جامعة دمشق
كلية الصيدلة
قسم الصيدلانيات و التكنولوجيا الصيدلية

مقرر: التكنولوجيا الصيدلية

السنة الثالثة

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powders

- **Most active and inactive pharmaceutical ingredients occur in the solid state as amorphous powders or as crystals of various morphologic structures.**
- **The term "powder" has more than one connotation in pharmacy. It may be used to describe the physical form of a material, that is, a dry substance composed of finely divided particles. Or, it may be used to describe a type of pharmaceutical preparation, that is, a medicated powder intended for internal (i.e., oral powder) or external (i.e., topical powder) use.**
- **Powders are intimate mixtures of dry, finely divided drugs and/or chemicals that may be intended for internal or external use.**

Micromeritics

- Micromeritics is the science of small particles;
- a particle is any unit of matter having defined physical dimensions.
- It is important to study particles because most drug dosage forms are solids, solids are not static systems, the physical state of particles can be altered by physical manipulation, and particle characteristics can alter therapeutic effectiveness.
- Micromeritics is the study of a number of characteristics, including particle size and size distribution, shape, angle of repose, porosity, true volume, bulk volume, apparent density, and bulkiness.

Important Powder Characteristics

- Size and size distribution
- Morphology and shape factor - microscopy and image analysis
- Specific Surface Area - gas adsorption (N_2 , >0.1 m^2/g)
- Porosity (internal structure) - adsorption-desorption of gas, mercury porosimetry
- Crystalline Phase - X-ray diffraction
- Chemical Composition (purity, additives)
- Homogeneity
- Density (absolute, apparent)* - poured, tapped, He pycnometry
- Internal friction* - angle of repose, shear tester Jenike cell
- Flowability and compressability* - Hall flow meter (Hausner ratio), pressing (414 MPa)

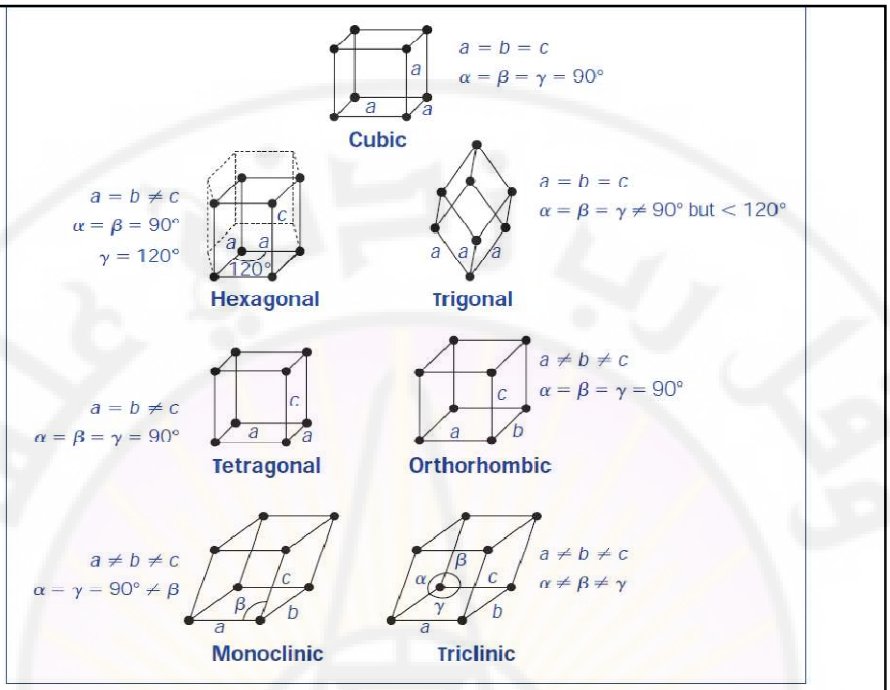
* L. Svarovsky, Powder Testing Guide, Elsevier Applied Science, London, 1987.

Table 1 Outline of the Potential Range of Solid Forms of Relevance to Pharmaceuticals and Their Composition

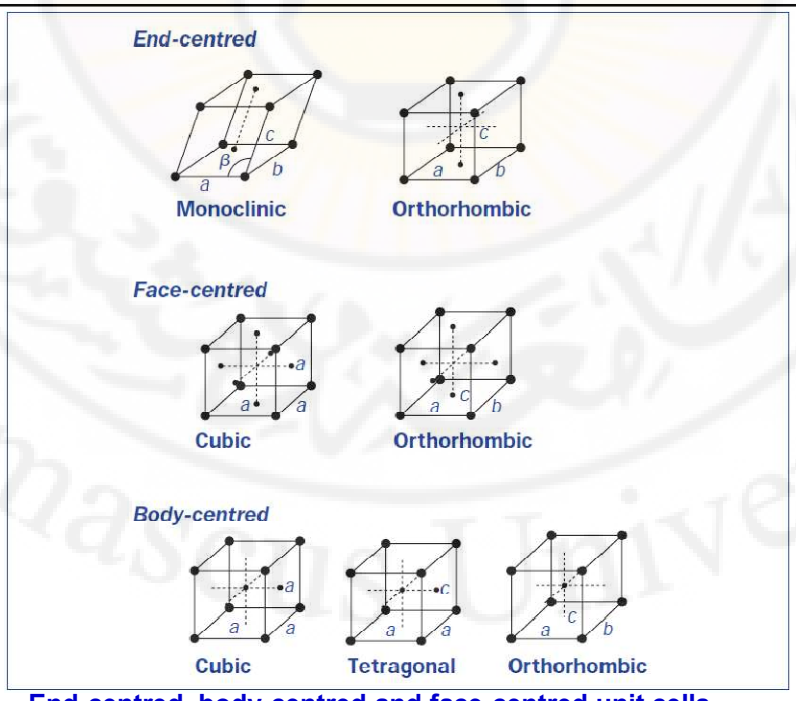
Type	Description	Composition
Crystalline solid	Solid form displaying highly ordered molecular packing. This long-range three-dimensionally ordered structure will give rise to Bragg diffraction of X-rays.	API
Polymorph	Chemically identical crystalline forms in which the constituent molecules adopt different packing arrangements.	API
Crystalline solvate	Solid in which solvent, typically from the crystallization process, is incorporated within the crystal lattice with the solute. The term "pseudopolymorph" is also sometimes used. Lattice comprises API plus solvent.	API plus solvent
Crystalline salt	Ionic crystalline solid formed between an ionized API and counterion.	Charged API plus counterion
Cocrystal	Crystalline solid comprising nonionized API plus additional chemical entity. Also termed "multicomponent crystal" (1) or molecular complex. As for solvate, though cocrystallizing agent is a solid itself at room temperature (2).	API plus cocrystal former
Amorphous	A noncrystalline solid that possesses no long-range order (i.e., crystal lattice) and so does not give rise to Bragg diffraction of X rays.	API

Polymorphism

Systems	Other names	Bravais lattices	Length of axes	Angles between axes
Regular	Cubic Octahedral Isometric Tesseral	Primitive cubic (*P*)	$a = b = c$	$\alpha = \beta = \gamma = 90°$
		Body-centred cubic (*I*)		
		Face-centred cubic (*F*)		
Tetragonal	Pyramidal Quadratic	Primitive tetragonal (*P*)	$a = b \neq c$	$\alpha = \beta = \gamma = 90°$
		Body-centred tetragonal (*I*)		
Orthorhombic	Rhombic Prismatic Isoclinic Trimetric	Primitive orthorhombic (*P*)	$a \neq b \neq c$	$\alpha = \beta = \gamma = 90°$
		Base-centred orthorhombic (*C*)		
		Body-centred orthorhombic (*I*)		
		Face-centred orthorhombic (*F*)		
Monoclinic	Monosymmetric Clinorhombic Oblique	Primitive monoclinic (*P*)	$a \neq b \neq c$	$\alpha = \beta = 90° \neq \gamma$
		Base-centred monoclinic (*C*)		
Triclinic	Anorthic Asymmetric	Primitive triclinic (P)	$a \neq b \neq c$	$\alpha \neq \beta \neq \gamma \neq 90°$
Trigonal	Rhombohedral	Rhombohedral (*R*)	$a = b = c$	$\alpha = \beta = \gamma \neq 90°$
Hexagonal	None	Hexagonal (*H*)	$a = b \neq c$	$\alpha = \beta = 90°$ $\gamma = 120°$

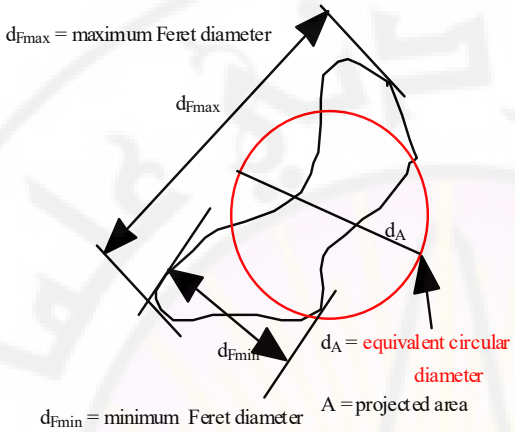


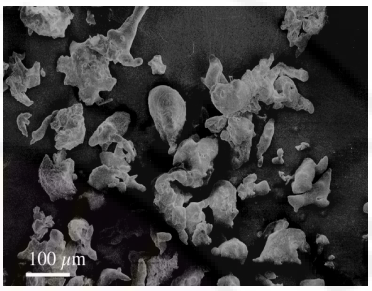
The seven possible primitive unit cells.



End-centred, body-centred and face-centred unit cells.

Diameters and distributions





Particle Diameters

Diameter		Definition
Stokes diameter	d_{st}	Diameter of free falling sphere which would fall at the same rate as the particle in a given fluid
Seive diameter	d_T	Minimum square aperture through which the particle will pass
Volume diameter	d_v	Diameter of the sphere with the same volume as the particle
Surface diameter	d_s	Diameter of the sphere that has the same surface area as the particle
Projected area diameter	d_A	Diameter of the circle which has the same area as the projected area of the particle
Feret's diameter	d_F	Distance between two parallel tangents which touch the outline of the particle projection
Average Feret diameter	d_{Fav}	Average Feret diameter from diameters measured over all angles between 0 and180°
Maximum Feret dia.	d_{Fmax}	Maximum distance between two parallel tangents which touch the outline of the particle projection
Minimum Feret dia.	d_{Fmin}	Minimum distance between two parallel tangents which touch the outline of the particle projection

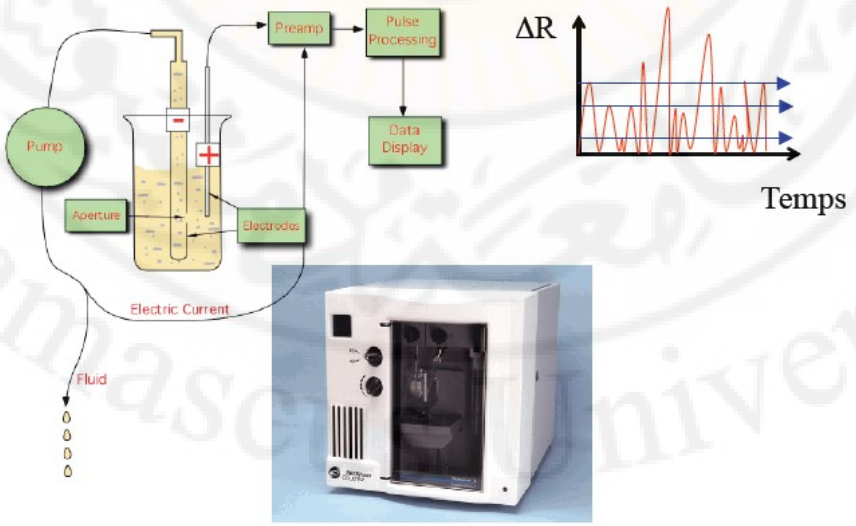
Table. Different definitions of particle diameters

