Pharmaceutical nanotechnology and nanomedicines

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Credit: Prof. Nizar Al-Zoubi 2023

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Introduction

- **Pharmaceutical nanotechnology** is a term applied to the design, characterization and production of pharmaceutical materials, structures and products that have one or more dimensions between approximately 1 and 100 nm.
- However an upper limit of 1000 nm is often considered.

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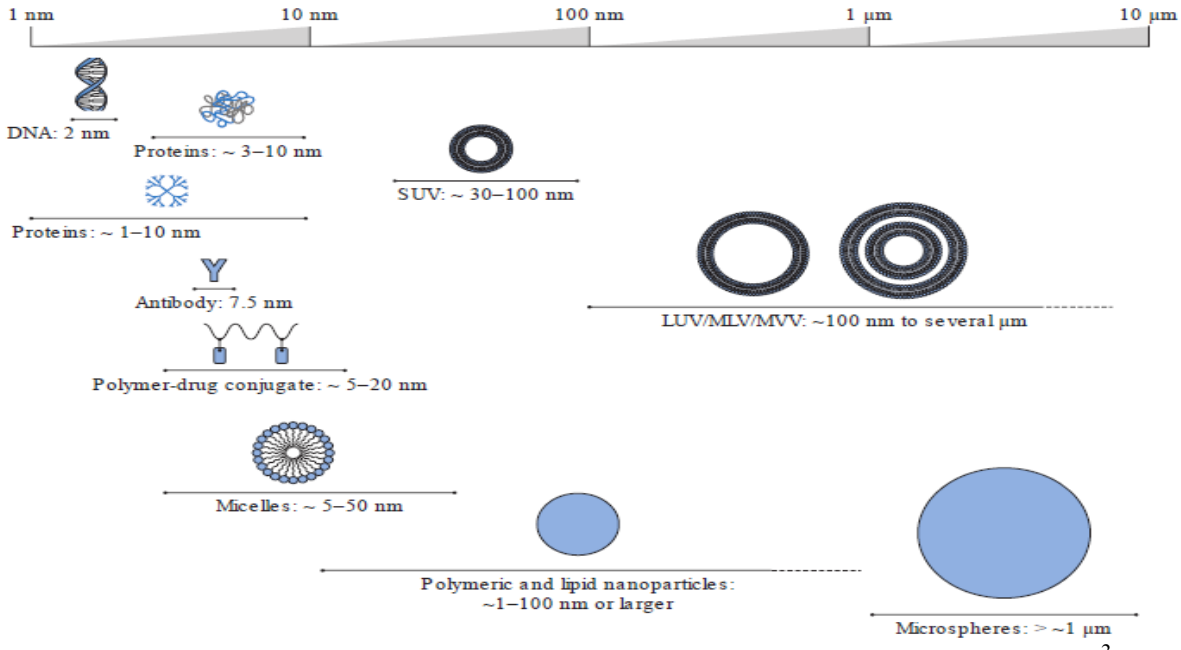

Fig. 45.1 • Approximate size range of various nanomedicines.

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Introduction to Nanoparticle (NPs) properties

- NPs offer unique properties as compared to micro or macroparticles. Main features include the following:
  1. Small size.
  2. High surface area.
  3. Easy to suspend in liquids.
  4. Deep access to cells and organelles.
  5. Variable optical and magnetic properties.
  6. Particles smaller than 200 nm can be easily sterilized by filtration with a 0.22-µm filter.

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Table 1 Typical Size of Various Objects

Object	Size (nm)
Carbon atom	0.1
DNA double helix (diameter)	3
Ribosome	10
Virus	100
Bacterium	1,000
Red blood cell	5,000
Human hair (diameter)	50,000
Resolution of unaided human eyes	100,000

Table 7 Number of Molecules in a Spherical Particle

Particle diameter	Particle volume (mL)	Number of molecules
0.58 nm	8.18×10^{-22}	1
1 nm	4.19×10^{-21}	5.05
10 nm	4.19×10^{-18}	5.05×10^{3}
100 nm	4.19×10^{-15}	5.05×10^{6}
500 nm	5.24×10^{-13}	6.31×10^{8}
1 μm	4.19×10^{-12}	5.05×10^{9}
5 μm	5.24×10^{-10}	6.31×10^{11}
1 mm	4.19×10^{-3}	5.05×10^{18}

Note: Drug molecular weight = 500 and solid density = 1 g/mL.

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Introduction to nanoparticle properties

Surface area

- For a spherical solid particle of diameter d and solid density, ρ_S, surface area per unit mass, S_g, is given as

$$S_g = \left(\frac{\pi d^2}{4}\right)\left(\frac{\pi d^3 \rho_s}{6}\right)^{-1} = \frac{3}{2d\rho_s}$$

- If the molecular diameter is σ, then the percentage of molecules on the surface monolayer is given as

$$\%\text{Surface molecules} = \frac{(4/3)\pi[d^3 - (d-\sigma)^3]}{(4/3)\pi[d^3]}100$$

$$= 100\left[\left(\frac{\sigma}{d}\right)^3 - 3\left(\frac{\sigma}{d}\right)^2 + 3\left(\frac{\sigma}{d}\right)\right]$$

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Table 2 % Surface Molecules in Particles

Particle size (nm)	Surface molecules (%)
1	100.00
10	27.10
100	2.97
1,000	0.30
10,000	0.03

- Because of the difference in the percentage of surface molecules, the **dissolution rate** is much higher for the NPs when compared to microparticles.

