



Technical Specification

Nanotechnologies — Vocabulary — Liposomes

Nanotechnologies — Vocabulaire — Liposomes

ISO/TS 4958

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Foreword

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This document was prepared by Technical Committee TC 229, *Nanotechnologies*.

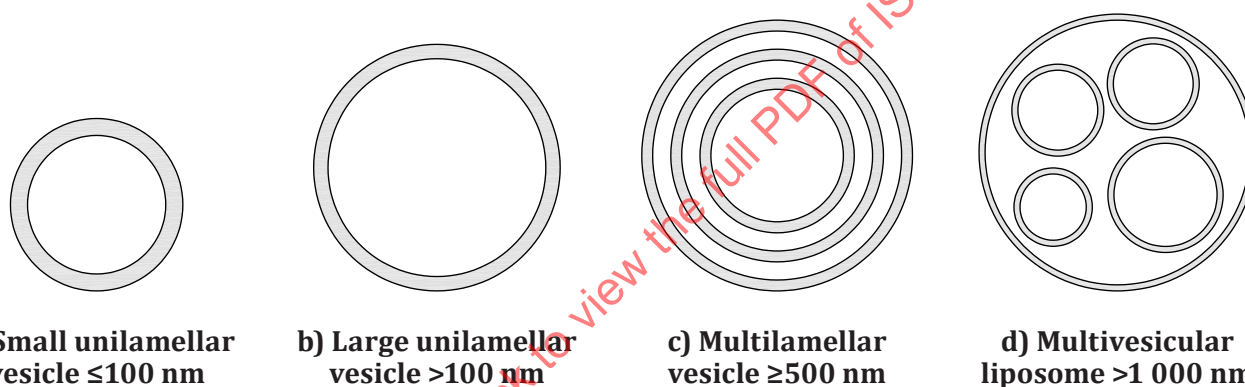
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Introduction

Lipid-based nanomaterials represent an important class of carriers for the in vivo transport and delivery of active pharmaceutical ingredients (APIs). By encapsulating the API inside a lipid-based structure, payloads can be protected from degradation while potent APIs can be delivered with reduced adverse physiological effects. These lipid-based carriers are carefully formulated to achieve specific properties and are generally well tolerated and biocompatible.

Lipid particles include different structural forms or subclasses that can be differentiated by structure, composition and chemistry (e.g. liposomes, solid lipid nanoparticles). The first lipid-based nanomaterial product to obtain regulatory approval in the US and EU was liposomal doxorubicin, approved in 1995 in the US for the treatment of ovarian cancer and AIDS-related Kaposi sarcoma. More recently, cationic lipid-containing nanoparticles complexed with mRNA were formulated as highly effective vaccines against the coronavirus SARS-CoV-2. This document aims to standardize the terminology associated with the most studied and mature form of lipid-based carriers, namely liposomes.

Liposomes are synthetic vesicles composed of a single bilayer (most common form for drug delivery) or of multiple concentric or non-concentric bilayers separated by aqueous compartments. [Figure 1](#) schematically illustrates these basic structural forms of liposome as used within a biomedical context. An example of pharmaceutical relevance (e.g. a drug product) is provided for each vesicle form defined in [3.2](#).



NOTE Images are not drawn to scale.

SOURCE Scientific Publications, Graphics and Media, Frederick National Laboratory for Cancer Research.

