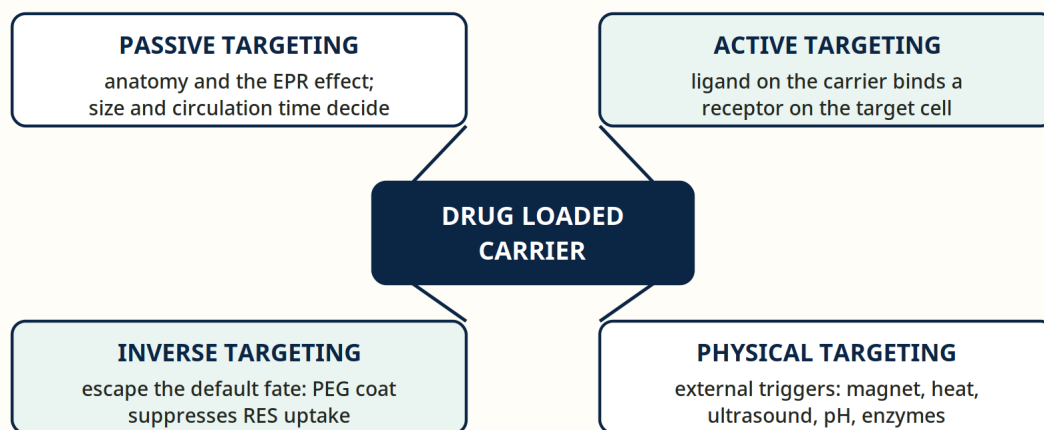


2.1 Targeting methods: a general introduction

The Four Targeting Methods



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Figure: The four general targeting methods. Every carrier in this subject is used in one or more of these ways.

The carriers in this subject are used in four general ways, and every preparation and evaluation method in the unit is a means of making one of these ways work.

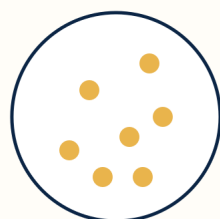
- **Passive targeting** uses the natural distribution of the carrier. It exploits anatomy and physiology: the fenestrations of the liver and spleen sinusoids, the leaky vasculature of tumours and inflamed tissue (the EPR effect, Unit 1), and the uptake of particles by phagocytic cells. No ligand is involved. The design variables are size, surface charge and circulation time.
- **Active targeting** attaches a ligand to the carrier surface so that it binds a specific receptor or antigen on the target cell and is taken up by receptor mediated endocytosis. Ligands used include monoclonal antibodies and their fragments, peptides, aptamers, and small molecules such as folic acid and galactose (for the asialoglycoprotein receptor of hepatocytes).
- **Inverse targeting** is the deliberate avoidance of the usual fate: instead of accepting RES uptake, the carrier surface is made hydrophilic (for example by PEGylation or poloxamer coating) so that opsonisation and macrophage uptake are suppressed and the carrier can reach non-RES targets. It is targeting by escaping the default destination.
- **Physical (and chemical) targeting** uses an external or local physical trigger: a magnet held over the site for magnetic carriers, local heating, ultrasound, or a stimulus at the site itself such as low pH or a specific enzyme that triggers release from a responsive carrier.

Whichever method is chosen, an ideal carrier has the same set of properties: it should carry enough drug, protect it during transit, be non toxic, non immunogenic and biodegradable or safely excreted, be stable during storage, release the drug at the required rate at the site, and be simple and cheap enough to manufacture reproducibly at scale. Preparation methods are judged by how close they come to this list, and

evaluation methods are the tests that measure it.

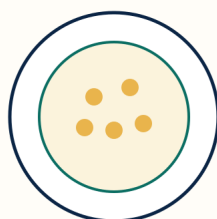
2.2 Nanoparticles: introduction and types

Types of Nanoparticles



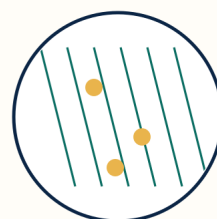
NANOSPHERE

drug dispersed in a solid matrix



NANOCAPSULE

drug in an oily core, polymer shell around it



SOLID LIPID NP

drug in a solid lipid matrix (SLN)

Size range of nanoparticles: about 10 to 1000 nm

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Figure: The three structural types of nanoparticle: matrix (nanosphere), reservoir (nanocapsule) and solid lipid nanoparticle.

Nanoparticles are solid colloidal particles in the approximate size range of 10 to 1000 nm that carry the drug dissolved, entrapped, encapsulated or adsorbed in or on a polymer or lipid matrix. Because of their small size they can pass through fenestrated endothelium, are taken up by cells, and can be given by several routes including intravenous, oral and ocular. Their large surface area to volume ratio also allows extensive surface modification with ligands and stealth coatings.