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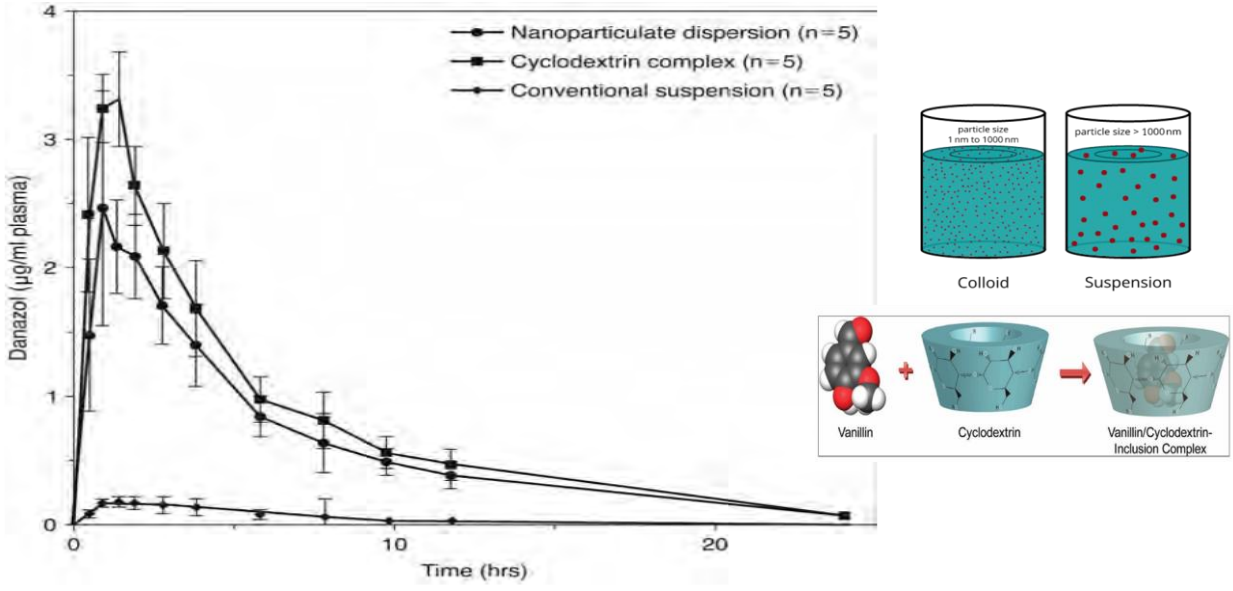

Figure 14 Danazol is a drug with an aqueous solubility of 10 µg/mL. Here danazol plasma levels are plotted as a function of time after administration of three forms of the drug including a nanoparticle dispersion and a hydroxypropyl-β-cyclodextrin complex, using a suspension with a mean particle size of 10 µm as a comparator. *Source*: From Ref. 83.

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Nanoencapsulation

Surface area

- NPs can show a strong adhesion because of the increased contact area for van der Waals attraction.
- For example, Lamprecht et al. (Pharm Res 2001; 18:788–793.) observed differential uptake/adhesion of polystyrene particle to inflamed colonic mucosa, with the deposition 5.2%, 9.1%, and 14.5% for 10-μm, 1000-nm, and 100-nm particles, respectively.

Introduction to nanoparticle properties

Surface area and aggregation

- Given their large surface to volume/weight ratios, NPs are prone to aggregate.
- Therefore additives are normally added to reduce aggregation.
- However, the formulation of a stable nanoparticle suspension in the laboratory is one thing, and the maintenance of the monodisperse state in vivo is another.

Introduction to nanoparticle properties

Surface area and aggregation

- Particles in blood, gut, nose, or lungs have moved from an aqueous-based medium to a more complex biological situation.
- **Aggregation**

1. changes the hydrodynamic size of the particles,
2. affects their diffusion and extravasation, and
3. reduces the effective surface area for interactions with receptors.

- The prevention of aggregation is sought by different approaches such as PEGylation (covalent attachment of PEG chains to the hydrophobic surface of the particles).

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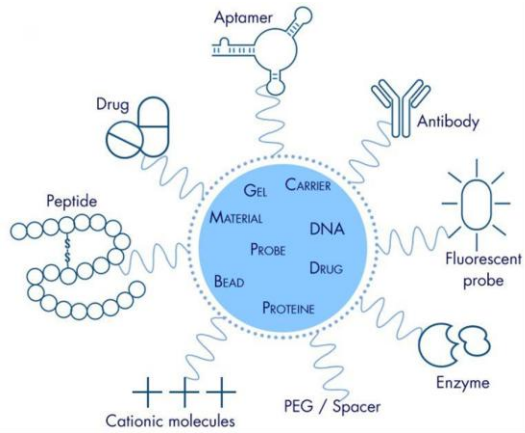

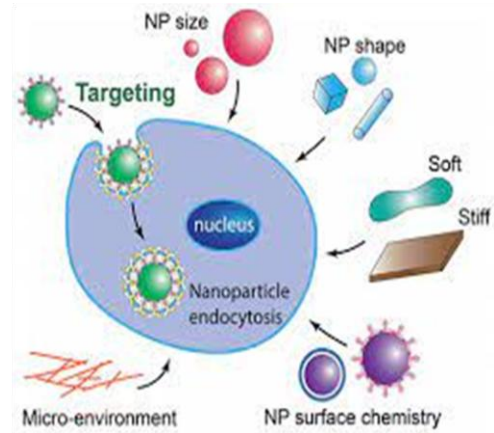

The Applications of PEGylation

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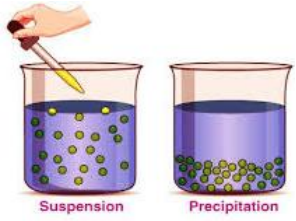
Introduction to nanoparticle properties

- Furthermore, particles below 1000 nm in size will not settle merely because of Brownian motion.
- This imparts an important property to NPs, that they can be easily kept suspended despite high solid density.
- Large microparticles easily settle out from suspension because of gravity