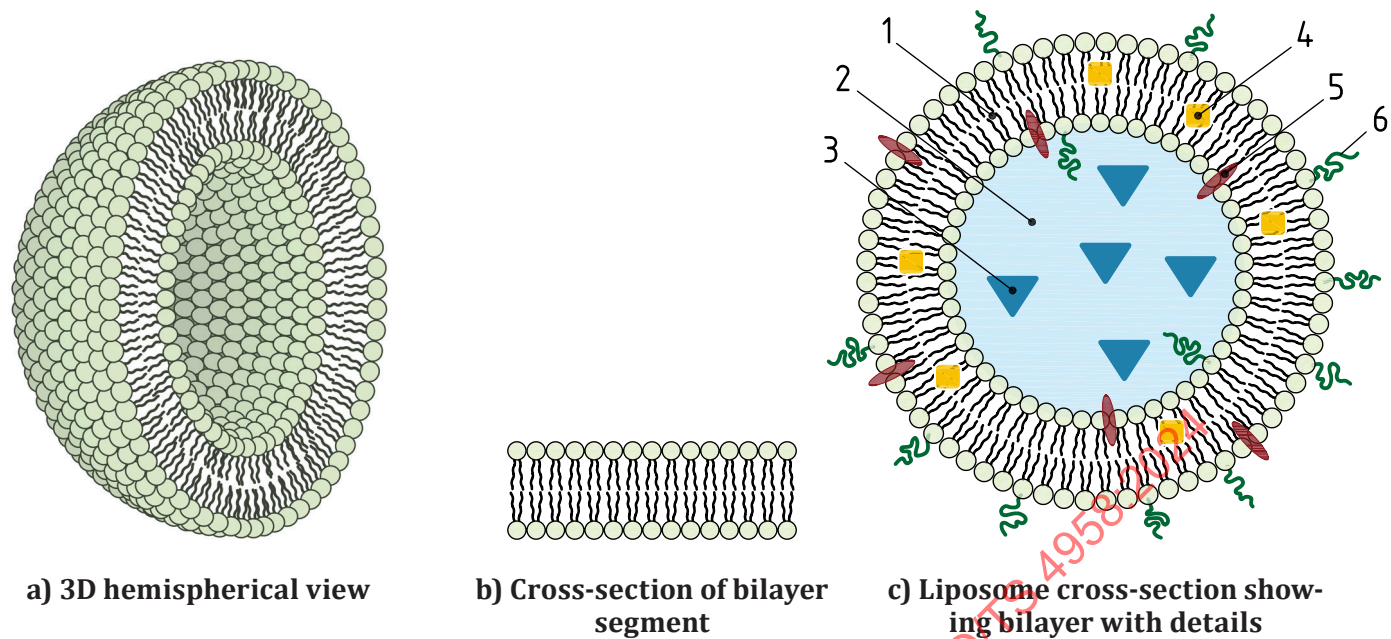
Figure 1 — Schematic illustration showing lamellar structure of different vesicle types

The bilayers are formed by amphipathic molecules, primarily phospholipids, but can include other molecular components necessary for membrane integrity (e.g. cholesterol) or avoidance of opsonization and reticuloendothelial clearance [e.g. polyethylene glycol (PEG)].

The size of liposomes can range from approximately 20 nm to over 1 000 nm, though therapeutic delivery most commonly involves particles in the 50 nm to 200 nm diameter range. Therefore, while not all liposomes are nano-objects as defined in this document, all liposomes consist of bilayers of nanoscale thickness and are therefore generally considered both nanomaterials and nanostructured materials.

[Figure 2](#) depicts a 3D cross-sectional perspective of an idealized unilamellar liposome, a lipid bilayer and a liposomal drug formulation showing the location of compartments and APIs.

[Figure 3](#) illustrates the three principal structural phases associated with lipid bilayers. These phases are principally dependent on composition and temperature, but other factors such as pH can also play a role.