Approximate Size Range of Methods for Particle Size Analysis

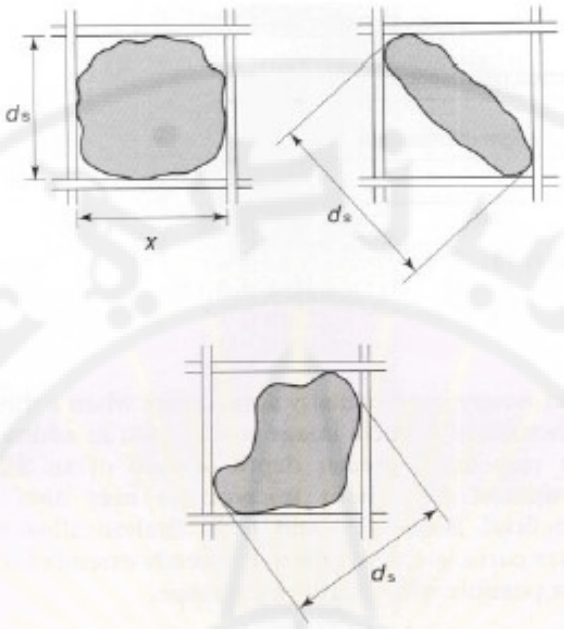
Particle sizing method	Size range (μm)	Comments
Optical microscopy (transmitted, reflected, polarized light, fluorescence, and confocal)	~0.5–600	Important tool for assessing particle size, shape, flocculation, aggregation, and coalescence, etc. Results are subjective and affected by sampling technique
Electron microscopy (transmission and scanning)	~0.01–10	High magnification and direct observation of particle size and shape. Samples need to be dry, coated, or frozen which can affect stability and size. Instrument is relatively expensive and difficult to operate
Electrical sensing zone (Coulter counter)	~0.5–500	Accurate but requires samples containing electrolyte to conduct current
Sedimentation	~1–500	Based on Stokes' law and applied to particles that settle in the dispersion medium by gravity without causing turbulence
Ultracentrifugation	~0.01–5	Based on Stokes' law and applied to particles that can be separated in the dispersion medium under centrifugal force
Sieving	~50–5000	No practical significance for colloidal dispersed systems
Dynamic light scattering (Malvern, Nicomp, Brookhaven)	~0.01–3	Commonly used particle size method for injectable dispersed systems. Upper size limit 3 μm
Static light scattering	~0.02–2000	Commonly used particle size method for injectable dispersed systems
X-ray and neutron scattering	~0.005–10	Similar to light scattering techniques but with better resolution
Size exclusive chromatography or field-flow fractionation	~0.05–20	Particles are separated according to size by interacting with the stationary phase or field force applied to samples (electric, magnetic, or thermal). Requires sample be stable under separating conditions
Optical sensing zone (HIAC, AccuSizer)	~0.5–500	Based on the principle of light blockage and is the commonly used method for coarse-dispersed systems

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Coulter counter



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ı. 10.7 Sieve diameter d_s for various shaped particles

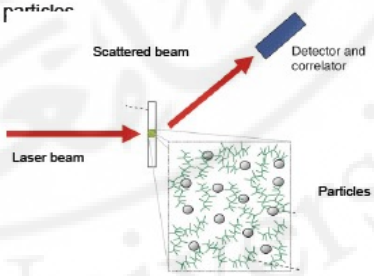
Size measurements

Photon correlation spectroscopy = quasi-elastic light scattering (QELS)
Brownian motion (movement in random direction)
Measurement as a function of time
Smaller particles move with higher velocity than larger particles

$D = K * T / 3\pi\eta d$

Stockes Estein

K : constant
T : temperature
d : diameter
η :viscosity



Adapted from : http://www.esrf.eu/UsersAndScience/Publications/Highlights/2005/SCM/SCM2

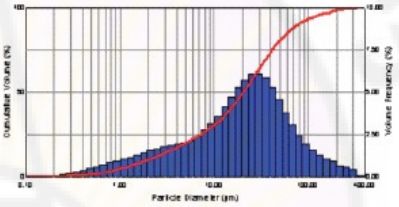
Diffraction of laser beam
Fluctuations in scattering intensity of the laser at a certain angle. Fluctuation depends on the speed which is related to the size
Calculation of the correlation function : diffusion coefficient
Conversion into particle size

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Laser diffraction particle sizing

The technique of laser diffraction is based around the principle that particles passing through a laser beam will scatter light at an angle that is directly related to their size. As the particle size decreases, the observed scattering angle increases logarithmically. The observed scattering intensity is also dependent on particle sizes and diminishes, to a good approximation, in relation to the particle's cross-sectional area. Large particles therefore scatter light at narrow angles with high intensity, whereas small particles scatter at wider angles but with low intensity.



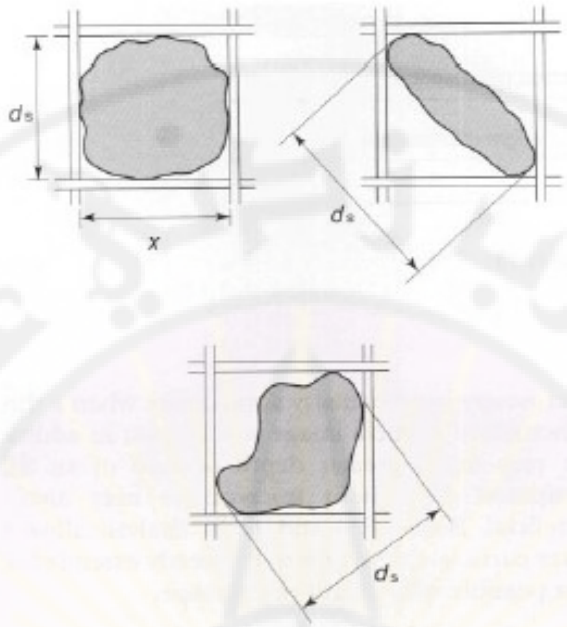


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Laser diffraction particle sizing



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ı. 10.7 Sieve diameter d_s for various shaped particles

Particle size classification of powders

(Ph. Eur. method 2.9.12, Sieve test) ***British Pharmacopoeia 2013***.
The following terms are used in the description of powders:

Coarse powder Not less than 95 per cent by mass passes through a number 1400 sieve and not more than 40 per cent by mass passes through a number 355 sieve.

Moderately fine powder Not less than 95 per cent by mass passes through a number 355 sieve and not more than 40 per cent by mass passes through a number 180 sieve.

Fine powder Not less than 95 per cent by mass passes through a number 180 sieve and not more than 40 per cent by mass passes through a number 125 sieve.

Very fine powder Not less than 95 per cent by mass passes through a number 125 sieve and not more than 40 per cent by mass passes through a number 90 sieve.

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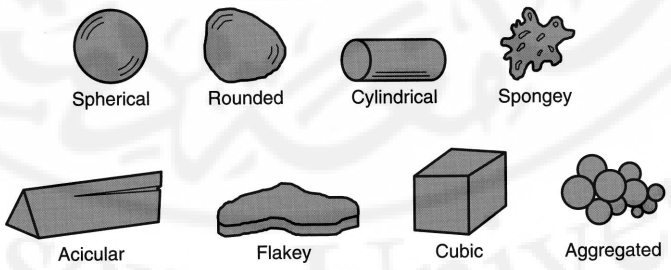
Additional points for monographs other than those of the European Pharmacopoeia

Moderately coarse powder Not less than 95% by weight passes through a number 710 sieve and not more than 40% by weight passes through a number 250 sieve.
Microfine powder Not less than 90% by weight passes through a number 45 sieve.
Superfine powder Not less than 90% by number of the particles are less than 10 µm in size.

Where the cumulative distribution has been determined by analytical sieving or by application of other methods, particle size may be characterised in the following manner:
x_{90} **= particle size corresponding to 90 per cent of the cumulative undersize distribution;**
x_{50} **= median particle size (i.e. 50 per cent of the particles are smaller and 50 per cent of the particles are larger);**
x_{10} **= particle size corresponding to 10 per cent of the cumulative undersize distribution.**

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Particle Shapes



Several of the possible (ideal) particle shapes in powder.

Particle shape

↑ angularity (irregularity) ⇒

Flowability?

↑ Angle of repose

↓ Flow rate through an orifice

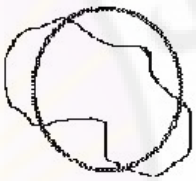
↓ Bulk density

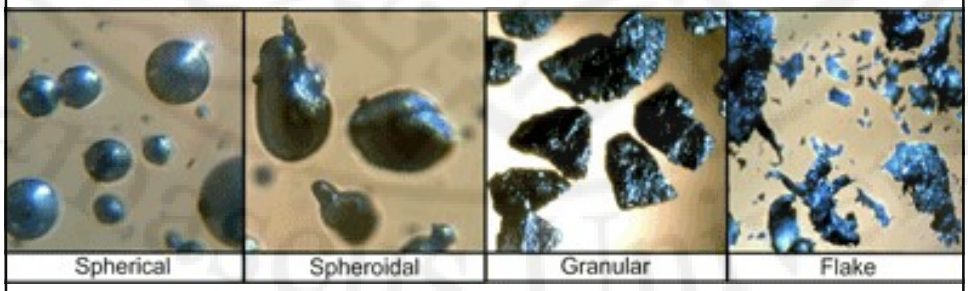
↑ Friction coefficient (griseofluvin Angular particles > rounded particles)

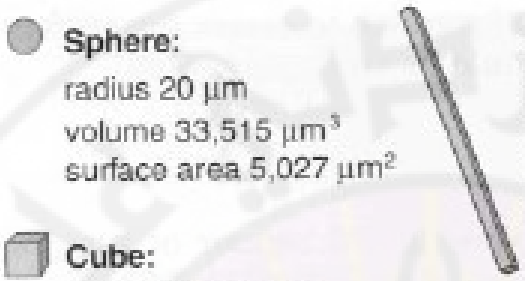
↑ Tablet strength due to ↑ particle interlock

↑ weight variation due to affecting the die-fill weight

↑ Dissolution rate







Sphere:
radius 20 μm
volume 33,515 μm^3
surface area 5,027 μm^2

Needle:
length 335 μm, width and thickness 10 μm
volume 33,500 μm^3
surface area 13,600 μm^2

Cube:
length, width and thickness 32.2 μm
volume 33,386 μm^3
surface area 6,221 μm^2

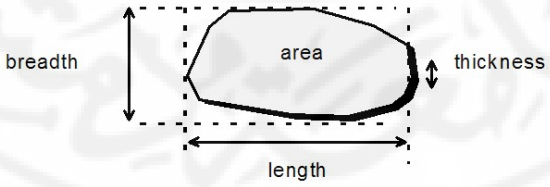
The relative surface areas of a sphere, a cube and a needle that each have similar volumes of material.

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⇒ Microscopy is the most valuable tool in measuring particle shape

⇒ Shape Coefficients

(based on actual particle measurements)



$$\text{elongation ratio} = \frac{\text{length}}{\text{breadth}} \qquad \text{flakiness ratio} = \frac{\text{breadth}}{\text{thickness}}$$

$$\text{bulkiness ratio} = \frac{\text{area}}{\text{length X breadth}}$$

⇒ Shape Factors

(based on measured equivalent diameters)

- sphericity ψ



$$\psi = \frac{\text{surface area of sphere having the same vo}}{\text{surface area of particle}}$$

if the particle is a sphere, $\psi = 1$

as ψ moves away from unity, the particle deviates from a sphere

all the particle sizing techniques refer to an equivalent sphere but measure different properties that are dependant on size.