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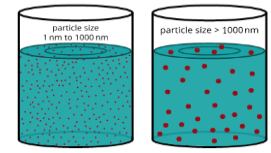
Stokes-Einstein equation:

$$D = \frac{1.38 \times 10^{-12} T}{3\pi\eta d} m^2 s^{-1}$$



- D = Brownian diffusion,
- T = absolute temperature,
- d = diameter,
- η= viscosity of liquid,

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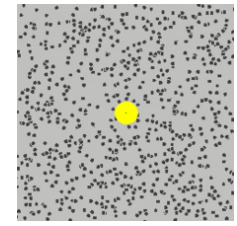

Table 4 Brownian Motion of the Particles

Particle size (nm)	Brownian displacement (nm in 1 sec)
1	54,250
10	17,155
100	5,425
1,000	1,716
10,000	543

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Introduction to nanoparticle properties

Biological transport of NPs

- For drug delivery, most of the sites are accessible through either microcirculation by blood capillaries or pores present at various surfaces and membranes.
- Most of the apertures, openings, and gates at cellular or subcellular levels are of nanometer size; hence, NPs are the most suited to reach the subcellular level.
- One of the prime requirements of any delivery system is its ability to move around freely in available avenues and by crossing various barriers that may come in the way.

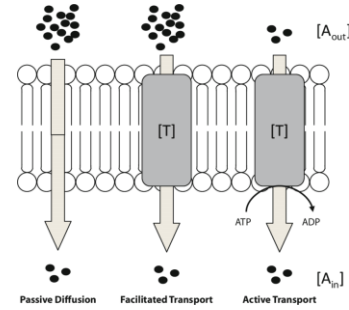

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Table 9 Approximate Sizes of Components in a Typical 20-μm Human Tissue Cell

Component	Size (nm)
Ribosomes	25
Golgi vesicles	30–80
Secretary vesicles	100–1000
Glycogen granules	10–40
Lipid droplets	200–5000
Vaults	55
Lysosomes	500–1000
Proteasomes	11
Peroxisomes	500–1000
Mitochondria	500–1000
Superfine filaments	2–4
Microfilaments	5–7
Thick filaments	15
Microtubules	25
Centrioles	150
Nuclear pores	70–90
Nucleosomes	10
Chromatin	1.9

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Solid NPs

- Solid NPs are solid constructs in the nanometer range, and can be prepared by a number of different manufacturing methods which generally involve either:
  - size reduction of particles (e.g. by milling) to within the nanoparticle range,
    - commonly used to prepare drug particles in the nanosize range where there is no carrier material added
  - molecular agglomeration (e.g. by precipitation methods) to form NPs
    - more commonly used to prepare nanoparticle carriers in which drug is loaded.

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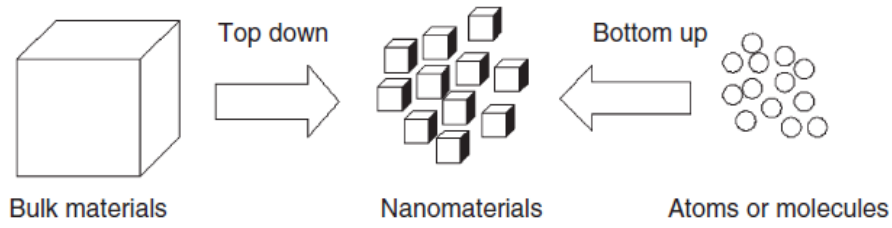
Solid NPs



Two basic methods to manufacture nanomaterials.

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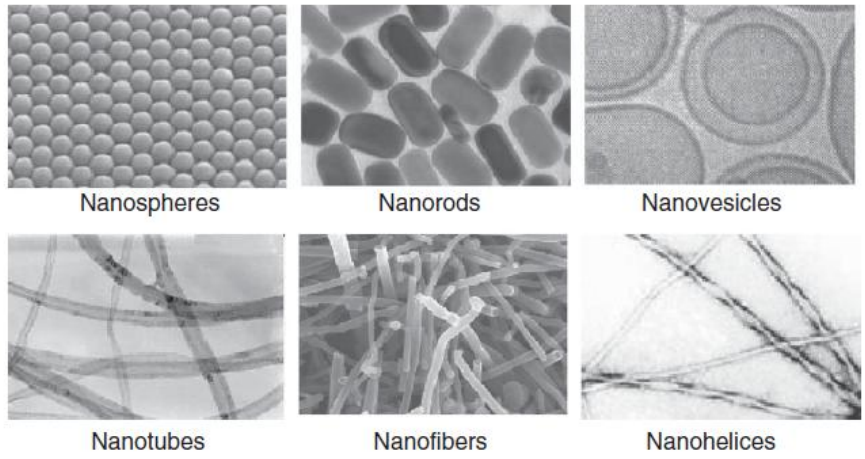
Solid NPs



Solid NP examples

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NPs by wet ball milling

- The preparation of pharmaceutical NPs by wet (bead, or media) milling reduces drug particles to a mean particle size less than 0.4 μm, more commonly 100 or 200 nm.
- In this process, drug is wet milled as a suspension in water, or in a medium such as safflower oil, ethanol, t-butanol, hexane.
- The milling dispersion also contains one or more surface modifiers which adsorb onto the freshly formed surfaces of the drug and prevent agglomeration through steric and/or electrostatic stabilization.

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Aspect	Electrostatic Stabilization	Steric Stabilization
Mechanism	Repulsion from surface charges plus electrical double layer	Physical barrier from adsorbed polymers/surfactants
Key Force	Coulombic (electrostatic)	**Entropic repulsion:** Overlap restricts polymer chain conformations **Osmotic repulsion:** Solvent molecules rush in to dilute it, pushing particles apart.
Common Stabilizers	Ionic surfactants, pH adjustment	Non-ionic polymers (e.g., PEG, PVP)
Advantages	Simple, effective in low-salt water	Robust in high-salt or non-aqueous media
Disadvantages	Sensitive to electrolytes	Requires thick polymer layer; higher cost
Best For	Aqueous systems with low ionic strength	High-salt, organic solvents, or broad pH

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NPs by wet ball milling

- Typical surface modifiers include low viscosity pluronics, PVPs, hydroxypropyl celluloses, hydroxypropyl methylcelluloses, PEGs, lecithin, dextran, aerosol OT, Tween 80, sodium lauryl sulfate, docusate sodium, and sodium deoxycholate.
- Drug concentrations as high as 40% can be milled, and milling time can range from minutes to days depending on the energy put into the milling process.
- Milling can be done under refrigerated conditions which minimize thermal degradation.