Key

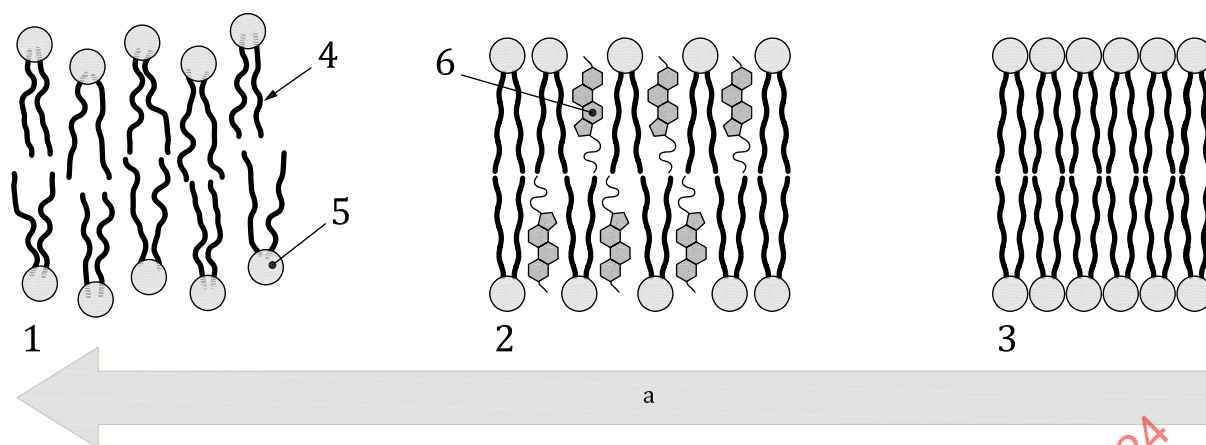
- 1 hydrophobic compartment (lipid bilayer)
- 2 hydrophilic compartment (aqueous phase core)
- 3 hydrophilic active pharmaceutical ingredient (API)
- 4 hydrophobic API
- 5 amphiphilic API
- 6 polyethylene glycol (PEG)

NOTE 1 Images are not drawn to scale.

NOTE 2 Polar headgroups are shown in green and hydrophobic tails are shown in black.

SOURCE Scientific Publications, Graphics and Media, Frederick National Laboratory for Cancer Research.

Figure 2 — Idealized unilamellar liposome showing phospholipid bilayer structure, internal compartments and representative details

**Key**

- 1 liquid disordered phase (above phase transition temperature)
- 2 liquid ordered phase (induced by cholesterol)
- 3 gel phase (below phase transition temperature)
- 4 phospholipid fatty acid tails
- 5 phospholipid polar headgroup
- 6 cholesterol
- ^a Increasing membrane fluidity.

NOTE Images are not drawn to scale.

SOURCE Scientific Publications, Graphics and Media, Frederick National Laboratory for Cancer Research.

Figure 3 — Idealized illustration of phospholipid bilayer structural phases

Due to their versatile nature, liposomes are promising materials in many industrial fields. In addition to therapeutics, liposome technologies have also been applied in products such as cosmetics and dietary supplements.

Additional terms that relate to the nano/bio interface and nanotechnologies related to diagnostics and therapeutics for healthcare are defined in ISO/TS 80004-5 and ISO/TS 80004-7, respectively.

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Nanotechnologies — Vocabulary — Liposomes

1 Scope

This document defines terms related to liposomes in nanotechnologies, within the context of biological systems and biomedical applications. In this context, liposomes are one form of lipid-based nanomaterials. This document does not address terms that can be relevant to other types of lipid-based particles (e.g. solid lipid nanoparticles).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1 Core terms related to liposomes

3.1.1

nanoscale

length range approximately from 1 nm to 100 nm

Note 1 to entry: Properties that are not extrapolations from a larger size are predominantly exhibited in this length range.

[SOURCE: ISO 80004-1:2023, 3.1.1, modified— Note 1 to entry has been added.]

3.1.2

nanomaterial

material with any external dimension in the *nanoscale* (3.1.1) or having an internal structure or surface structure in the *nanoscale*

Note 1 to entry: This term is inclusive of *nano-objects* (3.1.3) and *nanostructured materials* (3.1.4).

[SOURCE: ISO 80004-1:2023, 3.1.4, modified— Note 1 to entry has been replaced and Note 2 to entry has been deleted.]