Coulter Counter ⟶ volume of electrolyte solution

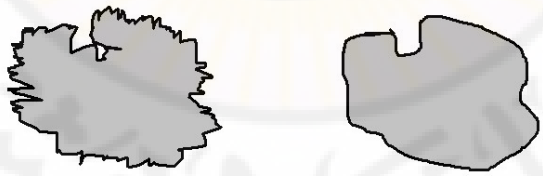
Andresen Pipette ⟶ rate of sedimentation

If the particle is spherical the measured size will be equal

e.g. Coulter - 6.4 μm sedimentation - 5.8 μm

ratio = $\frac{5.8}{6.4}$ = 0.906 ⟹ particles close to spheres

⇒ Fractal Geometry



two particles of the same "shape" but different fractal dimensions.

different ⇒ surface texture How?

⇒ flow properties

⇒ dissolution properties Why?

Particle Flow

For powders to be free-flowing, they need to be able to pass over each other.

Adhesive and cohesive forces tend to "stick" particles together. These forces tend to be short-range van der Waal's forces.

The intensity and quantity of adhesive and cohesive forces vary with:

- Area of Interaction which relates to particle size, particle shape, and surface topography.
- Surface free energy which relates to molecular arrangement and particle density.
- Humidity which relates to particle Hygroscopicity and relative humidity.
- Electrostatic Force

Observations

- Smaller particle sizes generally show greater friction and steeper angles
- Spherical shapes have the lowest interpartical friction
- As shape deviates from spherical, friction between particles tends to increase
- Easier flow of particles correlates with lower interparticle friction
- Lubricants are often added to powders to reduce interparticle friction and facilitate flow during pressing

Powder flow testing methods

1. ***Angle of Repose.***

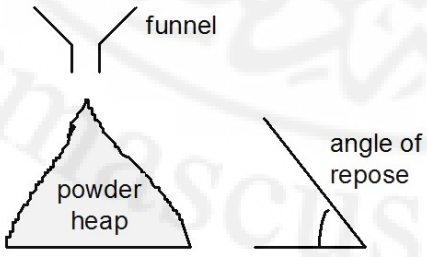
2. ***Bulk Density;***

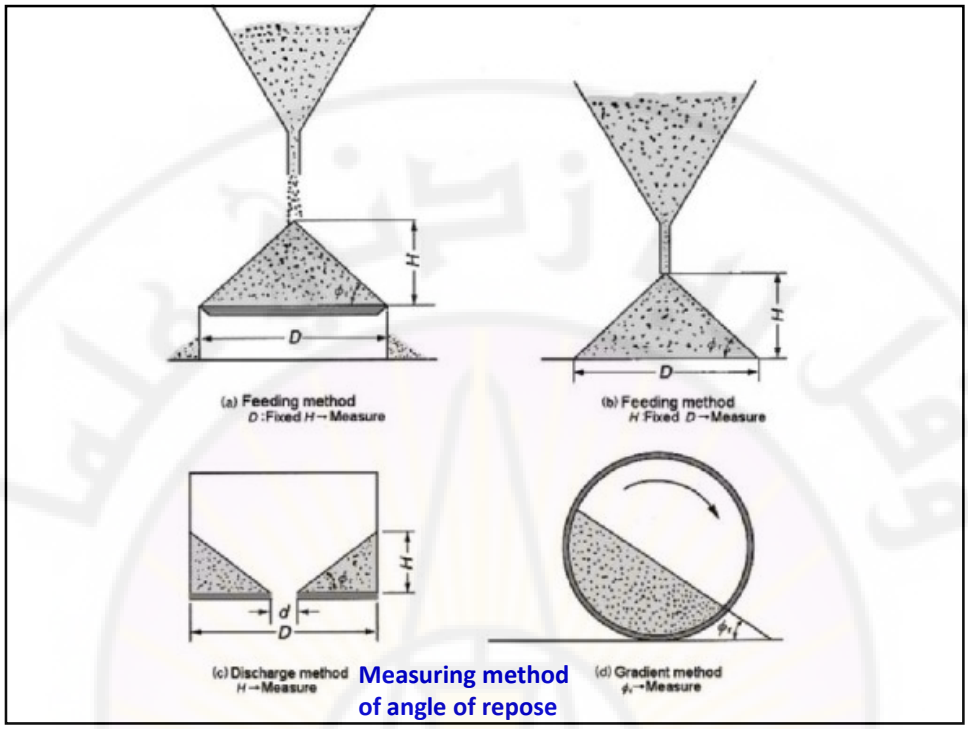
   - Hausner Ratio.
   - Carr Compressibility Index.

Angle of Repose

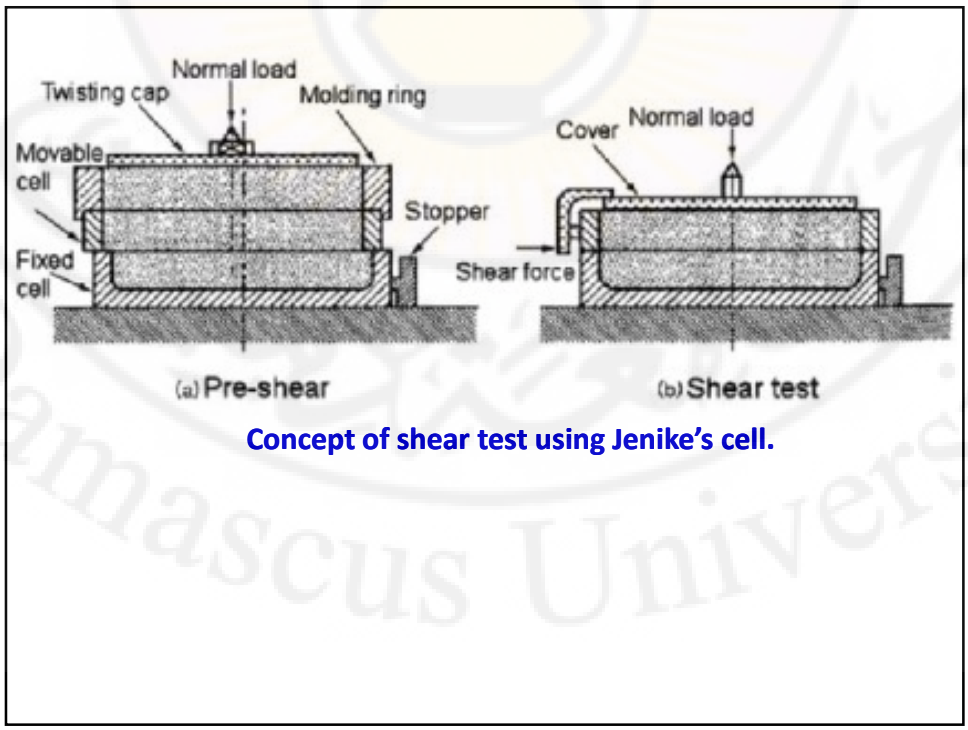
A particle bed will begin to flow if the tilt angle is large enough to overcome the frictional forces
A particle bed will stop flowing if the tilt angle is below that which is required to overcome adhesion/cohesion

After pouring particles into a heap, the angle of a powder bed at rest (repose) is indicative of the adhesive/cohesive forces within the powder bed.





Measuring method of angle of repose



Concept of shear test using Jenike's cell.

$$\tan(\alpha) = \frac{\text{height}}{0.5 \times \text{base}}$$

Table 2.9.36.-1. – *Flow properties and corresponding angles of repose*[2]

Flow property	Angle of repose (degrees)
Excellent	25-30
Good	31-35
Fair (aid not needed)	36-40
Passable (may hang up)	41-45
Poor (must agitate, vibrate)	46-55
Very poor	56-65
Very, very poor	> 66

Particle Density Measures

- True density - density of the true volume of the material
  - The density of the material if the powders were melted into a solid mass
- Bulk density - density of the powders in the loose state after pouring
  - Because of pores between particles, bulk density is less than true density

Bulk Density

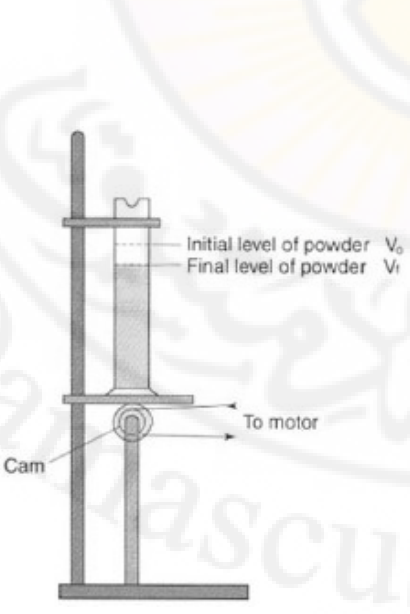
A particle has only one true density which is related to the internal molecular packing.

A collection of particles can have an infinite number of bulk densities depending on how the particles pack.

Bulk density, ρ_B, is the mass of powder, M, occupying a volume, V.

$$\rho_B = M/V$$

The bulk density is always less than the true density.





Mechanical tapping device
(joiting volumeter.).

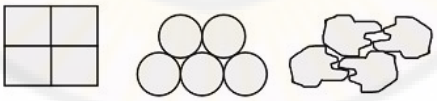


Tapped density tester. (Courtesy of Varian Inc.)

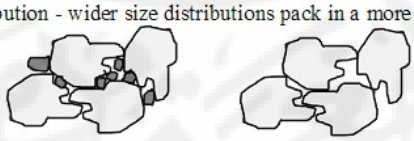
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Bulk density is affected by:

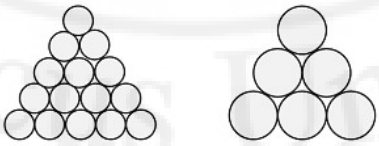
- Particle shape - different shaped particles will have different optimum packing geometry's.



- particle size distribution - wider size distributions pack in a more efficient manner



- Both large and small particles of the same shape have the same packing geometry.

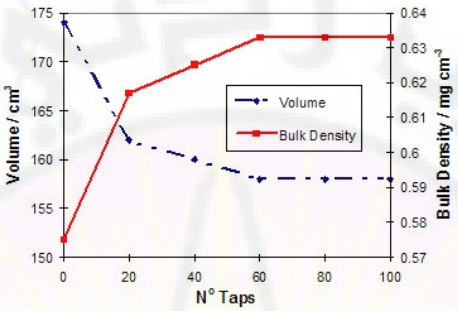


This means that particle size does not affect bulk density.

Measuring Particle Packing

⇒ Handling and processing of a powder will affect its packing geometry & therefore its bulk density.

By slight vibration, the particles can be mobilised and when the vibration is stopped, the particles will have adopted a different packing arrangement. In general, particles become more tightly packed upon tapping (vibration).



Poorly flowing powders initially adopt a non-optimum packing with a high fractional voidage

Good flowing powders initially adopt a packing which is close to optimum.

