draft guidance document.

**I. INTRODUCTION**

***Line 58:***

The draft guidance does not provide recommendations on bioequivalence studies or those to document sameness. We recommend that a guidance on these issues be developed.

***Line 59:***

The draft guidance does not apply to liposomal formulations of biologics. We recommend that a guidance on such products be developed.

**II. CHEMISTRY, MANUFACTURING, & CONTROLS**

***Lines 82-102: Physicochemical Properties***

The draft guidance suggests many physicochemical properties of liposomes for evaluation as part of drug product quality assessment. We believe that the majority of these properties are not relevant to liposomes, or are unreliable due to technical or other limitations. Our position on several of these properties is described below.

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### *Liposome lamellarity*

Electron-microscopic techniques have been used to characterize the lamellarity of liposome preparations. Results from these methods are qualitative in nature and could lead to misleading conclusions due to the limited number of observation fields that can be studied. These methods are also prone to potential artifacts introduced by the sample preparation procedure used. Liposome lamellarity has also been characterized qualitatively by spectroscopic measurements that rely on a bilayer impenetrable marker that alters the signal of the outer monolayer relative to all internal bilayer signals. However, many assumptions must be made regarding the internal morphology of the liposome to convert the spectroscopic measurement to a lamellarity value. Currently, there are no suitable quantitative measures of lamellarity. Due to these reasons, we believe that lamellarity is not a reliable parameter to use in the assessment of liposomal product quality.

### *Net charge*

Measurement of net charge cannot be directly assessed. Zeta potential, for example, may be measured, but a complex theoretical framework must be utilized to interpret the relationship between zeta potential and surface charge density. The value derived from a zeta potential measurement may vary significantly with measurement conditions, specifically the nature of the matrix in which the liposome is suspended. Since these measurements are best made at extremely low ionic strength, the measurement itself may affect properties of the liposomal drug product. These considerations often lead to challenges in obtaining precise and reproducible results, especially when liposomes with weak surface charges are involved. Furthermore, studies have shown that liposomes are rapidly coated with plasma components after *in vivo* administration and most liposomes, irrespective of their original charge, become anionic due to these interactions.<sup>1</sup> Therefore, we believe the utility of this property in the assessment of liposome quality is limited.

### *Volume of entrapment*

Measurements of entrapped volume currently rely on assessing the fraction of volume external to the liposome relative to the internal fraction ( $V_{out}/V_{in}$ ) by exclusion of a marker from the internal space of the liposome, or on methods that involve entrapping a radiolabeled marker during liposome formation. It is possible to use marker entrapment methods as characterization tools in research studies, but they cannot be used to assess the entrapment volume of the drug product lots manufactured for GLP, GMP and commercial use, as typical procedures require the use of an extrinsic marker. In addition, measurements of  $V_{out}/V_{in}$  do not measure the volume fraction of the aqueous space contained inside of the liposome, except in the specific case of unilamellar liposomes. When these measurements are performed

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<sup>1</sup> Senior JH: Fate and Behavior of Liposomes in vivo: A Review of Contributing Factors. Critical Reviews in Therapeutic Drug Carrier Systems 3:123-93, 1987.

on multilamellar liposomes or oligo-lamellar liposomes of relatively small sizes, the fraction of aqueous space occupied by additional internal lipid bilayers cannot be assigned unambiguously. Thus, entrapped volume measurements do not add any useful information beyond a prediction of entrapped volume that may be made based on liposome size distribution or one estimated from the percent encapsulation measurement of a drug substance that is predominately encapsulated in the intraliposomal aqueous space. For these reasons, we do not believe this parameter adds value to the quality assessment of liposomal products.

*Particle size (mean and distribution profile)*

Mean particle diameter may be measured by QELS for particles less than 1 micrometer average diameter or by laser diffraction for larger liposomes. Both methods can yield particle distribution profile information; however, they rely on extensive mathematical manipulation of the primary data, and do not always yield reproducible results. Although mean particle diameter can be measured on a routine basis for quality control, there are currently no suitable quality control methods that provide a measure of particle size distribution. Some research methods such as field flow fractionation and capillary hydrodynamic fractionation are currently under evaluation and may offer a suitable means of measuring particle size distribution useful for the characterization of liposome preparations. However, at this time no quality control method has been established that reliably and reproducibly measures particle size distribution.