3.1.3

nano-object

discrete piece of material with one, two or three external dimensions in the *nanoscale* (3.1.1)

[SOURCE: ISO 80004-1:2023, 3.1.5]

3.1.4

nanostructured material

material having internal *nanostructure* (3.1.5) or surface nanostructure

Note 1 to entry: This definition does not exclude the possibility for a *nano-object* (3.1.3) to have internal structure or surface structure. If external dimension(s) are in the *nanoscale* (3.1.1), the term nano-object is recommended.

[SOURCE: ISO 80004-1:2023, 3.1.7]

3.1.5**nanosurface**

surface or internal feature with one or more dimensions in the *nanoscale* (3.1.1)

Note 1 to entry: A feature includes but is not limited to *nano-objects* (3.1.3), structures, morphologies or other identifiable areas of nanoscale dimensions. For example, the nanosurface can be a nanopore or a solid feature on an object.

[SOURCE: ISO 80004-1:2023, 3.1.6]

3.1.6**nanoparticle**

nano-object (3.1.3) with all external dimensions in the *nanoscale* (3.1.1)

Note 1 to entry: If the dimensions differ significantly (typically by more than three times), terms such as nanofibre or nanoplate are preferred to the term nanoparticle.

[SOURCE: ISO 80004-1:2023, 3.3.4]

3.2 Terms related to lipid-bilayer vesicles**3.2.1****liposome**

synthetic *vesicle* (3.2.2) consisting of one or more *lipid bilayers* (3.3.3) that form both hydrophobic and hydrophilic *compartments* (3.3.4)

Note 1 to entry: Liposomes are principally composed of phospholipids (3.3.11) and *cholesterol* (3.3.10), and can contain both naturally-derived and synthetic *lipids* (3.3.1).

Note 2 to entry: The external dimensions of liposomes can range from tens of nanometres to micrometres, while the thickness of a typical *lipid bilayer* (3.3.3) is in the order of 5 nm.

3.2.2**vesicle**

structure in which a fluid phase is enclosed by a *lipid bilayer* (3.3.3)

Note 1 to entry: Vesicles are typically spheroidal when referring to *liposomes* (3.2.1).

3.2.3**small unilamellar vesicle****SUV**

vesicle (3.2.2) consisting of a single *lipid bilayer* (3.3.3) having external dimensions predominantly in the range 20 nm to 100 nm

EXAMPLE Liposomal doxorubicin hydrochloride is a chemotherapy drug product.

3.2.4**large unilamellar vesicle****LUV**

vesicle (3.2.2) consisting of a single *lipid bilayer* (3.3.3) having external dimensions predominantly greater than 100 nm

Note 1 to entry: LUVs are typically between 100 nm and 1 000 nm.

EXAMPLE Liposomal amikacin is a drug product that treats refractory lung infection.

3.2.5**multilamellar vesicle****MLV**

vesicle (3.2.2) consisting of two or more concentric *lipid bilayers* (3.3.3)

Note 1 to entry: The exterior dimensions of MLVs have been reported from approximately 0,5 µm to 30 µm.

EXAMPLE Liposomal clodronate disodium is a drug product for targeted macrophage depletion.

3.2.6

multivesicular liposome

MVL

liposome (3.2.1) consisting of multiple non-concentric *lipid bilayers* (3.3.3)

EXAMPLE Liposomal bupivacaine is a local long-lasting anaesthetic drug product.

3.3 Terms related to the components and regions of liposomes

3.3.1

lipid

diverse class of organic compounds that are insoluble in water but soluble in nonpolar solvents and that consist principally of triacylglycerols, fatty acids, phospholipids and sterols

Note 1 to entry: Many lipids are *amphiphilic* (3.3.2) and can form *bilayers* (3.3.3).

Note 2 to entry: Lipids are the principal structural components of *liposomes* (3.2.1) and cell membranes.

Note 3 to entry: Although lipids are principally of biological origin or their derivatives, lipids can also be synthesized.

Note 4 to entry: Petroleum derived waxes and aromatic compounds are not considered lipids.