⇒ Small change in bulk density ⇨ good flow

⇒ Large change in bulk density ⇨ bad flow

The particle flowability is mathematically described using the bulk density values as following indices:

$$\text{Hausner Ratio} = D_f / D_0$$

$$\text{Carr Compressibility Index} = [(D_f - D_0 / D_f) \times 100]$$

D_o is the initial (poured, fluff) bulk density, and D_f is the final (equilibrium, tapped) bulk density

$$Compressibility\ Index = 100 \times \frac{V_0 - V_f}{V_0}$$

$$Hausner\ Ratio = \frac{V_0}{V_f}$$

$$Compressibility\ Index = 100 \times \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}}$$

$$Hausner\ Ratio = \frac{\rho_{tapped}}{\rho_{bulk}}$$

Table 2.9.36.-2. – *Scale of flowability*(2)

Compressibility index (per cent)	Flow character	Hausner ratio
1-10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
> 38	Very, very poor	> 1.60

خواص الانسيابية و الانضغاطية:

الجدول من دستور الأدويةUSP 34:

Flow properties	Angle of repose (º)	Compressibility index (%)	Hausner ratio
Excellent	25-30	<10	1.0-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	45-46	26-31	1.35-1.45
Very poor	46-56	32-37	1.46-1.59
Very very poor	>56	>38	>1.60

Consequences of poor flow

Poor flow from hoppers into tablet or capsule filling machines

* reduces the weight uniformity (dose uniformity) of the product.
* affects the mechanical properties of tablets (breakage).

Approaches for improving flow

⇒ Alter the particle shape/size/texture: (smooth spherical particles have the best flow).

⇒ Processing conditions: lower humidity, hopper shape.

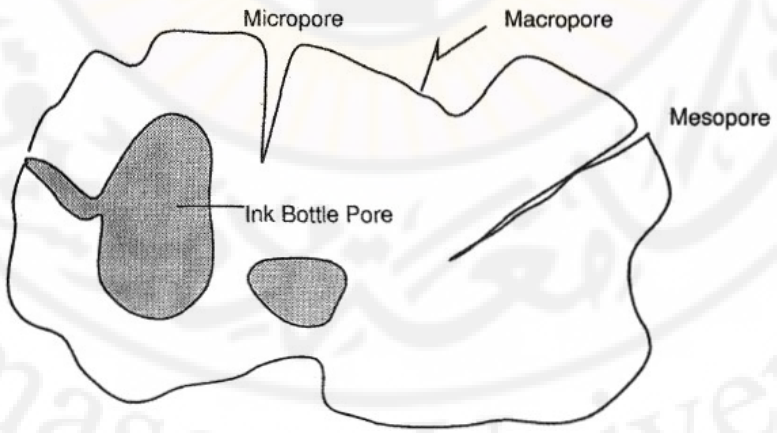
⇒ Addition of additives (glidants/lubricants): talc, maize starch, magnesium stearate.

Danger! Altering one process can adversely affect other processes. An increase in the magnesium stearate content needed to improve powder flow can produce softer tablets, decrease the disintegration time and increase the dissolution time.

Packing Factor

Bulk density divided by true density

- Typical values for loose powders range between 0.5 and 0.7
- If powders of various sizes are present, smaller powders will fit into spaces between larger ones, thus higher packing factor
- Packing can be increased by vibrating the powders, causing them to settle more tightly
- Pressure applied during compaction greatly increases packing of powders through rearrangement and deformation of particles



S chematic representation of pores in a granule.

Porosity

Ratio of volume of the pores (empty spaces) in the powder to the bulk volume

- In principle

  Porosity + Packing factor = 1.0

- The issue is complicated by possible existence of closed pores in some of the particles
- If internal pore volumes are included in above porosity, then equation is exact

الكثافة والمسامية

الكثافة والمسامية Density and Porosity

الكثافة الحقيقية True density: وزن بلورة مسحوق بحجم ١ سم^3 .

الكثافة الظاهرية Apparent density: وزن مسحوق حجمه ١ سم^3 تم ذره بشكل حر

الكثافة الربتية Tapped density: وزن مسحوق حجمه ١ سم^3 بعد الهز والنقر

كثافة التعبئة Bulk Density : وزن مسحوق حجمه ١ سم^3 بعد الهز والنقر بشكل حر على آلات التعبئة.

المسامية Porosity

للمسحوق الصيدلاني الناعم له حجم تعبئة V يتألف من حجمين :

-Va - حجم الهواء أو المسام بين الأجزاء.

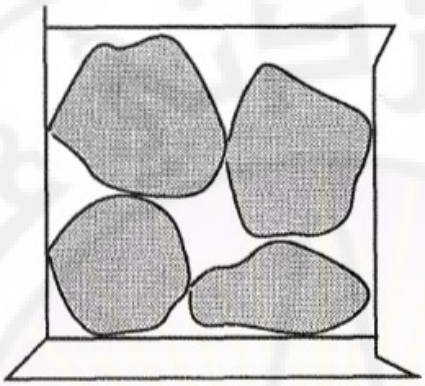
- Vs - حجم المادة الصلبة.

- V = Va + Vs

-مسامية المسحوق (ε)هي النسبة بين حجم الهواء Va وبين الحجم العام للمسحوق V

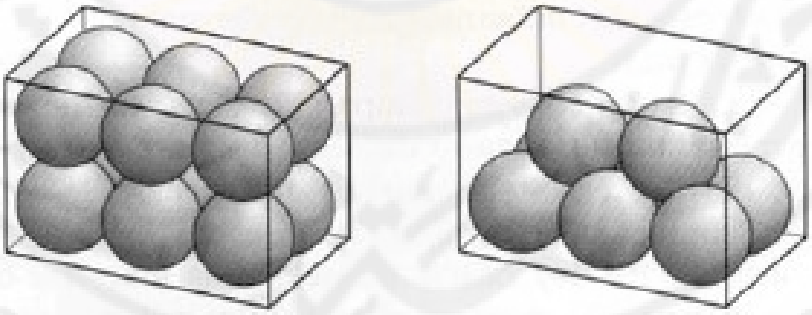
$$\varepsilon = Va/V$$

الكثافة والمسامية



Total Volume V
Volume of Solid V_s

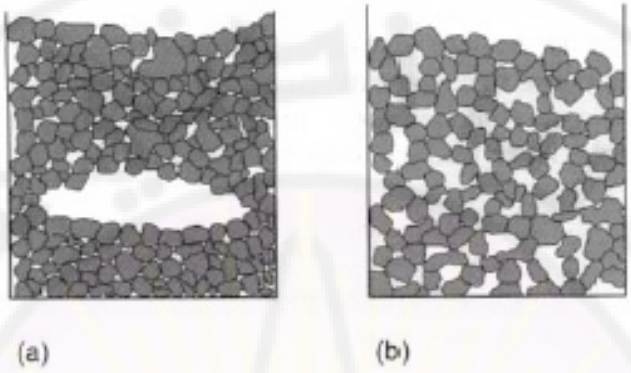
Schematic for definition of fractional volume and apparent density.



(a) (b)

Different geometric packings of spherical particles: (a) cubic packing; (b) rhombohedral packing.

تعبئة مختلفة لأجزاء أو جسيمات كروية الشكل



Two equidimensional powders having the same porosity but different packing geometries.

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Porosity المسامية

العلاقة بين الكثافة الحقيقية ρ و الكثافة الظاهرية ρ' و هي:

$$\rho' / \rho = Vs / V$$

العلاقة بين الكثافة الحقيقية ρ و الكثافة الظاهرية ρ' و المسامية ε كما يلي:

$$W = \rho' . V \qquad W = \rho . Vs$$

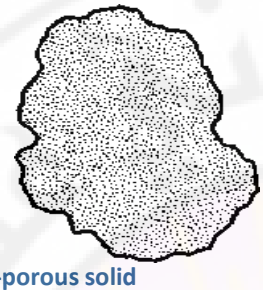
$$\rho' = \rho . Vs / V \qquad \rho' . V = \rho . Vs$$

$$(V - Vs) / V = 1 - \rho' / \rho \qquad \rho' / \rho = Vs / V$$

$$\varepsilon = 1 - \rho' / \rho \qquad \varepsilon = Va / V$$

W : وزن المادة الصلبة

What are Porous Materials?



Non-porous solid
- Low specific surface area
- Low specific pore volume



Porous solid
- High specific surface area
- High specific pore volume

Porous materials have highly developed internal surface area that can be used to perform specific function.
Almost all solids are porous except for ceramics fired at extremely high temperatures

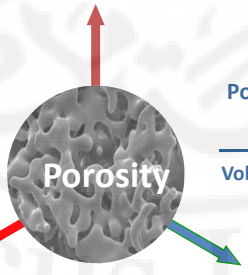
F. Rouquerol, J. Rouquerol, K. S. W. Sing, Adsorption by Powders and Porous Solids, Academic Press, 1-25, 1999

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Measure of Porosity

There are three parameters used as a measure of porosity; specific surface area, specific pore volume or porosity, and pore size and its distribution.

$$\text{Specific Surface Area, } m^2/g = \frac{\text{Total surface area, } m^2}{\text{Mass of the solid, g}}$$



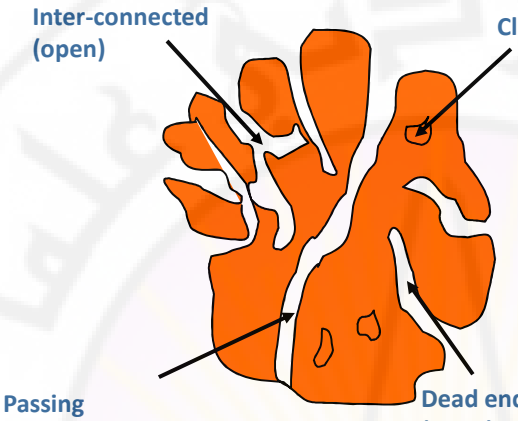
$$\text{Porosity, \%} = \frac{\text{Volume of pores}}{\text{Volume of solid (including pores)}} \times 100$$

Pore size and its distribution

$$\text{Specific Pore volume, } cm^3/g = \frac{\text{Total pore volume, } cm^3}{\text{Mass of the solid, g}}$$

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Concept of Porosity: Open vs. Closed Pores



Open pores are accessible whereas closed pores are inaccessible pores. Open pores can be inter-connected, passing or dead end.

F. Rouquerol, J. Rouquerol, K. S. W. Sing, Adsorption by Powders and Porous Solids, Academic Press, 1-25, 1999

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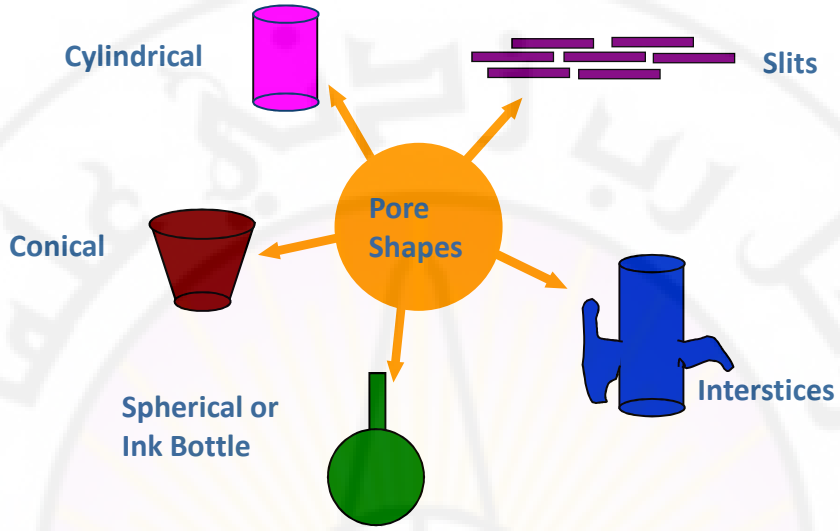
Size of Pores (IUPAC Standard)



Porous material are classified according to the size of pores: material with pores less than 15 Å are called micropores, materials with pores between 15 and 1000 Å are called mesopores, and material with pores greater than 50 nm are macrospores

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Shapes of Pores



F. Rouquerol, J. Rouquerol, K. S. W. Sing, Adsorption by Powders and Porous Solids, Academic Press, 1-25, 1999

Porosity

Porosity is the void space in a material and it can be calculated using data from a number of following techniques

- Density
- Gas adsorption
- Water displacement
- Porosimetry

Pore size measurements provide information about the actual pore structure of a material, including the pore radii and volume.