*Phase transition temperature*

Although phase transition temperature can be reproducibly measured by differential scanning calorimetry (DSC) for single lipid components, phase transition temperature data for complex lipid mixtures are complicated and often difficult to interpret. As an example, there is no discernable transition temperature for complex lipid mixtures in liposome bilayers, such as ALZA's cholesterol-containing, mononuclear phagocyte system (MPS)-evading liposomes. For some lipid mixtures in liposome bilayers, the DSC profile may vary depending on the thermal exposure history of the preparation. The situation could be further complicated when a significant fraction of the drug substance actively interacts with the lipid bilayer. Therefore, this parameter may be of limited value for many liposomal products.

*Spectroscopic data*

Spectroscopic data on intact liposomes, as applicable, requires further definition, in part due to the multitude of spectroscopic techniques that are available. We request the agency clarify their position on this parameter.

*In vitro release*

Measuring *in vitro* release rate of drug substance from liposomes can provide useful information on lot-to-lot consistency of liposomal products. In some cases, designing a discriminating and reproducible method for this measurement can be

very challenging. For example, MPS-evading liposomes are designed to remain stable in circulation and they do not readily release contents, even in the presence of serum or simulated physiological medium at elevated temperatures. Exposure of these liposomes to adverse conditions, such as treatment with significant concentrations of surfactants or organic solvents, often leads to release rates that are too rapid to provide useful information. In addition, because of the complexity of the *in vivo* mechanism of action of liposomal products, it is extremely difficult to provide any correlation between an *in vitro* drug release method and *in vivo* performance of the liposomes.

#### *Osmotic properties*

Liposomes can exhibit a change in particle diameter or release their contents when exposed to media of different osmotic strengths. We believe that osmotic property studies of liposomal products should focus on the evaluation of their particle diameter and content release characteristics in relevant osmotic environments (e.g., infusion bag fluids and plasma).

#### *Light Scattering Index*

Light Scattering Index is currently characterized by Rayleigh scattering, using spectrophotometry. This measurement would indicate changes in sample turbidity, and is useful as a diagnostic measurement of how a formulation may change over time during a stability study. While it is difficult to correctly interpret a turbidity measurement, significant change in this value signifies agglomeration/aggregation/crystallization occurring in a liposomal product during long-term storage. Because of the relative ease to develop a reliable and reproducible method for turbidity measurement, it could be included in routine characterization of stability samples. It can also be useful in the assessment of lot-to-lot consistency of a liposomal product with respect to particle size when the optical density value is normalized to the concentration of liposomes in the drug product.

#### ***Lines 121-122, Control of Excipients: Lipid Components***

“...Information concerning the CMC of the lipid components should be provided at the same level of detail expected for a drug substance...”

#### ***Lines 169-170: Lipid Specifications***

“...The level [of impurities] that would warrant identification and qualification will be determined on a case-by-case basis.”

Taken together, these sections are ambiguous and contradictory. If the lipid components are to be treated as drug substance, then ICH guidance on impurities in active pharmaceutical ingredients (APIs) would apply<sup>2</sup>. However, later in the

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<sup>2</sup> Guidance for Industry: *ICH Q3A, Impurities in New Drug Substances*, January 1996.

passage, it states that lipid impurity levels that warrant identification would be determined on a case-by-case basis, suggesting that the ICH guidance would not be applied to lipids. In addition, it is not clear whether other API requirements might be applied to lipids. Unless there is a specific characteristic of lipids that should be evaluated to the same level of detail as for an API, then the lipid should not be treated differently than other excipients in regards to the level of detail for information provided. Furthermore, we believe that the relative biological inertness of typical lipids used in the manufacture of liposomal products does not support viewing them in the same light as active drug substances.