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The mill can be operated in a batch or a recirculation mode.

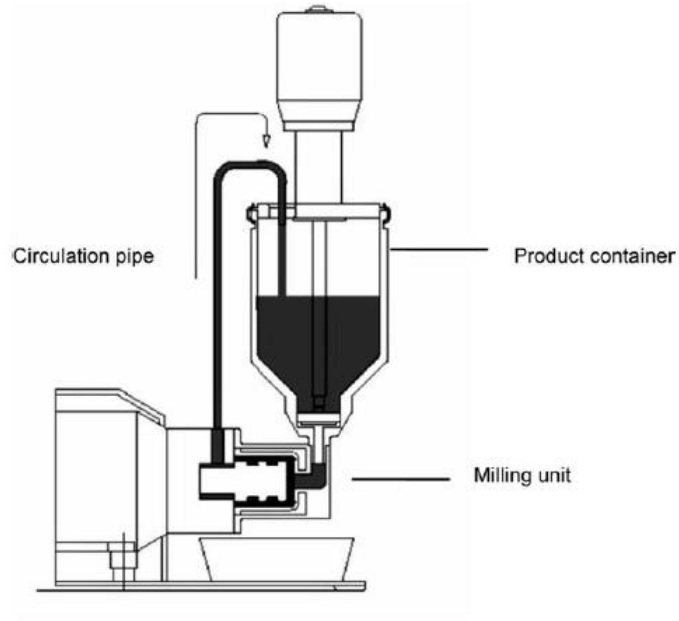

Figure 1 DISPERMAT® SL: schematic view of a bead mill using recirculation method. *Source*: From Ref. 21.

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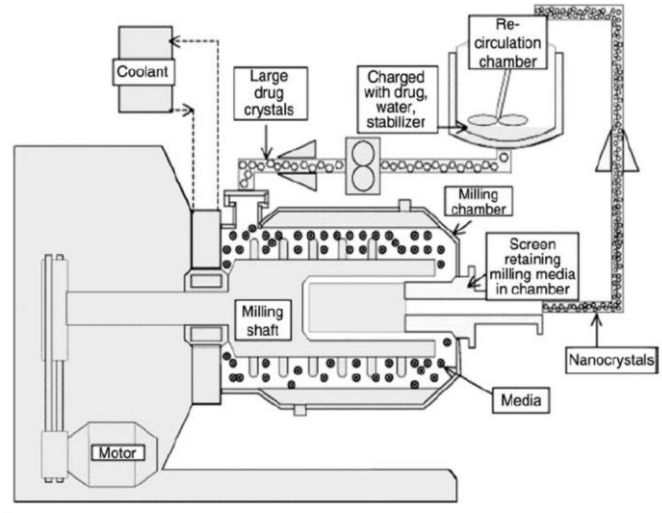
The media milling process

- The milling chamber charged with polymeric media is the active component of the mill.
- A crude slurry consisting of drug, water, and stabilizer is fed into the milling chamber and processed into a nanocrystalline dispersion.
- The typical residence time required to generate a nanometersized dispersion with a mean diameter <200 nm is 30–60 min



. (From Liversidge, E.M.; Liversidge, G.G.; Cooper, E.R. Eur. J. Pharm. Sci. 2003, 18, 113–120).

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Table 1. Marketed products containing drug nanocrystals.

Trade name	INN name	FDA approval	Nanosizing technology	Company	Adminstration route
Rapamune®	Sirolimus	2000	NanoCrystal® (WBM)	Pfizer (Wyeth)	Oral
Emend®	Aprepitant	2003	NanoCrystal® (WBM)	Merck	Oral
Tricor® Lyphantyl®	Fenofibrate	2004	NanoCrystal® (WBM)	Fournier Pharma, Abbott Laboratories	Oral
Triglide®	Fenofibrate	2005	IDD-P® (HPH) high- pressure homogenization	Sciele, Shionogi Pharma Inc.	Oral
Megace® ES	Megestrole acetate	2005	NanoCrystal® (WBM)	PAR Pharmaceuticals	Oral
Invega® Sustenna® Xeplion®	Paliperidone palmitate	2009	NanoCrystal® (WBM)	Janssen	Parenteral, Intramuscular

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Solid polymeric NPs

- Solid NPs can be formed from polymers with the drug incorporated within the polymer matrix or associated onto the particle surface.
- Polymeric NPs are generally formulated from natural or synthetic polymers with the most commonly studied polymers being those which are biodegradable, such as:
  - poly(lactide-co-glycolide) (PLGA),
  - polylactic acid (PLA),
  - polycaprolactone (PCL)
  - chitosan

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Solid polymeric NPs

Several nanoencapulation methods that have been adapted to pharmaceutical use.

Example: Emulsion evaporation method

- Drug and polymer are dissolved in a volatile organic solvent.
- The mixture is emulsified in an aqueous solution to yield very small droplets size (e.g. by high pressure homogenization).
- The resulting emulsion is stirred until most of the organic solvent evaporates, leaving solid NPs that may be washed with water and freeze-dried.
- To facilitate solvent evaporation, the emulsion is often heated slightly above the boiling point of the solvent. For example, when methylene chloride (boiling point: 39.8 °C) is used as an organic solvent, the emulsion is heated to ~40 °C.