These measurements are usually obtained using

- Gas adsorption
  - Limited to pore radii < 1000 Å
- Mercury porosimetry
  - Much larger pores (e.g. interparticle spaces)
  - More applicable to many pharmaceutical applications

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Porosity

The pore structure of a solid can contribute to

- Disintegration
- Dissolution
- Adsorption
- Diffusion

Classification system of pore sizes

Pore designation	Pore size (Å)
Macropores	> 1000
Transitional (Mesopores)	15-1000
Micropores	< 15

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Porosimetry Theory and Equations

The affinity of a liquid for a solid can be described as wetting.

Spreads spontaneously
Wetting liquid

Stationary and appears spherical
Nonwetting liquid

A measure of the degree of wetting is the Contact angle (θ)



(a) Wetting liquid, $\theta < 90°$;

$\theta < 90°$ is considered a wetting liquid

(b) nonwetting liquid, $\theta > 90°$

$\theta > 90°$ is considered a nonwetting liquid

The measured contact angle of mercury on various samples can range from 112 to 170°

For most applications the average value of 140° is used

What about accuracy?

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Accuracy of porosimetry

The accuracy of the pore radii measurement

is limited by the accuracy of the contact angle measurement

Contact angles can readily be measured on flat surfaces or compacts of powders

using Washburn equation

$$r = \frac{-2\gamma \cos\theta}{p}$$

r = pore radius (μm)

γ = surface tension of the liquid

θ = contact angle between the liquid and the sample

p = applied pressure (psi)

What is the relationship (r / p)?

For mercury

γ = 480 dyne/cm

θ = 140°

The problem of accuracy (same sample, different samples)?

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Table 2.9.5.-1

Pharmaceutical Form	Average Mass	Percentage deviation
Tablets (uncoated and film-coated)	80 mg or less	10
	More than 80 mg and less than 250 mg	7.5
	250 mg or more	5
Capsules, granules (uncoated, single-dose) and powders (single-dose)	Less than 300 mg	10
	300 mg or more	7.5
Powders for parenteral administration* (single-dose)	More than 40 mg	10
Suppositories and pessaries	All masses	5
Powders for eye-drops and powders for eye lotions (single-dose)	Less than 300 mg	10
	300 mg or more	7.5

* When the average mass is equal to or below 40 mg, the preparation is not submitted to the test for uniformity of mass but to the test for uniformity of content of single-dose preparations (*2.9.6*).

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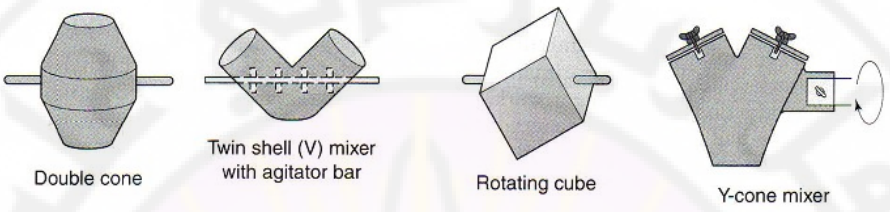
EFFERVESCENT POWDERS

Effervescent powders are presented as single-dose or multidose preparations and generally contain acid substances and carbonates or hydrogen carbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

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Using a combination of citric and tartaric acids rather than either acid alone avoids certain difficulties. When tartaric acid is used as the sole acid, the resulting granules readily lose their firmness and crumble.
Citric acid alone results in a sticky mixture difficult to granulate.

Mixing



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Conclusions

- Particles – just like people come in all shapes and sizes



Characterise

Characterise

Characterise

NANOPARTICLES - PREPARATION AND APPLICATIONS

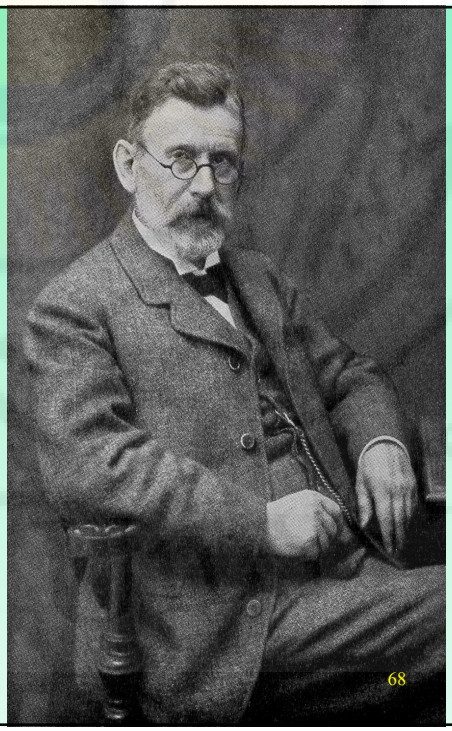
الجسيمات النانومترية – تحضير و تطبيقات

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DRUG TARGETING

توجيه الدواء

PAUL EHRLICH



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DEFINITION OF NANOPARTICLES

تعريف الجسيمات النانومترية

Nanoparticles are solid polymeric particles of a size between 10 and 1000 nm into which drugs or biologically active materials are incorporated, surface adsorbed or chemically bound.

- الجسيمات النانومترية عبارة عن جسيمات بلمرية أي من بلمرات (و قد تكون من مصدر آخر غير البلمرات) ذات حجم يتراوح بين ١٠ – ١٠٠٠ نانومتر، قادرة على أن تشتمل أو تضم في بنائها على الأدوية أو المواد الفعالة حيوياً، و يتم ذلك من خلال الإدمصاص على سطحها أو بتشكيل روابط كيميائية

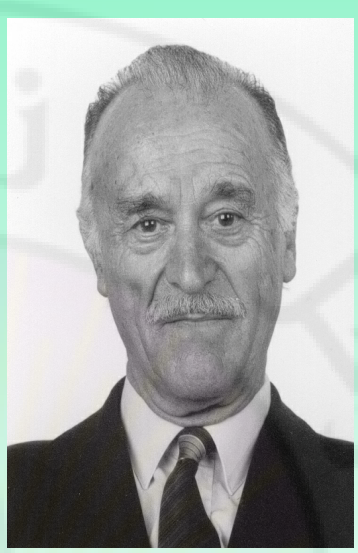
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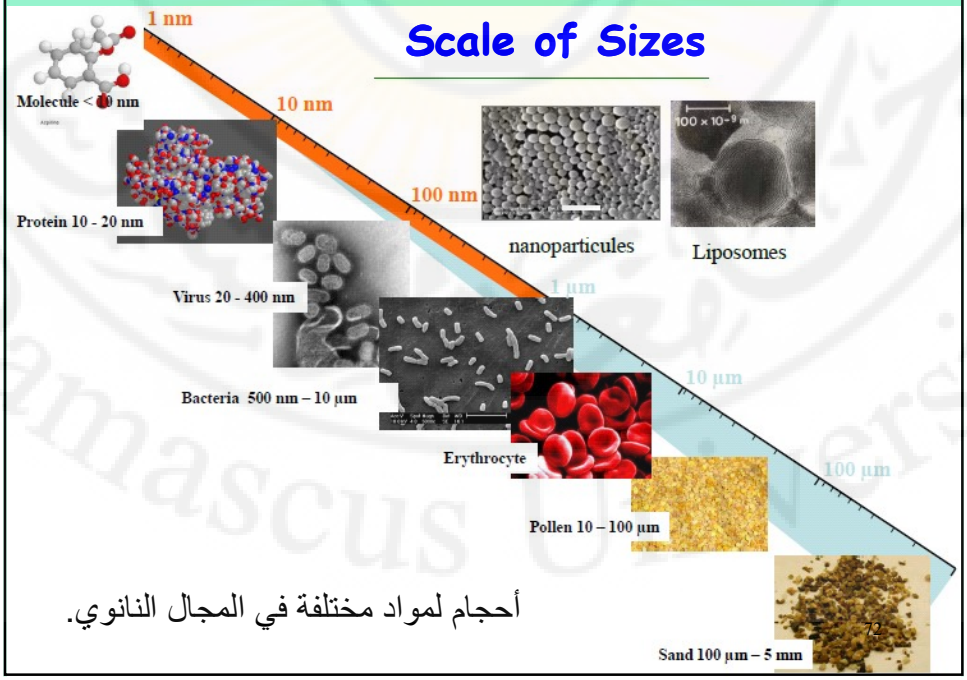
NANO-PARTICLES

PETER SPEISER



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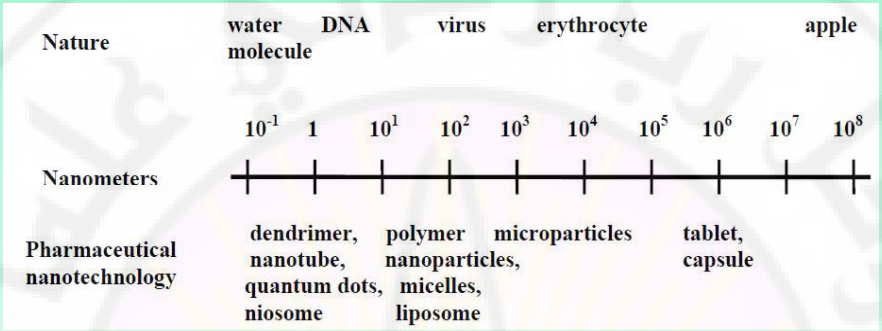
Scale of Sizes



أحجام لمواد مختلفة في المجال النانوي.

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Scale of Sizes



أحجام لمواد مختلفة في المجال النانوي.

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FEW GENERAL REMARKS
ملاحظات عامة

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Why do we need a pharmaceutical carrier?

لماذا نحتاج النواقل الصيدلانية

1. Protect a drug from the body

لحماية الدواء من الجسم.

2. Protect the body from the drug

لحماية الجسم من الدواء.

3. Adjust pharmacokinetics, i.e. distribution and clearance

لضبط حركية الدواء مثل: التوزع والتصفية.

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من أهم ميزات الحجم النانوي Nano sizing

- **زيادة مساحة السطح.**
- **زيادة الذوبانية.**
- **زيادة معدل الذوبان.**
- **زيادة التوافر البيولوجي الفموي.**
- **بداية أسرع للفعل العلاجي.**
- **استخدام كمية أقل من الجرعة المطلوبة.**
- **إنقاص الفروقات العلاجية في حال أخذ الدواء قبل الطعام (حالة الصيام)/بعد الطعام.**
- **إنقاص فروقات الجرعة من مريض لآخر.**

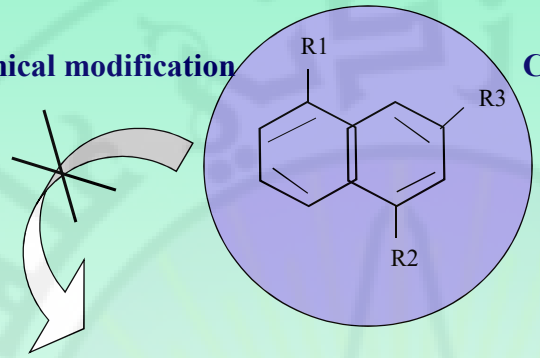
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•علم النانو:
يعريف على أنه دراسة ظواهر المواد على المستوى الذري والجزيئي و فوق الجزيئي والتعامل معها.
•التكنولوجيا النانوية:
وهي التكنولوجيا المتعلقة بتحديد المواصفات البُنى والأجهزة والنظم و إنتاجها وتطبيقاتها من خلال التحكم في الشكل والحجم ضمن أبعاد النانومتر.
•التكنولوجيا النانوية الصيدلانية:
تتضمن تطبيقات علم النانو في الصيدلة كالمواد النانوية والأجهزة النانوية مثل نظم إيتاء الدواء، أجهزة التشخيص، التصوير، والحساسات البيولوجية biosensor.
•الدواء النانوي:
يعرف على أنه واحدات بأبعاد أقل من واحد ميكرومتر، ويستخدم لعلاج النظم البيولوجية، و تشخيص، إصاباتها، و مراقبتها، و التحكم بها

توفر التكنولوجيا النانوية الصيدلانية نوعين أساسين من الأدوات النانوية:
١) المواد النانوية Nanomaterials
٢) والأجهزة النانوية Nanodevices
إن الأجهزة النانوية هي أجهزة صغيرة جداً ذات أبعاد نانومترية وتتضمن النظم الكهرو ميكانيكية الدقيقة nano- and micro-electromechanical systems (NEMS/ MEMS)، الخلايا التنفسية (الكريات الحمراء الصنعية)، والمنظومات المكروية microarrays (تقوم بأشكال مختلفة من المعايرات البيولوجية مثل معايرة الدنا والبروتينات والخلايا والأضداد).