***Lines 141-145: Manufacture of Lipids***

This section indicates that the source of any starting materials should be identified. This is more stringent than the requirements in the referenced guideline for drug substances, *Submitting Supporting Documentation for the Manufacture of Drug Substances*, which does not mention sources of starting materials. It also seems more stringent than BACPAC I<sup>3</sup>, which indicates that the FDA need not be informed of changes to the supplier of a starting material for an API intermediate. It is inappropriate to have a more stringent requirement for a lipid excipient than for an API.

***Lines 147-155: Manufacture of Lipids***

This section indicates that the specifications should be provided for raw materials, solvents and reagents used in the manufacture of lipids. This is more stringent than the requirements in the referenced guideline for drug substances, which permit a statement of quality in place of a specification. It is inappropriate to have a more stringent requirement for a lipid excipient than for an API.

***Lines 193-197: Stability of Lipids***

Stability testing is performed on lipids. However, the potential degradants in the lipids are not as critical as the potential degradation products of the API, and therefore the stress testing requirements for the lipids should be in-line with other excipients rather than with the API.

***Line 222: Stability of Liposome Drug Products***

Stability testing on unloaded liposomes may not be predictive of stability studies performed on the drug product, since incorporation of the drug substance is likely to alter the properties of the unloaded liposomes. Therefore, its usefulness as a routine quality assessment is limited.

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<sup>3</sup> FDA Guidance for Industry: *BACPAC I: Intermediates in Drug Substance Synthesis Bulk Actives Postapproval Changes: Chemistry, Manufacturing, and Controls Documentation*, February 2001.

**Line 230: Stability of Liposome Drug Products**

It is not generally accepted that small unilamellar liposomes are more susceptible to size changes than multilamellar liposomes. While it is true that kinetically trapped, highly strained small unilamellar liposomes such as those formed by prolonged ultrasonication do undergo liposome-liposome fusion with concomitant size growth, this is a phenomenon observed for liposomes below 50 nm diameter. These smaller sonicated liposomes have limited utility as drug carriers since their internal volume, hence, drug carrying capacity, is limited. Multilamellar liposomes, on the other hand, may be prone to breakage and subsequent changes in size distribution. However, since size and drug retention are usually primary physicochemical measures of liposome integrity that are performed routinely, there are no issues in detecting changes in these parameters.

**III. HUMAN PHARMACOKINETICS AND BIOAVAILABILITY****Lines 313-314: In Vivo Integrity of Liposome**

“The liposome is considered stable *in vivo* if, over the time course of the single-dose study, the:

- Drug substance, when in circulation, remains substantially in the encapsulated form
- Ratio of unencapsulated to encapsulated drug substance remains constant”

Clarification is needed as to whether BOTH bullet points are required simultaneously in order to satisfy *in vivo* stability, or only ONE OR THE OTHER is sufficient. Three different scenarios are presented below:

- 1) The liposome is considered stable *in vivo* if, over the time course of the single-dose study, the drug substance, when in circulation, remains substantially in the encapsulated form, however, the ratio of unencapsulated to encapsulated drug substance may or may not remain constant. Therefore, liposomal drug with the following characteristics is considered stable *in vivo*:

	Time Since Drug Administration (h)			
	0	2	10	24
% Encapsulated	80	75	70	65
% Unencapsulated	20	25	30	35
Ratio of unencapsulated / encapsulated drug	0.25	0.33	0.43	0.54

- 2) The liposome is considered stable *in vivo* if, over the time course of the single-dose study when in circulation, the ratio of unencapsulated to encapsulated drug substance remains constant, however, the drug substance may or may not substantially remain in the encapsulated form. Therefore, liposomal drug with the following characteristics is considered stable *in vivo*:

	Time Since Drug Administration (h)			
	0	2	10	24
% Encapsulated	35	35	35	35
% Unencapsulated	65	65	65	65
Ratio of unencapsulated / encapsulated drug	1.86	1.86	1.86	1.86

- 3) The liposome is considered stable *in vivo* if, over the time course of the single-dose study, the drug substance, when in circulation, remains substantially in the encapsulated form AND ratio of unencapsulated to encapsulated drug substance remains constant. Therefore, liposomal drug with the following characteristics is considered stable *in vivo*:

	Time Since Drug Administration (h)			
	0	2	10	24
% Encapsulated	80	80	80	80
% Unencapsulated	20	20	20	20
Ratio of unencapsulated / encapsulated drug	0.25	0.25	0.25	0.25

The above three scenarios all describe stable liposomes, but only #3 fulfills both criteria stated in the draft guidance. Based on the other two examples given, it should be acceptable for a liposome to meet one or the other, but not necessarily both, of the criteria in order to be considered stable *in vivo*.