3.3.2

amphiphile

molecule having both hydrophilic and hydrophobic regions

3.3.3

lipid bilayer

lamella

self-assembled structure composed of two stacked *lipid* (3.3.1) layers

Note 1 to entry: Bilayers typically have a central hydrophobic region and polar headgroups oriented outwards.

3.3.4

compartment

<liposome> defined region within a liposome (3.2.2)

Note 1 to entry: Hydrophobic regions are typically located within *lipid bilayers* (3.3.3) and hydrophilic regions typically refer to aqueous cores.

3.3.5

trapping agent

<liposome> molecule that facilitates the sequestration or *active loading* (3.4.5) of therapeutic agents

3.3.6

liquid ordered phase

<liposome> *lipid bilayer* (3.3.3) phase with properties intermediate between a fluid phase (3.3.7) and an ordered *gel phase* (3.3.8)

Note 1 to entry: The liquid ordered phase is generally associated with the addition of sterol to the bilayer leading to tighter packing of the liquid phase structure.

3.3.7

liquid disordered phase

<liposome> fluid phase of a *lipid bilayer* (3.3.3)

Note 1 to entry: The liquid disordered phase exists when temperature exceeds the *phase transition temperature* (3.4.13).

3.3.8**gel phase**

<liposome> solid phase of a *lipid bilayer* (3.3.3)

Note 1 to entry: The gel phase can form when a *lipid bilayer* (3.3.3) is cooled below the *phase transition temperature* (3.4.12).

Note 2 to entry: The term 'gel' used to describe *lipids* (3.3.1) should not be confused with the term 'gel' used to describe colloids.

3.3.9**fusogen**

molecule contained within a *liposome* (3.2.1) or *vesicle* (3.2.2) that promotes fusion between opposing *bilayers* (3.3.3)

3.3.10**cholesterol**

<liposome> sterol with the formula $C_{27}H_{46}O$ present in all eukaryotic cell membranes, commonly used as a component of *lipid bilayers* (3.3.3) in the formulation of *liposomes* (3.2.1)

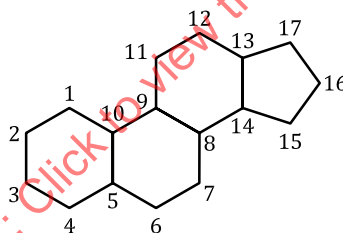
Note 1 to entry: Cholesterol is classified as a *lipid* (3.3.1).

Note 2 to entry: Cholesterol impacts fluidity, permeability and flexibility of *lipid bilayers* (3.3.3).

Note 3 to entry: Cholesterol is obtained from both natural sources and as a synthetic product.

3.3.11**sterol**

organic compound derived from the tetracyclic steroid nucleus (17 carbon skeleton, as shown below) with a hydroxyl group at the C-3 position



Note 1 to entry: In a *lipid bilayer* (3.3.3), the 3-hydroxyl associates with the polar head groups of the *phospholipids* (3.3.12), while the tetracyclic region associates with the hydrophobic region inside the bilayer.

Note 2 to entry: Sterols are a subgroup of steroids and vary in their degree of hydrogenation and presence of side groups. There is often an alkyl group at C-17.

[SOURCE: Reference [3]]

3.3.12**phospholipid**

lipid (3.3.1) containing phosphoric acid as mono- or di-esters, including phosphatidic acids and phosphoglycerides

Note 1 to entry: A phospholipid is an *amphiphilic* (3.3.2) molecule integral to the structure and function of *liposomes* (3.2.1) and contains a hydrophilic headgroup covalently attached to a pair of hydrophobic fatty acids.

[SOURCE: Reference [7]]

3.4 Terms related to the characteristics and formation of liposomes**3.4.1****bilayer thickness**

distance measured across two stacked hydrated *lipids* (3.3.1)

3.4.2

lamellarity

number of concentric *lipid bilayers* (3.3.3) in a *multilamellar vesicle* (3.2.5) or *liposome* (3.2.1)

Note 1 to entry: Lamellarity influences *encapsulation efficiency* (3.4.9) and drug release kinetics.