PRINCIPALES OF TARGETTING

Chemical modification



Carrier utilisation

pKa Lipophilicity Fixation to proteines	→	Distribution Elimination
Size Lipophilicity Charge Stability	→	Distribution Elimination

PHARMACEUTICAL NANOCARRIERS

النواقل النانومترية

soluble polymers البلمرات المنحلة cyclodextrines السيكلودكسترينات

microcapsules المحافظ الميكرونية dendrimers البلمرات المتغصنة

microparticles الجسيمات الميكرونية

carbon nanotubes الأنابيب النانومترية للكربون

cells الخلايا fullerenes الفوليرن

cell ghosts خَلايا شَبَحِيَّة drug nanocrystals البلورات النانومترية للدواء

liposomes الليبوزومات

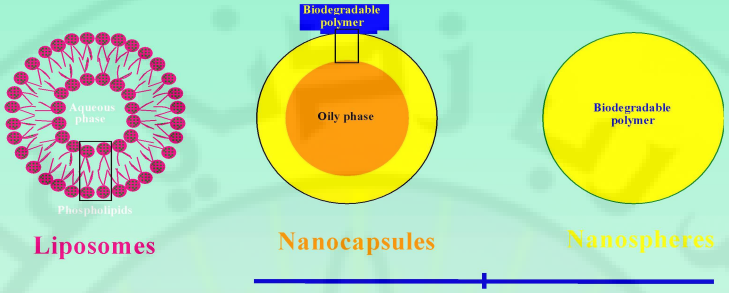
gold nanoparticles جسيمات الذهب النانومترية

micelles المذيلات Ca phosphate

niosomes النيوزوم silica nanoparticles الجسيمات النانومترية للسيليكا

solid lipid particles الجسيمات الشحمية الصلبة lipoproteins الليبوبروتينات

PHARMACEUTICAL NANOCARRIERS



Liposomes Nanocapsules Nanospheres

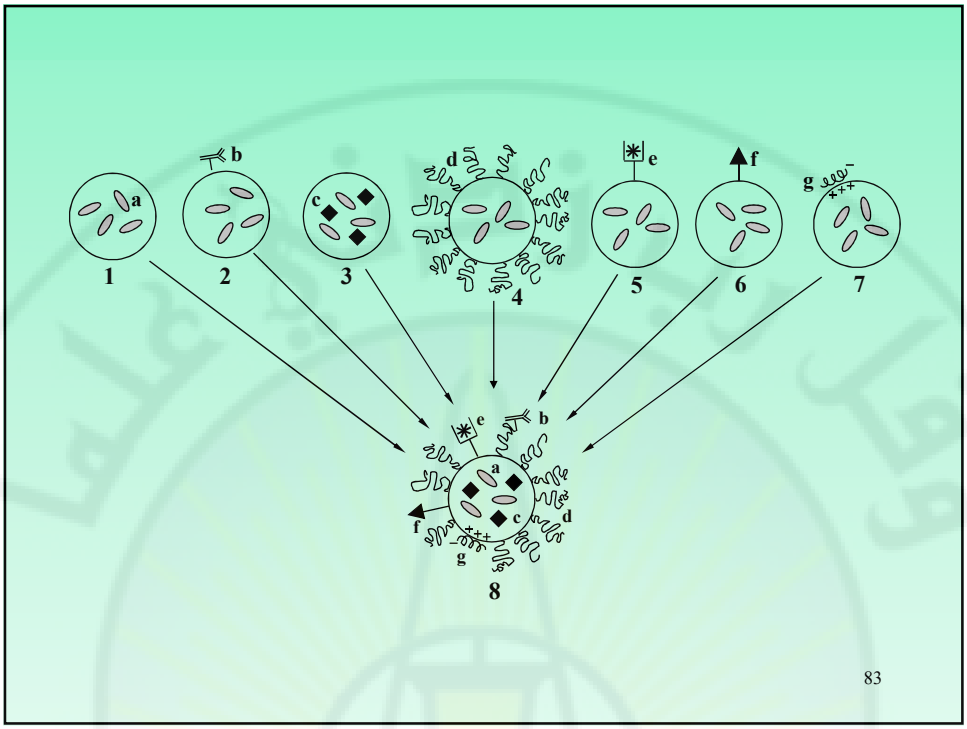
Nanoparticles



INDIVIDUAL PROPERTIES OF PHARMACEUTICAL NANOCARRIERS THAT CAN BE COMBINED IF REQUIRED:

- size and size distribution — الحجم وتوزع الحجم
- Charge — الشحنة
- stability at storage conditions and in vivo — الثباتية في شروط التخزين وفي العضوية الحية
- longevity in the circulation — طول زمن الدوران الدموي
- Biodegradability — التدرّك الحيوي
- Targetability — قابلية الاستهداف
- ability to carry a sufficient cargo — القدرة على حمل شحنة كافية (من الدواء)
- ability to release a cargo — القدرة على تحرير الحمولة
- ability to carry a reporter (contrast) moiety — القدرة على حمل جزء كاشف كعوامل التباين
- sensitivity towards magnetic field — حساسية تجاه الحقل المغناطيسي
- ability to complex DNA — قدرة على صنع معقد مع الـ DNA
- ability to escape from endosomes — القدرة على الإفلات من الجُسَيمّات الداخِلِيّة
- ability to penetrate inside cells — القدرة على النفاذية إلى داخل الخلايا

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COMMERCIALLY AVAILABLE NANOPARTICLES
الجسيمات النانومترية المتوفرة تجارياً

- **Abraxane®** **Abraxis Oncology, albumin NP Paclitaxel-loaded Los Angeles, USA**

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COMMERCIALLY AVAILABLE NANOPARTICLES (Diagnostics)
الجسيمات النانومترية المتوفرة تجارياً (المستخدمة في التشخيص)

- Nanocol® Albumin-NP for ^{99m}Tc-labelling — Amersham Amersham, UK
- Quantison® Air-filled albumin NP — Andaris
- Albunex® Air-filled albumin NP — Mallinckrodt, St. Louis, USA
- Optison® Perfluorocarbon-filled albumin NP — Mallinckrodt, St. Louis, USA

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COMMERCIALLY AVAILABLE NANOPARTICLES (Diagnostics)
الجسيمات النانومترية المتوفرة تجارياً (المستخدمة في التشخيص)

Table 1. Nanoparticle-based drugs that have been approved or are being tested in the clinic.

Commercial name	Platform	Targeting ligand	Active pharmaceutical ingradient	Indication	Status
Nanoparticle systems without targeting ligands					
DaunoXome	liposomes		daunorubicin	Kaposi's sarcoma	approved
Myocet	liposomes		doxorubicin	combinational therapy of recurrent breast, ovarian cancer	approved
Doxil/Caelyx	PEG-liposomes		doxorubicin	refractory Kaposi's sarcoma, recurrent breast, ovarian cancer	approved
Onco TCS	liposomes		vincristine	relapsed aggressive non-Hodgkin's lymphoma	approved
Abraxane	albumin-bound paclitaxel nanoparticles		paclitaxel	metastatic breast cancer	approved
Genexol-PM*	PLA-PEG micelle		paclitaxel	metastatic breast cancer	approved

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Clinically approved nanoparticle-based therapeutics

Table 1 Clinically approved nanoparticle-based therapeutics

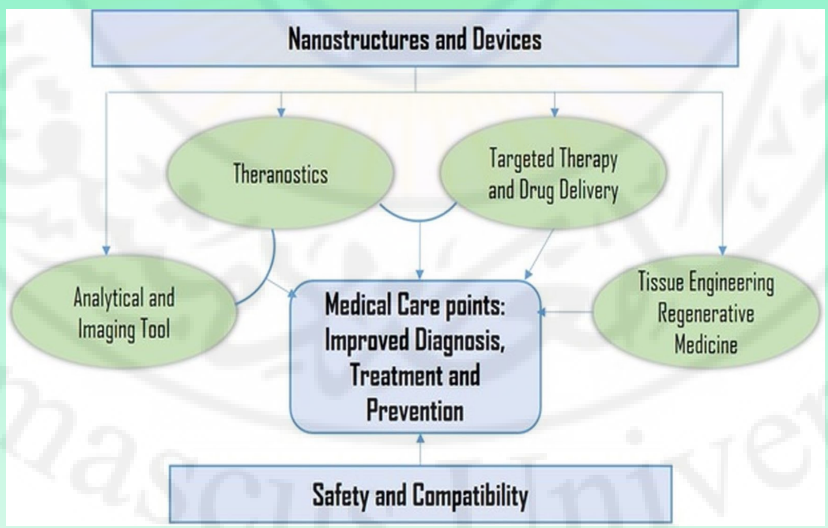
Composition	Trade name	Company	Indication
Liposomal platforms			
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal amphotericin B	AmBisome	Gilead Sciences	Fungal and protozoal infections
Liposomal cytarabine	DepoCyt	SkyePharma	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma
Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer
Liposomal IRIV vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal IRIV vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia
Liposomal verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome-PEG doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy

Clinically approved nanoparticle-based therapeutics

Polymeric platforms			
L-Glutamic acid, L-alanine, L-lysine, and L-tyrosine copolymer	Copaxone	TEVA Pharmaceuticals	Multiple sclerosis
Methoxy-PEG-poly(D,L-lactide) taxol	Genexol-PM	Samyang	Metastatic breast cancer
PEG-adenosine deaminase	Adagen	Enzon	Severe combined immunodeficiency disease associated with ADA deficiency
PEG-anti-VEGF aptamer	Macugen	OSI Pharmaceuticals	Age-related macular degeneration
PEG-α-interferon 2a	Pegasys	Nektar, Hoffmann-La Roche	Hepatitis B, hepatitis C
PEG-GCSF	Neulasta	Amgen	Neutropenia associated with cancer chemotherapy
PEG-HGF	Somavert	Nektar, Pfizer	Acromegaly
PEG-L-asparaginase	Oncaspar	Enzon	Acute lymphoblastic leukemia
Poly(allylamine hydrochloride)	Renagel	Genzyme	End-stage renal disease