**Lines 327-335: Protein Binding**

“...The protein (including lipoprotein) binding of the drug substance and liposome drug product should be determined over the expected therapeutic concentration range. The major binding proteins should be identified.”

It is possible that liposome stability can be adversely affected by interaction with proteins in blood. It is also possible that drug release can be triggered by such interactions. The possibility of protein-induced drug release from a liposome-drug product can be addressed directly by measuring drug release in medium designed to mimic *in vivo* conditions, using a validated analytical method. Pharmacokinetic measurements of released vs encapsulated drug over time following administration to animals or human subjects would also provide insight into any drug-release issues that might impact safety.

We do not believe identifying and/or measuring protein binding to liposomes will add value to the understanding of the *in vivo* behavior of liposomal products. Indeed, given the large number of plasma proteins that have a tendency to bind to

liposomes, analytical identification and/or quantification of them all would be virtually impossible. Although an early report<sup>4</sup> suggests a relationship between liposome surface charge, total protein binding and stability, no consensus has emerged in the scientific literature over the past 15 years on the identity of proteins that may influence stability. Moreover, the affinity (or functional avidity) of plasma proteins for liposome surfaces will likely vary widely, rendering analytical separations problematic.<sup>5,6,7,8,9</sup>

**Lines 339-340: *In Vitro* Stability**

"A validated *in vitro* test method should be established that uses an appropriate simulated physiological medium and/or human plasma..."

Human plasma is not a suitable reagent for a quality control method. In our experience, unacceptable lot-to-lot variations in plasma samples occur, and significant changes in plasma composition occur on storage. In addition, human plasma is considered a biohazard, requiring special handling and disposal procedures. Therefore, the use of simulated physiological media is preferred. However, as it was pointed out earlier in this document (CMC – Physiochemical Properties-*in vitro* release), identifying an appropriate medium could represent a significant challenge for certain liposomal products. In some cases, non-physiological conditions may have to be employed to obtain an assay with acceptable precision and discriminating capabilities.

**Lines 355-373: Mass Balance Study**

The guidance should define whether mass balance studies are recommended in the situation where there is no approved nonliposomal drug product with the active moiety.

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<sup>4</sup> Hernandez-Caselles T, Villalain J and Gomez-Fernandez JC: Influence of liposome charge and composition on their interaction with human blood serum proteins. *Mol Cell Biochem* 120:119-26, 1993.

<sup>5</sup> Lee KD, Pitas RE and Papahadjopoulos D: Evidence that the scavenger receptor is not involved in the uptake of negatively charged liposomes by cells. *Biochim Biophys Acta* 1111:1-6, 1992.

<sup>6</sup> Maruyama K, Mori A, Bhadra S, et al.: Proteins and peptides bound to long-circulating liposomes. *Biochim Biophys Acta* 1070:246-52, 1991.

<sup>7</sup> Oja CD, Semple SC, Chonn A, et al.: Influence of dose on liposome clearance: critical role of blood proteins. *Biochim Biophys Acta* 1281:31-7, 1996.

<sup>8</sup> Oku N, Tokudome Y, Namba Y, et al.: Effect of serum protein binding on real-time trafficking of liposomes with different charges analyzed by positron emission tomography. *Biochim Biophys Acta* 1280:149-54, 1996.

<sup>9</sup> Torchilin VP, Omelyanenko VG, Papisov MI, et al.: Poly(ethylene glycol) on the liposome surface: on the mechanism of polymer-coated liposome longevity. *Biochim Biophys Acta* 1195:11-20, 1994.

**Line 385: Mass Balance Study**

“Both parent drug substance and any metabolites should be monitored as appropriate.”