The main types are:

- **Nanospheres:** matrix systems in which the drug is dispersed uniformly through a solid polymer or lipid matrix and is released by diffusion and by erosion of the matrix.
- **Nanocapsules:** reservoir systems with a liquid or oily core containing the drug, surrounded by a thin polymer shell; release is controlled mainly by the shell.
- **Polymeric nanoparticles:** made from biodegradable polymers such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(caprolactone) and poly(alkyl cyanoacrylates), or from natural polymers such as albumin, gelatin and chitosan.
- **Solid lipid nanoparticles (SLN):** the liquid oil of an emulsion is replaced by a lipid that is solid at room and body temperature (for example triglycerides, waxes or fatty acids), stabilised by surfactants. They combine the tolerability of lipids with solid matrix release control, and are used for drugs such as vaccines, peptides and poorly soluble compounds, and in cosmetics and brain delivery research.
- **Nanocrystals:** pure drug in crystalline nanoparticle form, stabilised by surfactants or polymers, used to raise the dissolution rate and bioavailability of poorly soluble drugs.

Feature	Nanosphere	Nanocapsule
Structure	Solid matrix throughout	Core surrounded by a shell
Drug location	Dispersed in the matrix	Dissolved in the core (often oily)
Release control	Diffusion plus matrix erosion	Membrane (shell) controlled diffusion
Drug loading	Limited by matrix capacity	Often higher for lipophilic drugs in an oily core

2.3 Preparation of nanoparticles

The methods fall into two families: building particles from monomers (polymerisation) and forming particles from a preformed polymer or lipid.

Polymerisation methods

- **Emulsion polymerisation:** the monomer (for example an alkyl cyanoacrylate) is dispersed in an aqueous phase containing surfactant; polymerisation starts in the micelles or droplets and nanoparticles form directly in the dispersion. The drug may be added before polymerisation (incorporated) or afterwards (adsorbed).
- **Interfacial polymerisation:** monomer and drug are dissolved in an organic phase that is emulsified into water; polymerisation occurs at the oil water interface and produces nanocapsules with an oily core.

Methods from preformed polymers

- **Nanoprecipitation (solvent displacement):** the polymer and drug are dissolved in a water miscible organic solvent (acetone or ethanol); this phase is poured into water containing a stabiliser, the solvent diffuses out rapidly, and the polymer precipitates as nanoparticles. The method is simple and reproducible, works best for lipophilic drugs, and is the most common laboratory method for PLA and PLGA particles.
- **Emulsification and solvent evaporation:** polymer and drug are dissolved in a water immiscible volatile solvent (dichloromethane or ethyl acetate), emulsified into an aqueous phase (oil in water, or water in oil in water double emulsion for hydrophilic drugs), and the solvent is removed by evaporation under stirring or reduced pressure, hardening the droplets into particles.
- **Emulsification and solvent diffusion:** a partially water miscible solvent is used; on dilution with excess water the solvent diffuses out of the droplets and the polymer precipitates.
- **Salting out:** the polymer solution in a water miscible solvent is emulsified into an aqueous phase rich in salts (for example magnesium chloride) and stabiliser that temporarily prevents solvent diffusion; dilution with water then allows diffusion and particle formation. No chlorinated solvent is needed and no heating is used, which suits labile drugs.
- **Supercritical fluid methods:** a supercritical fluid (usually carbon dioxide) is used as solvent or antisolvent to produce fine, solvent free particles; equipment cost limits routine use.
- **High pressure homogenisation (for SLN):** the drug is melted or dissolved in molten lipid and dispersed in hot surfactant solution; the pre emulsion is passed through a high pressure homogeniser and, on cooling, the droplets solidify into solid lipid nanoparticles (hot homogenisation). A cold variant homogenises solid drug lipid microparticles for heat sensitive drugs.

After preparation, the particles are separated and purified (centrifugation, ultrafiltration or dialysis), and are often freeze dried with a cryoprotectant such as trehalose or mannitol for storage.