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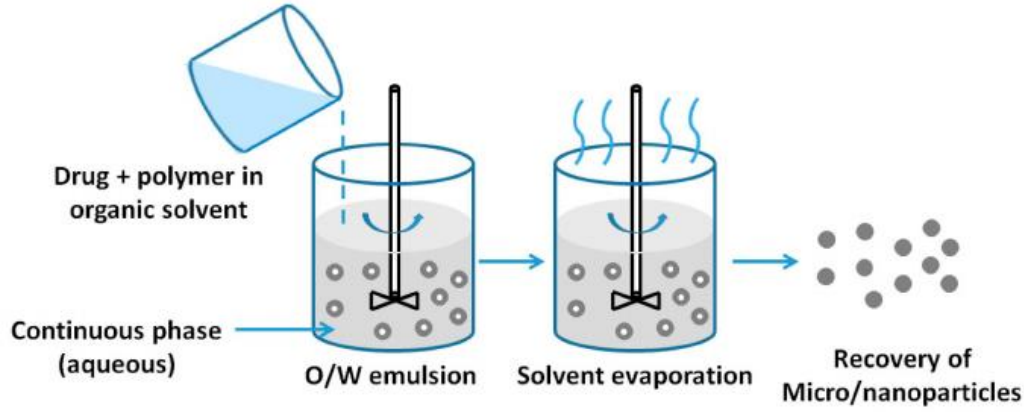

Emulsion evaporation method

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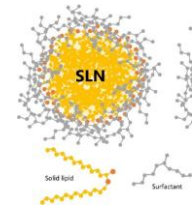
Solid lipid NPs

- These are NPs made from solid (high melting point) lipids dispersed in an aqueous phase.
- Examples of lipids used include:
  - solid triglycerides,
  - saturated phospholipids
  - fatty acids



- The drug is incorporated within the lipid matrix of the particle or by attaching the drug to the lipid nanoparticle surface.
- Solid lipid nanoparticle dispersions have been developed for parenteral, oral, ocular, dermal and cosmetic applications.
- They can be prepared on a large-scale by homogenization to disperse the lipid into an aqueous environment.

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Protein NPs

- The first commercial product based on protein nanotechnology was Abraxane® (paclitaxel).
- Abraxane® consists of 130 nm particles of albumin-bound paclitaxel.
- The drug, paclitaxel, has a low water solubility and requires addition of solubility-enhancing agents to allow its clinical use.
- The albumin functions to coat the paclitaxel and provide colloidal stabilization to the drug and avoid the toxicity issues associated with the use of solubilizers.

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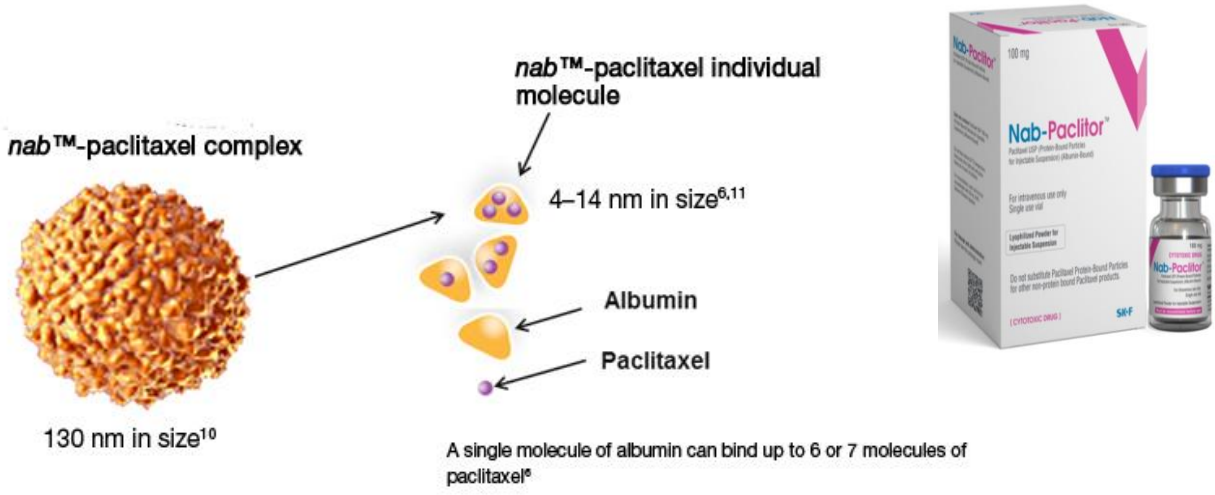

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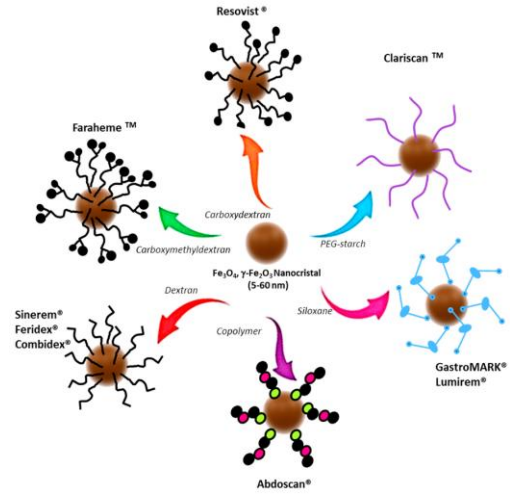
Inorganic NPs

- NPs can be fabricated from inorganic materials including metal oxides, metal sulphides, carbon nanotubes, calcium phosphate and ceramics.
- Disadvantage: they are not biodegradable and so have a more limited application.
- Example: Abdoscan® is an iron oxide nanoparticle formulation which is administered orally and can be used for magnetic resonance imaging (MRI) diagnostics of the bowel, as it is a superparamagnetic.