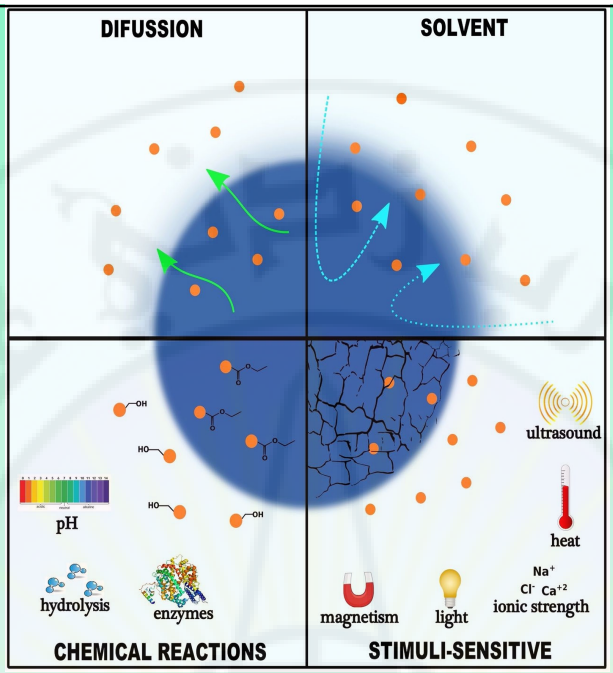
Clinically approved nanoparticle-based therapeutics

Other platforms			
Albumin-bound paclitaxel	Abraxane	Abraxis BioScience, AstraZeneca	Metastatic breast cancer
Nanocrystalline aprepitant	Emend	Elan, Merck	Antiemetic
Nanocrystalline fenofibrate	Tricor	Elan, Abbott	Anti-hyperlipidemic
Nanocrystalline sirolimus	Rapamune	Elan, Wyeth Pharmaceuticals	Immunosuppressant

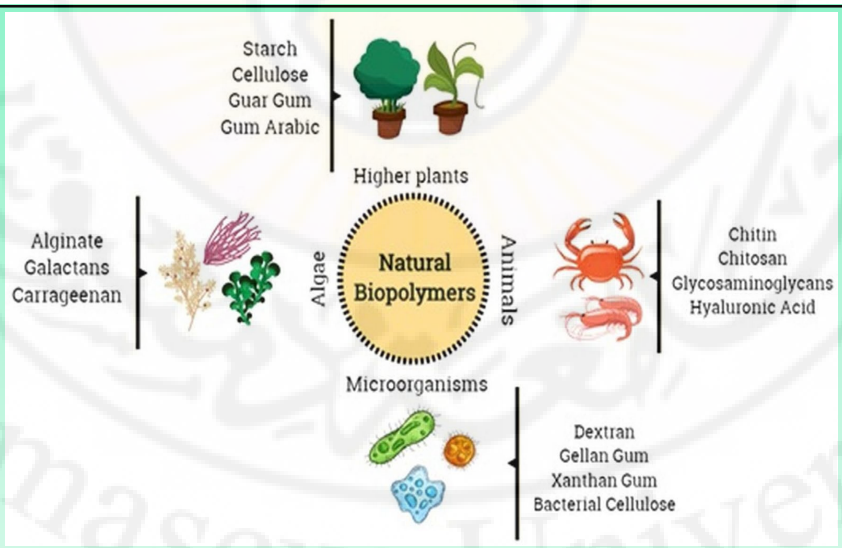


Application and goals of nanomedicine in different sphere of biomedical research

Patra *et al. J Nanobiotechnol (2018) 16:71*

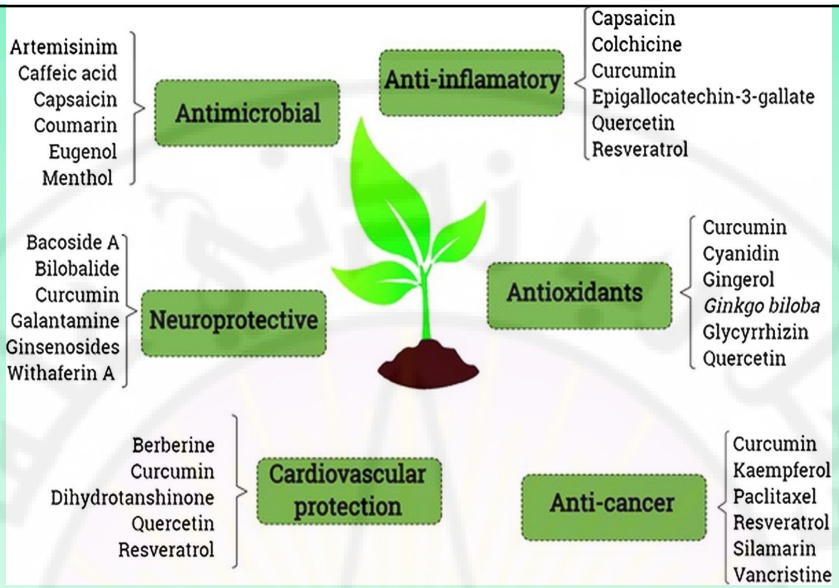


Mechanisms for controlled release of drugs using different types of nanocarriers
Patra *et al. J Nanobiotechnol (2018) 16:71*

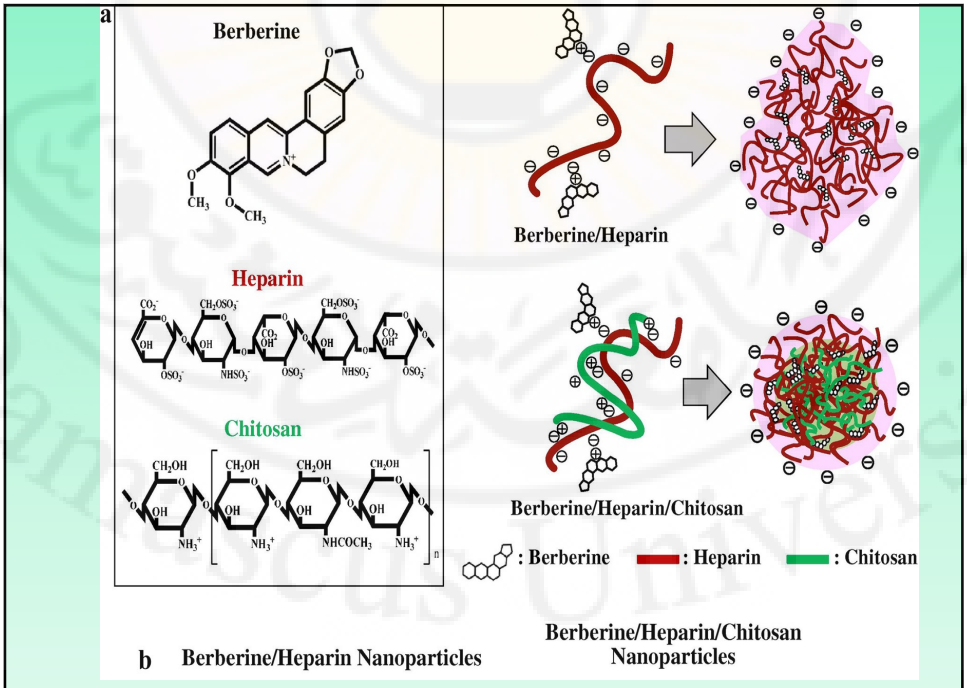


Different sources of natural biopolymers to be used in nanomedicine applications. Natural biopolymers could be obtained from higher plants, animals, microorganisms and algae

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Examples of natural compounds extracted from higher plants used in anomedicine aiming different approaches. Some of these extracts are already being marketed, others are in clinical trials and others are being extensively studied by the scientific community



Structure of berberine/heparin based nanoparticles and berberine/heparin/chitosan nanoparticles.

Table 1 Approved drugs commonly referred to as "nanopharmaceuticals"

Name	Description	Mechanism of action	Approval/indication
1) Liposomes			
AmBisome®	Amphotericin B encapsulated in liposomes (60–70 nm) composed of hydrogenated soy phosphatidylcholine, cholesterol, and distearoyl phosphatidylglycerol (2/0.8/1 molar)[15]	MPS targeting: Liposomes preferentially accumulate in organs of the MPS. Negative charge contributes to MPS targeting. Selective transfer of the drug from lipid complex to target fungal cell with minimal uptake into human cells has been postulated[16,17]	FDA 1997 Systemic fungal infections (IV)
DaunoXome®	Daunorubicin citrate encapsulated in liposomes (45 nm) composed of distearoyl phosphatidylcholine and cholesterol (2/1 molar)[18,19]	Passive targeting via EPR effect: Concentration of available liposomal drug in tumors exceeds that of free drug. Liposomal daunorubicin persists at high levels for several days[20]	FDA 1996 HIV-related KS (IV)
DepoCyt®	Cytarabine encapsulated in multivesicular liposomes (20 μm; classified as nanopharmaceutical based on its individual drug containing "chambers") made from dioleoyl lecithin, dipalmitoyl phosphatidylglycerol, cholesterol, and triolein[21]	Sustained release: This formulation of cytarabine maintains cytotoxic concentrations of the drug in the cerebrospinal fluid for more than 14 days after a single 50 mg injection[22]	FDA 1999/2007 Lymphomatous malignant meningitis (IV)
DepoDur®	Morphine sulfate encapsulated in multivesicular liposomes (17–23 μm; per se not a nanopharmaceutical – classified as such based only on its individual drug containing "nano-sized chambers") made from dioleoyl lecithin cholesterol, dipalmitoyl phosphatidylglycerol, tricaprylin, and triolein	Sustained release: After the administration into the epidural space, morphine sulfate is released from the multivesicular liposomes over an extended period of time[23,24]	FDA 2004 For treatment of chronic pain in patients requiring a long-term daily around-the-clock opioid analgesic (administered into the epidural space)
Doxil®	Doxorubicin hydrochloride encapsulated in Stealth® liposomes (100 nm) composed of N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero3-phosphoethanolamine sodium, fully hydrogenated soy phosphatidylcholine, and cholesterol[9]	Passive targeting via EPR effect: Extravasation of liposomes by passage of the vesicles through endothelial cell gaps present in solid tumors. Enhanced accumulation of doxorubicin in lesions of AIDS-associated KS after administration of PEG-liposomal doxorubicin[25]	FDA 1995 AIDS-related KS, multiple myeloma, ovarian cancer (IV)
Inflexal® V	Influenza virus antigens (hemagglutinin, neuraminidase) on surface of 150 nm liposomes	Mimicking native antigen presentation: Liposomes mimic the native virus structure, thus allowing for cellular entry and membrane fusion.[26] Retention of the natural presentation of antigens on liposomal surface provides for high immunogenicity[27,28]	Switzerland 1997 Influenza vaccine
Marqibo®	Vincristine sulfate encapsulated in sphingomyelin/cholesterol (60/40, molar) 100 nm liposomes	Passive targeting via EPR effect: Extravasation of liposomes through fenestra in bone marrow endothelium	FDA 2012 Acute lymphoid leukemia, Philadelphia chromosome-negative, relapsed or progressed (IV)
Mepact™	Mifamurtide (synthetic muramyl tripeptide-phosphatidylethanolamine) incorporated into large multilamellar liposomes composed of 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine and 1,2-dioleoyl-sn-glycero-3-phospho-L-serine[29]	MPS targeting: The drug, an immune stimulant, is anchored in negatively charged liposomal bilayer membrane	Europe 2009 Non-metastasizing resectable osteosarcoma (IV)
Myocet®	Doxorubicin encapsulated 180 nm oligolamellar liposomes composed of egg phosphatidylcholine/cholesterol (1/1, molar)	MPS targeting: Forms "MPS depot", slow release into blood circulation resembles prolonged infusion[30]	Europe 2000 Metastatic breast cancer (IV)
Visudyne®	Verteporfin in liposomes made of dimyristoyl-phosphatidylcholine and egg phosphatidylglycerol (negatively charged); lyophilized cake for reconstitution	Drug solubilization: Rendering drug biocompatible and enhancing ease of IV administration. No other apparent function of liposomes. Liposomal formulation instable in the presence of serum. Fast transfer of verteporfin from Visudyne® to lipoproteins[31]	FDA 2000 Photodynamic therapy of wet age-related macular degeneration, pathological myopia, ocular histoplasmosis syndrome (IV)