2.4 Evaluation of nanoparticles

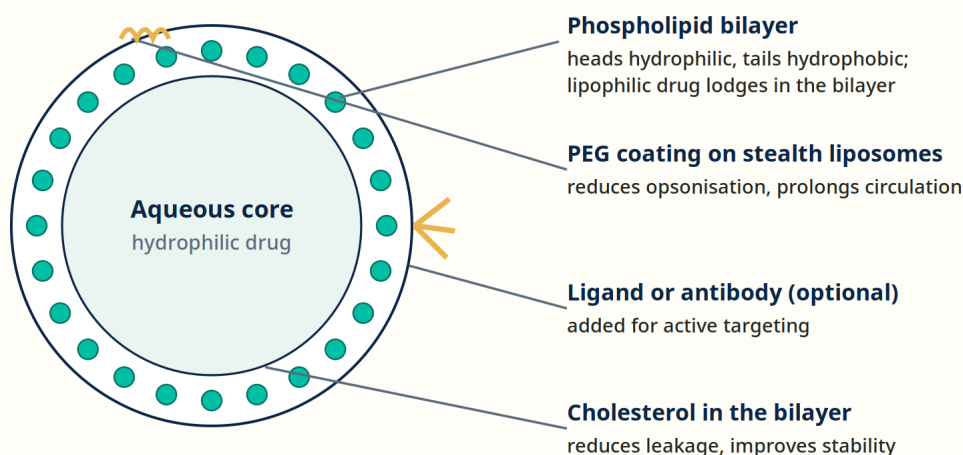
Evaluation answers four questions: what size and surface the particles have, how much drug they carry, how they release it, and whether they stay that way.

- **Particle size and size distribution:** measured by photon correlation spectroscopy (dynamic light scattering), which gives the mean size and the polydispersity index; a narrow distribution (low polydispersity index) is wanted for reproducible biodistribution. Laser diffraction and Coulter counting cover larger particles.

- **Morphology and surface:** scanning and transmission electron microscopy show shape and confirm size; atomic force microscopy gives surface detail.
- **Surface charge (zeta potential):** measured from electrophoretic mobility. A zeta potential magnitude of about 30 mV or more (positive or negative) generally gives good electrostatic stability against aggregation; near zero charge, particles aggregate quickly unless sterically stabilised.
- **Drug content and encapsulation efficiency:** the drug is extracted from a known quantity of particles and assayed; encapsulation efficiency is the percentage of the drug taken that is actually entrapped, and drug loading is the drug mass as a percentage of the particle mass.
- **In vitro release:** studied in a release medium at body temperature, usually by dialysis bag or sample and separate methods, and fitted to release models (zero order, first order, Higuchi, Korsmeyer-Peppas) to identify the mechanism.
- **Stability:** size, charge, drug content and release are followed on storage, including after freeze drying and reconstitution.
- **Biological evaluation:** sterility and pyrogen testing for injectables, haemolysis and cell culture studies in development, and animal biodistribution studies to prove targeting.

2.5 Liposomes: introduction and structure

Structure of a Liposome



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Figure: Structure of a liposome: the bilayer holds lipophilic drugs, the aqueous core holds hydrophilic drugs, and the surface can be PEGylated or fitted with a targeting ligand.

Liposomes are microscopic vesicles in which an aqueous compartment is enclosed by one or more bilayers of phospholipid. They were described by Bangham in the 1960s and remain the most successful carrier family in clinical use. The structure explains their versatility: hydrophilic drugs are held in the aqueous core, lipophilic drugs lodge in the bilayer, and amphiphilic drugs sit at the interface, so one carrier suits all three classes. Liposomes are made of the same phospholipids as cell membranes (phosphatidylcholine, phosphatidylethanolamine and related lipids, usually with cholesterol), so they are biocompatible and biodegradable, and their surface can be modified with PEG (stealth liposomes) or with ligands and antibodies (immunoliposomes). Their limitations are physical and chemical instability (leakage, fusion, oxidation and hydrolysis of the lipids), rapid RES clearance of conventional vesicles, and cost of manufacture.

2.6 Types of liposomes

Liposomes are classified in two main ways.

By size and lamellarity (number of bilayers)

- **Multilamellar vesicles (MLV):** several concentric bilayers like an onion, typically about 0.5 to 5 micrometres and heterogeneous; high lipid content, suited to lipophilic drugs, but cleared rapidly.
- **Large unilamellar vesicles (LUV):** a single bilayer enclosing a large aqueous volume, about 100 to 1000 nm; high aqueous trapped volume, good for hydrophilic drugs.
- **Small unilamellar vesicles (SUV):** a single bilayer, about 20 to 100 nm; small and uniform, longer circulating than MLV, but low trapped volume per unit lipid.

By composition and behaviour

- **Conventional liposomes:** neutral or negatively charged phospholipid with cholesterol; rapidly taken up by the RES.
- **Stealth (long circulating) liposomes:** surface coated with PEG, which forms a hydrated steric barrier, reduces opsonisation and greatly prolongs circulation, allowing EPR based tumour accumulation (Doxil is the model product).
- **Targeted liposomes and immunoliposomes:** carry a ligand or antibody on the surface for active targeting (Unit 1).
- **Cationic liposomes:** made with positively charged lipids; they bind negatively charged nucleic acids and cell surfaces and are the standard nonviral gene delivery carrier (Unit 5).
- **Stimulus sensitive liposomes:** pH sensitive or temperature sensitive compositions that destabilise and release their contents at the target site.