3.4.3

drug loading

<liposome> process of encapsulation of a drug in a *liposome* (3.2.1)

3.4.4

drug load

<liposome> amount of drug encapsulated in a *liposome* (3.2.1)

3.4.5

active loading

remote loading

<liposome> facilitated encapsulation of active pharmaceutical agent inside a *liposome* (3.2.1) *compartment* (3.3.4)

Note 1 to entry: Active loading involves processes other than those associated with passive loading.

Note 2 to entry: Active loading is typically carried out using pre-formed *liposomes* (3.2.1) through the application of transmembrane gradients (e.g. as induced by pH or concentrations of trapping agents).

3.4.6

passive loading

<liposome> unfacilitated encapsulation of active pharmaceutical agent inside a *liposome* (3.2.1) by physical entrainment concurrent with liposome formation or by simple diffusion using pre-formed liposomes

Note 1 to entry: Passive loading indicates the absence of *active loading* (3.4.5).

Note 2 to entry: Passive loading by diffusion generally requires modulation of bilayer permeability.

3.4.7

drug encapsulation

<liposome> entrapment of a drug within a *compartment* (3.3.4) of a *liposome* (3.2.1)

3.4.8

entrapment volume

encapsulation volume

<liposome> volume of an identified *compartment* (3.3.4) of a *liposome* (3.2.1) in which the active pharmaceutical agent is contained

3.4.9

encapsulation efficiency

<liposome> percentage of active pharmaceutical agent that is encapsulated by a liposome relative to the total quantity of said agent present during the encapsulation process

Note 1 to entry: This value can be determined by multiple methods and approaches. A common method is to measure the total mass of active agent (target molecule) present (free + encapsulated) and the mass of encapsulated active agent (after removal of free active agent).

3.4.10

functionalized liposome

liposome (3.2.1) modified to enable new or enhanced capability

Note 1 to entry: Modifications can be made to the surface or internal to the *lipid bilayer* (3.3.3) and can be of physical or chemical origin (e.g. *PEGylation* (3.4.11) of a bilayer component).

Note 2 to entry: Capabilities include, for instance, targeting, imaging, increased systemic circulation time, biocompatibility or bilayer structural integrity.

3.4.11**PEGylation**

<liposome> chemical or physical association of polyethylene glycol (PEG) chains with a *lipid bilayer* (3.3.3)

Note 1 to entry: Ideally, PEG chains are anchored to lipids and extend outward from the external surface of the *lipid bilayer* (3.3.3). However, it is also possible for PEG to orient such that it faces the internal aqueous *compartment* (3.3.4).

Note 2 to entry: The principal purpose of PEG is to prolong the systemic circulation time by shielding the liposome from protein adsorption, opsonization and phagocytosis. This property is frequently referred to as “stealth” and is an example of a *functionalized liposome* (3.4.10).

3.4.12**phase transition**

<liposome> phenomenon where *lipid bilayer* (3.3.3) structure changes from a *liquid ordered phase* (3.3.7) to a *gel phase* (3.3.8) or vice versa

Note 1 to entry: Phase transition occurs at the *phase transition temperature* (3.4.13), which is influenced by changes in *lipid bilayer* (3.3.3) composition or fluid properties.

3.4.13**phase transition temperature**

<liposome> temperature at which a *lipid bilayer* (3.3.3) changes from a *liquid ordered phase* (3.3.7) to a *gel phase* (3.3.8) or vice versa

3.4.14**fluidity**

<liposome> property that describes the lateral movement of *lipids* (3.3.1) in the *lipid bilayer* (3.3.3)

Note 1 to entry: The fluidity involves biophysical properties such as membrane dynamics, local viscosity and order.

[SOURCE: Reference [8]]