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Polymer-drug conjugates

- To improve the drug solubility and/ or the delivery of drugs, drug molecules may be conjugated to polymers producing *polymer-drug conjugates*.
- These polymer-drug conjugates are considered as new chemical entities in their own right and, as their overall size is generally below 100 nm, these systems can be classified within the general area of nanotechnology.

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Polymer-drug conjugates

A polymer-drug conjugate can be described as being built of three basic components:

1) A water soluble polymer backbone.

Examples: PEG, poly(ethyleneimine) (PEI), PVP, Polyvinylalcohol (PVA), poly(glutamic acid) (PGA) hydroxypropylmethacrylate (HPMA) copolymers.

2) A linker group.

- to help avoid the therapeutic action of the drug being blocked by the polymer
- can also be designed to be cleaved under certain conditions, such as changes in pH, enzymatic degradation or hydrolysis.

3) Drug

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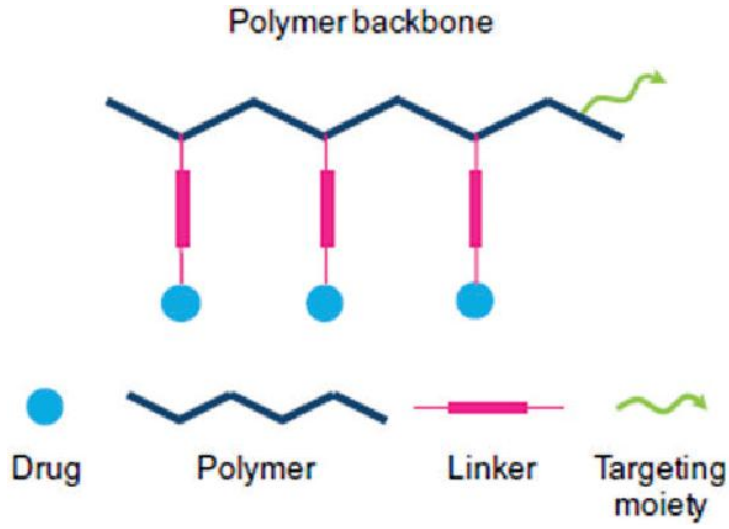

Polymer-drug conjugate

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Polymer-drug conjugates

Water soluble polymer backbone

Examples:

- **Synthetic**: poly(ethyleneglycol) (PEG),
- poly(ethyleneimine) (PEI),
- Poly (vinylpyrrolidone) (PVP),
- Polyvinylalcohol (PVA),
- poly(glutamic acid) (PGA)
- hydroxypropylmethacrylate (HPMA) copolymers.
- **Natural** : dextran, chitosans, hyaluronic acid and proteins can be used.

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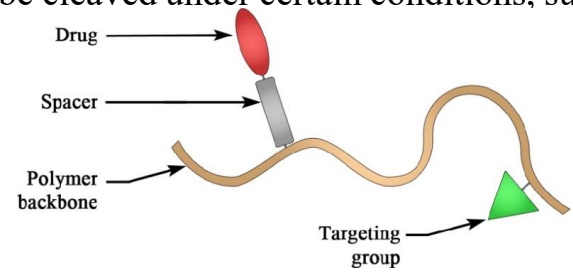
Polymer-drug conjugates

A linker group

- Whilst a drug can be directly covalently bonded to a polymer, it is more common to attach the drug via a *linker* or *spacer group*, to help avoid the therapeutic action o the drug being blocked by the polymer.
- The linker can also be designed to be cleaved under certain conditions, such as:

1. changes in pH,
2. enzymatic degradation or
3. hydrolysis.



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Polymer-drug conjugates

A linker group

- This property can be used to promote the triggered release of the drug from the polymer conjugate under suitable conditions, thereby enhancing drug targeting.
- Examples of linker groups that can be used include:
  - amine
  - carbamate
  - ester groups,
  - amide (the most common option)

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Polymer-drug conjugates

Drug

- Anti-cancer chemotherapeutic agents e.g. doxorubicin and paclitaxel
  - polymer conjugates can improve delivery and reduce unwanted side effects or these drugs which have narrow therapeutic windows.
- Proteins e.g. L-asparaginase or interferons.
  - By conjugating proteins to polymers it is possible to increase their half-life by protecting the proteins from enzyme degradation and reducing clearance rates.

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Liposomes and bilayer vesicles

- Liposomes are closed spherical vesicles consisting of an aqueous core surrounded by one or more bilayer membranes (lamellae) alternating with aqueous compartments.
- These bilayer membranes can be composed of natural or synthetic amphiphilic lipids, and commonly phospholipids are employed for the formulation of liposomes, however a range of amphiphilic lipids can be used.

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Liposomes and bilayer vesicles

- Liposomes form when the lipids (which are surface active with a hydrophilic head group and a hydrophobic chain(s) at opposing ends) are exposed to an aqueous environment.
- Under appropriate lipid-to-water ratio and temperature, the lipids will arrange into bilayer vesicles.
- Unlike micelle formulation, which form spontaneously, energy must be added to the system to drive the formation of liposomes.