Cholesterol deserves a note of its own: inserted into the bilayer, it reduces membrane fluidity and permeability, decreases leakage of water soluble drugs, and improves stability in plasma; most practical formulations contain it.

2.7 Preparation of liposomes

- **Thin film hydration (Bangham method):** the lipids are dissolved in an organic solvent (chloroform or chloroform methanol) in a round bottom flask, and the solvent is removed under vacuum to leave a thin dry lipid film on the flask wall. The aqueous phase (buffer, with hydrophilic drug dissolved in it) is added and the flask is shaken or rotated above the lipid transition temperature; the film hydrates and peels off as MLVs. Lipophilic drugs are dissolved with the lipids in the organic phase. This is the standard laboratory method; the MLVs are then sized down.

```

Thin film hydration (Bangham) method
Lipids and cholesterol dissolved in chloroform
    |
    v
Solvent removed under vacuum: thin lipid film on flask wall
    |
    v
Aqueous phase added, hydrate above transition temperature
    |
    v
Multilamellar vesicles (MLV) formed
    |
    v
Size reduction: sonication, extrusion or homogenisation
    |
    v
Separation of untrapped drug; sterilisation; storage

```

- **Reverse phase evaporation:** lipids are dissolved in ether and the aqueous phase is added; the mixture is sonicated to a water in oil emulsion and the ether is evaporated under vacuum, so the emulsion collapses into LUVs with a large trapped volume and high encapsulation of hydrophilic drugs.
- **Solvent injection:** in the ether injection method, the lipid solution in ether is injected slowly into warm aqueous phase and the ether evaporates, forming vesicles; in the ethanol injection method, the ethanolic lipid solution is injected into water and the ethanol is later removed, a method that is simple and suitable for scale up.
- **Detergent removal (dialysis):** lipids and detergent are mixed into micelles; as the detergent is dialysed away, the micelles grow and close into uniform unilamellar vesicles.
- **Size reduction methods:** probe or bath sonication (gives SUVs but risks drug and lipid degradation and metal contamination), membrane extrusion through polycarbonate filters of defined pore size (gives reproducible LUVs of the filter size and is the standard sizing step), the French pressure cell, and microfluidisation or high pressure homogenisation for larger scale.
- **Active (remote) loading:** for weak base drugs such as doxorubicin, an ammonium sulphate or pH gradient across the bilayer of preformed liposomes is used to draw the drug in and trap it, giving very high encapsulation; this is how the commercial liposomal anticancer products are loaded.

Untrapped drug is removed by dialysis, gel filtration or centrifugation, and injectable liposomes are sterilised by filtration where size permits or prepared aseptically.

2.8 Evaluation of liposomes

- **Vesicle size and distribution:** dynamic light scattering for the mean size and polydispersity; microscopy for the larger MLVs.
- **Lamellarity and morphology:** electron microscopy (freeze fracture and negative staining), and phosphorus NMR methods in research settings.
- **Trapped (encapsulated) volume and encapsulation efficiency:** the liposomes are separated from free drug and the entrapped drug is assayed after disrupting the vesicles with a detergent or solvent; the trapped volume (aqueous volume per unit lipid) is measured with an entrapped marker.
- **Surface charge:** zeta potential, as for nanoparticles.
- **Bilayer properties:** the phase transition temperature by differential scanning calorimetry, and membrane fluidity in research studies.
- **Drug leakage and stability:** leakage of an entrapped marker or drug on storage and in plasma, lipid oxidation and hydrolysis, and size growth by fusion or aggregation.

- **In vitro release and in vivo performance:** release in buffer and plasma, then pharmacokinetic and biodistribution studies comparing free and liposomal drug.
- **Quality tests for the finished product:** sterility, pyrogen (endotoxin) content, and residual solvent, because production uses organic solvents.

2.9 Applications and marketed products

Nanoparticle and liposome systems are applied mainly in cancer chemotherapy, fungal and intracellular infections, vaccine delivery, and the delivery of peptides, proteins and nucleic acids. Products that reached the market show what the methods of this unit can achieve: Doxil (PEGylated liposomal doxorubicin, the first approved nanodrug, 1995) and DaunoXome (liposomal daunorubicin) in oncology, AmBisome (liposomal amphotericin B) for systemic fungal infection and visceral leishmaniasis, DepoCyt (liposomal cytarabine) for lymphomatous meningitis, and Abraxane (albumin bound paclitaxel nanoparticles, about 130 nm) in oncology. Each succeeds because the carrier changes the biodistribution or the toxicity profile of a drug whose free form was limited, which is the practical test of every design in this unit.

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