Clarification is needed regarding the scope of metabolites that are required to be monitored. Other FDA Guidance documents, such as *Guideline for the Format and Content of the Human Pharmacokinetics and Bioavailability Section of an Application* (February 1987) and *In Vivo Drug Metabolism/Drug Interaction Studies - Study Design, Data Analysis, and Recommendations for Dosing and Labeling* (November 1999) refer to **major** metabolites, **active** metabolites, **key** metabolites, and **important active** metabolites in discussions of the determination of human pharmacokinetics and/or metabolism of drugs. Therefore, replacing the word “any” in the current guideline with “relevant,” “major,” or “active” would be more meaningful. Furthermore, it is important to define the time course of any active metabolites.

**Lines 390-414: Pharmacokinetic Studies**

The guidance should define whether inclusion of a nonliposome formulation is recommended in ADME studies in the situation where there is no approved nonliposome drug product with the active moiety.

**Lines 427-432: Food-Effect Studies**

We suggest deletion of the recommendation to perform food-effect studies because we do not believe food is a major factor in the disposition of liposome drug products.

There are a number of mechanisms that affect the disposition of liposome drug products *in vivo* following intravenous administration. These include release of drug from liposomes, structural disruption of liposomes and uptake of liposome drug product by the organs of the mononuclear phagocyte system (MPS) including liver, spleen and bone marrow. The disposition depends on physicochemical properties of the drug substance, lipid composition of the liposomes and the method used to encapsulate the drug in the liposome drug product. Plasma proteins and two major classes of human plasma lipoproteins, low-density lipoproteins (LDL) and high-density lipoproteins (HDL), have been implicated in playing a significant role either directly or indirectly.<sup>10,11</sup>

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<sup>10</sup> Chonn A, Semple S, and Cullis PR: Beta 2 glycoprotein I is a major protein associated with very rapidly cleared liposomes *in vivo*, suggesting a significant role in the immune clearance of “non-self” particles. *J Biol Chem* 270:25845-9, 1995.

<sup>11</sup> Wasan KM, Brazeau GA, Keyhani A, et al.: Roles of liposome composition and temperature in distribution of amphotericin B in serum lipoproteins. *Antimicrob Agents Chemother* 37:246-50, 1993.

Although food intake results in changes of lipid composition in plasma, these changes are largely due to the elevation of triglyceride-rich lipoproteins (TRLs), e.g. chylomicrons and very low-density lipoproteins (VLDL).<sup>12,13</sup> Plasma proteins, LDL and HDL are affected very little or not at all by food intake. In addition, the measurement of plasma triglyceride to apolipoprotein B ratio has led to a conclusion that the increase in TRL triglyceride is largely due to an increase in the size of particles more than an increase in the number of particles present.<sup>13</sup> In normal humans, the TRLs are rapidly catabolized in hours through the action of lipoprotein lipases in extrahepatic tissues and further metabolized by interaction with liver.

Based on the above observations, we do not believe a transient increase of TRLs following a food intake would have major impact on the disposition of liposome drug products.

#### **IV. LABELING**

##### ***Lines 455-462: Product Name***

Please clarify the proposed descriptive terminology to distinguish liposomal formulations. Adoption of such terminology (in particular with regards to Type A, B and C liposomal products) was discussed at an April 19, 2001 joint FDA/USP/AAPS workshop on controlled release parenteral drug products; however, there is no evidence that such nomenclature has been formally adopted.

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<sup>12</sup> Redgrave TG and Carlson LA: Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man. J Lipid Res 20:217-29, 1979.

<sup>13</sup> Pott JL, Humphreys SM, Coppack SW, et al.: Fasting plasma triacylglycerol concentrations predict adverse changes in lipoprotein metabolism after a normal meal. Br J Nutr 72:101-9, 1994.

Once again, ALZA appreciates the opportunity to provide comments on this draft guidance document. Please feel free to contact us if there are any questions or need for further clarification.

Sincerely yours,  
ALZA Corporation

A handwritten signature in black ink, appearing to read "Bridget Binko". The signature is written in a cursive style with a large initial "B".

Bridget Binko  
Sr. Director, Regulatory Affairs