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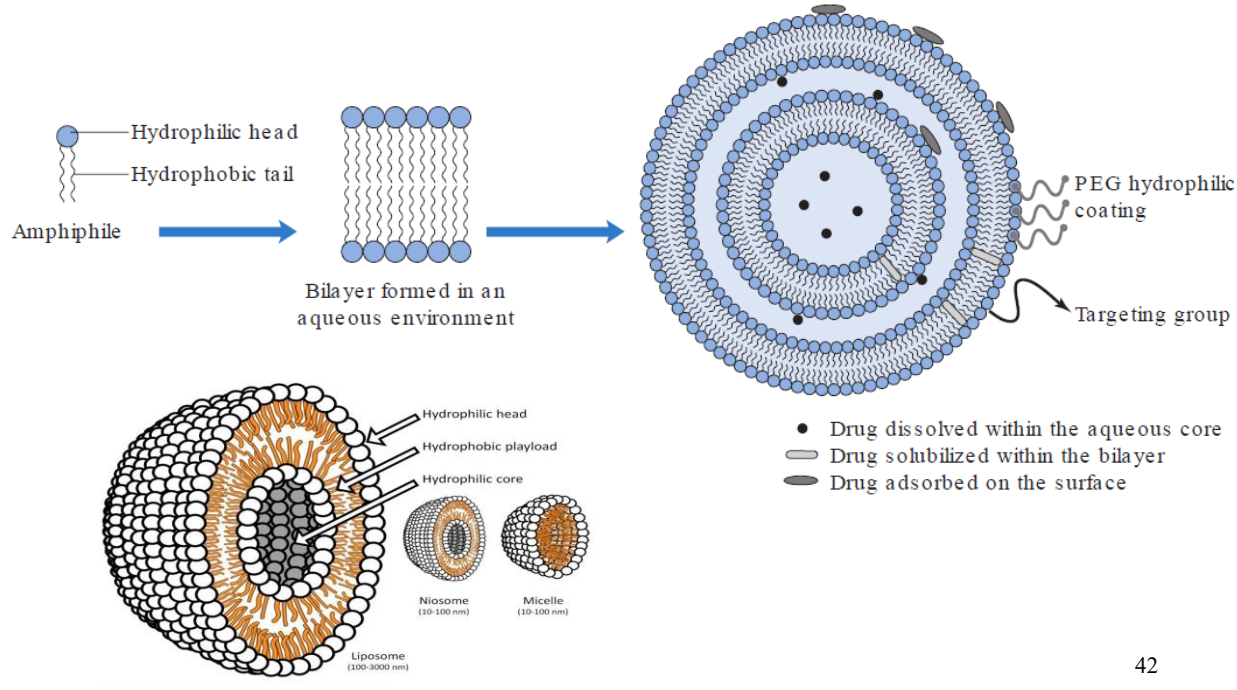

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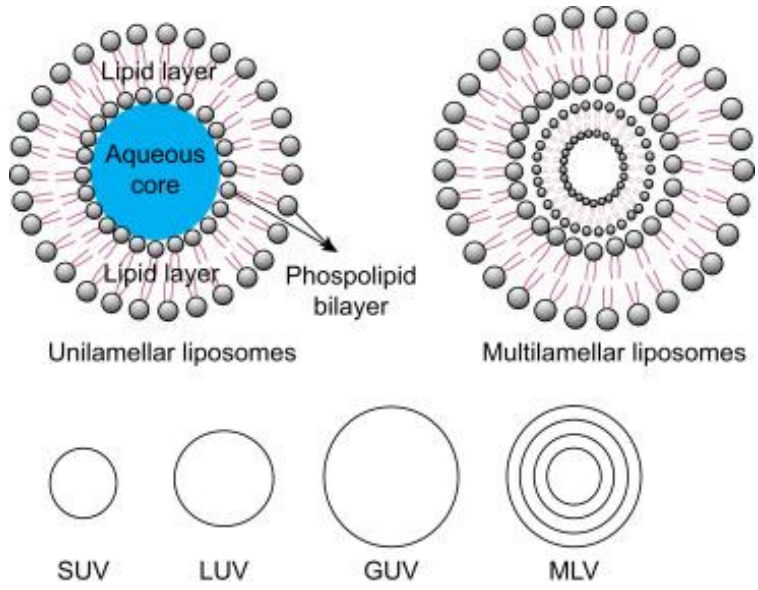

S: Single, L: Large G: Giant U: Uni M: Multi , L: Lamellar V: Vesicle

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Liposomes and bilayer vesicles

- Due to their nature, liposomes are able to carry both water-soluble and lipophilic moieties, with the water-soluble drugs being incorporated within the aqueous compartments and lipid-soluble drugs incorporated within the bilayer.
- In addition, some drugs and molecules can be adsorbed onto the surface of the liposomes through electrostatic interactions.
- Liposomes can be formulated in a range of diameters from around 30 nm up to several micrometres, therefore they can be considered as nanotechnology.

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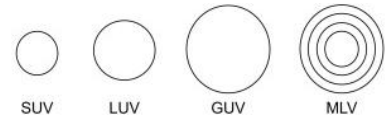
Liposomes and bilayer vesicles

Small unilamellar vesicles (SUV)

- These are single-bilayer vesicles, around 30 to 100 nm in size.
- These are generally easier to prepare in a homogeneous size range compared to other types of vesicles and are the most commonly used in clinically approved products.
- Due to their small size there is a low ratio of internal aqueous volume per mole of lipid.



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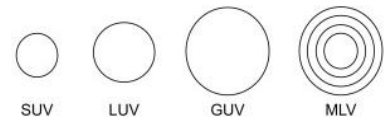
Liposomes and bilayer vesicles

Large unilamellar vesicles (LUV)

- These are large single-bilayer vesicles of 100 nm and greater.
- These vesicles offer a larger aqueous compartment compared with SUVs.

Multilamellar vesicles (MLV)

- These vesicles have multiple concentric bilayers and are 100 nm to several micrometers in size, depending on their composition and their method of preparation.
- Their low aqueous volume (due to the multiple bilayers) reduces their capacity or carrying water-soluble drugs.[



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Liposomes and bilayer vesicles

Examples on clinical applications

- Cancer chemotherapy (e.g. Caelyx® and DaunoXome®)
- Treatment of systemic fungal infections (e.g. AmBisome®)
- Delivery of vaccines (e.g. Inflexal V®)
- Sustained drug release (DepoCyte®and DepoDur®)

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Examples of clinically approved liposome products

Product name	Drug	Formulation	Clinical indications
AmBisome®	Amphotericin B	SUV liposomes. The vesicles are less than 100 nm in size. Amphotericin B is intercalated within the liposome membrane.	An antifungal agent given intravenously for the treatment of systemic fungal infections
DepoCyte®	Cytarabine	Multivesicular vesicles. The vesicles are 3 to 30 µm in size. Cytarabine is entrapped in the aqueous compartments of the liposomes.	Intrathecal treatment of lymphomatous meningitis.
Myocet®	Doxorubicin	SUV liposomes around 45 nm in size. Doxorubicin is loaded into the aqueous core of the liposomes where it forms a doxorubicin citrate complex	First-line treatment of metastatic breast cancer in women.

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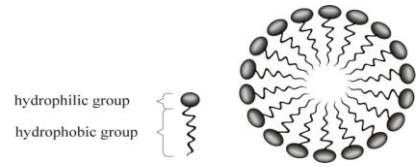
Micelle systems

- Micelles are widely used to formulate low solubility drugs in colloidal solutions.
- Their size (often < 100 nm) means that micelles can be considered within the nanotechnology classification.
- Fugizone® is a mixed micellar formulation which is employed to solubilize Amphotericin B, an antifungal agent used to treat invasive fungal infections, such as systemic candidiasis and histoplasmosis.



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Dendrimers

- Dendrimers are highly branched polymeric, star-shaped macromolecules which can be prepared in the nanosize range.
- There are three main elements to dendrimers:
  1. A central core
  2. The internal dendritic structure, which is composed of the branched polymeric structure built onto the central core
  3. The exterior surface of the dendrimer.
- These branched polymeric structures are synthesized by step-wise addition of layers of polymer branching, referred to as generations (termed G1, G2, etc.)

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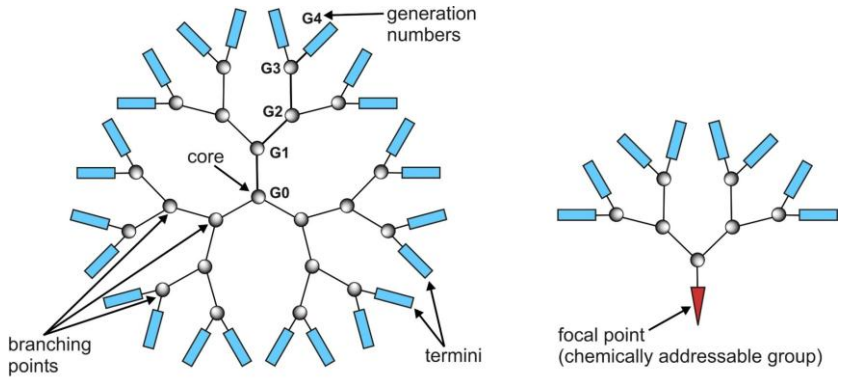

A dendrimer (coloured) with 4 generations labelled G1 to 4 respectively. Drug molecules and targeting groups can be conjugated to the exterior of the dendrimer. PEG can also be added to the exterior surface to provide a hydrophilic coating.

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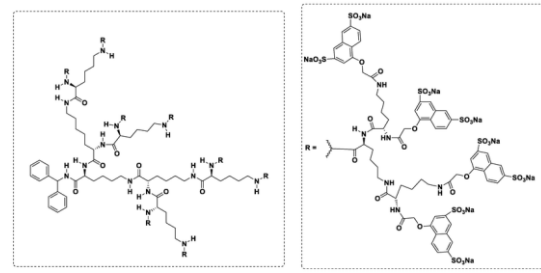
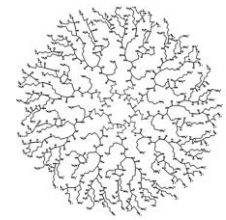
Dendrimers

- **VivaGel®** is a microbicide gel which uses dendrimers.
- In this formulation, the dendrimer is the active ingredient in its own right
- The dendrimer has antiviral properties due to its ability to bind to viruses and thereby blocking their ability to infect cells.





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Nanoencapsulation

- Nanoencapsulation of drugs involves forming drug loaded particles with diameters ranging from 1 to 1000 nm.
- Owing to their small size, NPs exhibit interesting properties, making them suitable for a variety of drug delivery applications.
- One can distinguish two types of NPs:
  - nanomatrices, which are matrix systems (some times termed also nanospheres); and
  - nanocapsules, which are reservoir systems composed of a polymer membrane surrounding an oily or aqueous core.

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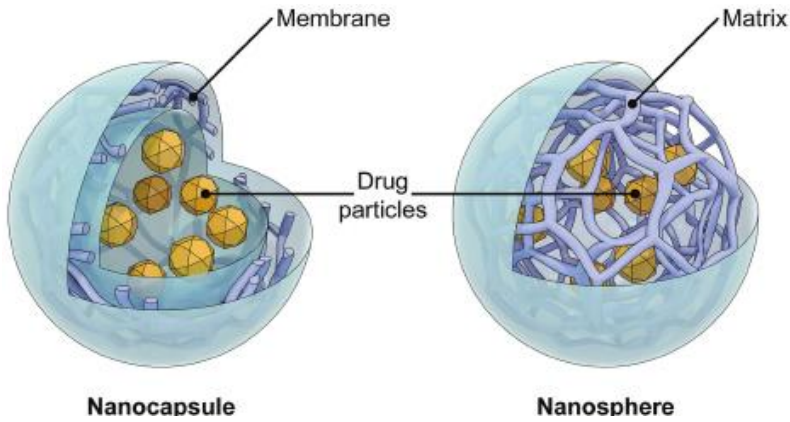

Schematic representation of a nanocapsule (left). nanomatrix (right)
In Nanocapsules, a core-shell structure with a liquid core surrounded by a polymer shell. In nanospheres (matrix), the whole particle consists of a continuous polymer network.

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