Name	Description	Mechanism of action	Approval/indication
2) Lipid-based (non-liposomal) formulations			
Abelcet®	Amphotericin B complex 1:1 with DMPC and DMPG (7:3), >250 nm, ribbon like structures of a bilayered membrane[15]	MPS targeting: Selective transfer of drug from lipid complex to fungal cell with minimal uptake into human cells has been postulated[32,33]	FDA 1995 and 1996 Marketed outside USA as Amphocil® Systemic fungal infections (IV)
Amphotec®	Amphotericin B complex with cholesteryl sulfate (1:1). Colloidal dispersion of disc-like particles, 122 nm ×4 nm[15]	MPS targeting	

3) PEGylated proteins, polypeptides, aptamers			
Adagen®	PEGylated adenosine deaminase[34] One enzyme molecule is modified with up to 17 strands of PEG, MW 5,000, 114 oxymethylene groups per strand	Increased circulation time and reduced immunogenicity PEGylation generally increases hydrodynamic radius, prolongs circulation and retention time, decreases proteolysis, decreases renal excretion, and shields antigenic determinants from immune detection without obstructing the substrate-interaction site[35,36]	FDA 1990 Adenosine deaminase deficiency – severe combined immunodeficiency disease
Cimzia®	PEGylated antibody (Fab' fragment of a humanized anti-TNF-alpha antibody)		FDA 2008 Crohn's disease, rheumatoid arthritis
Neulasta®	PEGylated filgrastim (granulocyte colony-stimulating factor)		FDA 2002 Febrile neutropenia, In patients with nonmyeloid malignancies; prophylaxis (SC)
Oncaspar®	PEGylated L-asparaginase		FDA 1994 Acute lymphoblastic leukemia
Pegasys®	PEGylated interferon alfa-2b		FDA 2002 Hepatitis B and C
PegIntron®	PEGylated interferon alfa-2b		FDA 2001 Hepatitis C
Somavert®	PEGylated human growth hormone receptor antagonist		FDA 2003 Acromegaly, second-line therapy
Macugen®	PEGylated anti-VEGF aptamer		FDA 2004 Intravitreal Neovascular age-related macular degeneration
Mircera®	PEGylated epoetin beta (erythropoietin receptor activator)		FDA 2007 Anemia associated with chronic renal failure in adults

4) Nanocrystals			
Emend®	Aprepitant as nanocrystal	Increased bioavailability due to increased dissolution rate:	FDA 2003 Emesis, antiemetic (oral)
Megace ES®	Megestrol acetate as nanocrystal	Below 1,000 nm, the saturation solubility becomes a function of the particle size	FDA 2005 Anorexia, cachexia (oral)
Rapamune®	Rapamycin (sirolimus) as nanocrystals formulated in tablets	leading to an increased saturation solubility of nanocrystals, which in turn increases the	FDA 2002 Immunosuppressant (oral)
Tricor®	Fenofibrate as nanocrystals	concentration gradient between gut lumen	FDA 2004
Triglide®	Fenofibrate as insoluble drug-delivery microparticles	and blood, and consequently the absorption by passive diffusion[37]	Hypercholesterolemia, hypertriglyceridemia (oral)
5) Polymer-based nanoformulations			
Copaxone®	Polypeptide (average MW 6.4 kDa) composed of four amino acids (glatiramer)	No mechanism attributable to nanosize. Based on its resemblance to myelin basic protein, glatiramer is thought to divert as a "decoy" an autoimmune response against myelin	FDA 1996/2014 Multiple sclerosis (SC)
Eligard®	Leuprolide acetate (synthetic GnRH or LH-RH analog) incorporated in nanoparticles composed of PLGH copolymer (DL-lactide/glycolide; 1/1, molar)	Sustained release[38]	FDA 2002 Advanced prostate cancer (SC)
Genexol®	Paclitaxel in 20–50 nm micelles[39] composed of block copolymer poly(ethylene glycol)-poly(D,L-lactide)	Passive targeting via EPR effect	South Korea 2001 Metastatic breast cancer, pancreatic cancer (IV)

Name	Description	Mechanism of action	Approval/indication
Opaxio®	Paclitaxel covalently linked to solid nanoparticles composed of polyglutamate	Passive targeting via EPR effect: Drug release inside solid tumor via enzymatic hydrolysis of polyglutamate	FDA 2012 Glioblastoma
Renagel®	Cross-linked poly allylamine hydrochloride,[40] MW variable	No mechanism attributable to nano size. Phosphate binder	FDA 2000 Hyperphosphatemia (oral)
Zinostatin stimalamer®	Conjugate protein or copolymer of styrene-maleic acid and an antitumor protein NCS.[41] Synthesized by conjugation of one molecule of NCS and two molecules of poly(styrene-co-maleic acid)[42]	Passive targeting via EPR effect[43]	Japan 1994 Primary unresectable hepatocellular carcinoma
6) Protein–drug conjugates			
Abraxane®	Nanoparticles (130 nm) formed by albumin with conjugated paclitaxel[44,45]	Passive targeting via EPR effect: Dissociation into individual drug-bound albumin molecules, which may mediate endothelial transcytosis of paclitaxel via albumin-receptor mediated pathway[46,47]	FDA 2005 Metastatic breast cancer, non-small-cell lung cancer (IV)
Kadcyla®	Immunoconjugate. Monoclonal antibody (against human epidermal growth factor receptor-2)–drug (DM1, a cytotoxin acting on microtubule) conjugate, linked via thioether	No mechanism attributable to nano size	FDA 2013 Metastatic breast cancer
Ontak®	Recombinant fusion protein of fragment A of diphtheria toxin and subunit binding to interleukin-2 receptor	Fusion protein binds to interleukin-2 receptor, followed by receptor-mediated endocytosis; fragment A of diphtheria toxin then released into cytosol where it inhibits protein synthesis[48]	FDA 1994/2006 Primary cutaneous T-cell lymphoma, CD25-positive, persistent or recurrent disease

7) Surfactant-based nanoformulations			
Fungizone® (also referred to as "conventional AMB")	Lyophilized powder of amphotericin B with added sodium deoxycholate. Forms upon reconstitution colloidal (micellar) dispersion	Drug solubilization: Rendering drug biocompatible and enhancing ease of administration after IV injection No other apparent function of micelles, which dissociate into monomers following dilution in circulation	FDA 1966 Systemic fungal infections (IV)
Diprivan®	Oil-in-water emulsion of propofol in soybean oil/glycerol/egg lecithin	Drug solubilization: Rendering drug biocompatible and enhancing ease of administration after IV injection	FDA 1989 Sedative–hypnotic agent for induction and maintenance of anesthesia (IV)
Estrasorb™	Emulsion of estradiol in soybean oil, polysorbate 80, ethanol, and water	Drug solubilization	FDA 2003 Hormone replacement therapy during menopause (transdermal)
8) Metal-based nanoformulations			
Feridex®	Superparamagnetic iron oxide nanoparticles coated with dextran. Iron oxide core 4.8–5.6 nm, hydrodynamic diameter 80–150 nm	MPS targeting: 80% taken up by liver and up to 10% by spleen within minutes of administration. Tumor tissues do not take up these particles and thus retain their native signal intensity[36]	FDA 1996 Liver/spleen lesion MRI (IV) Manufacturing discontinued in 2008
Feraheme™ (Ferumoxytol)	Superparamagnetic iron oxide nanoparticles coated with dextran. Hydrodynamic diameter >50 nm	MPS targeting: Iron released inside macrophages, subsequently enters into intracellular storage iron pool, or is transferred to plasma transferrin	FDA 2009 Treatment of iron deficiency anemia in adults with chronic kidney disease
NanoTherm®	Aminosilane-coated superparamagnetic iron oxide 15 nm nanoparticles	Thermal ablation: Injecting iron oxide nanoparticles exposed to alternating magnetic field causing the nanoparticles to oscillate, generating heat directly within the tumor tissue	Europe 2013 Local ablation in glioblastoma, prostate, and pancreatic cancer (intratumoral)

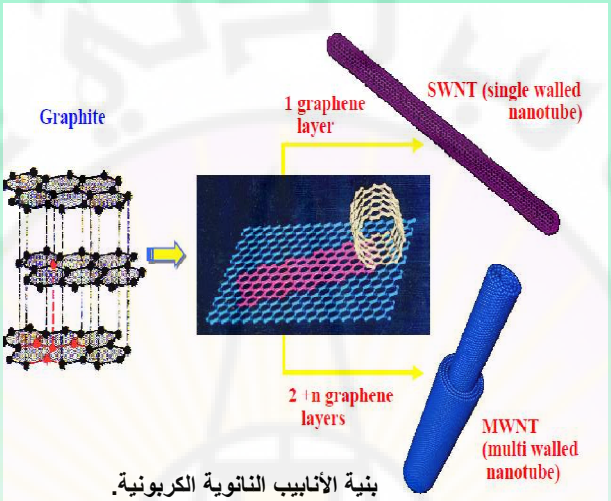
Name	Description	Mechanism of action	Approval/indication
9) Virosomes			
Gendicine®	Recombinant adenovirus expressing wildtype-p53 (rAd-p53)	"[…] the adenoviral particle infects tumor target cells and delivers the adenovirus genome carrying the therapeutic p53 gene to the […] nucleus […] The expressed p53 gene appears to exert its antitumor activities"[49]	People's Republic of China 2003 Head and neck squamous cell carcinoma
Rexin-G®	Gene for dominant-negative mutant form of human cycline G1, which blocks endogenous cyclin-G1 protein and thus stops cell cycle, inserted into retroviral core (replication-incompetent retrovirus) devoid of viral genes. About 100 nm particle	Targeted gene therapy: This retrovirus-derived particle targets specifically exposed collagen, which is a common histopathological property of metastatic tumor formation[50,51]	Philippines 2007 For all solid tumors

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; EPR, enhanced permeability and retention; FDA, US Food and Drug Administration; GnRH, Gonadotropin-releasing hormone; IV, intravenous; KS, Kaposi's sarcoma; LH-RH, Luteinizing hormone-releasing hormone; MPS, Mononuclear phagocyte system; MRI, magnetic resonance imaging; MW, molecular weight; NCS, neocarzinostatin; PEG, polyethylene glycol; PLGH, poly-(D,L-lactide-co-glycolide); SC, subcutaneous.

1- الأنابيب النانوية الكربونية (CNT) Carbon nanotubes:

هي شبكات سداسية مؤلفة من ذرات كربون، يمكن اعتبارها كطبقة من الغرانيت مطوية على شكل اسطوانة قطرها ١ نانومتر وطولها يتراوح بين ١-١٠٠ نانومتر.

هناك نوعان من الأنابيب النانوية: أنابيب نانوية أحادية الجدار(SWNTs) وأنابيب نانوية متعددة الجدار(MWNTs)



بنية الأنابيب النانوية الكربونية.

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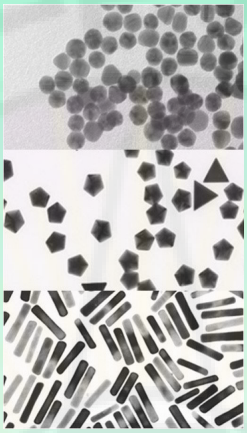
1- الأنابيب النانوية الكربونية
Carbon nanotubes (CNT):

وهي جزيئات كبروية صغيرة small macromolecules تتمتع بخصائصها فريدة من حيث الحجم، الشكل، فضلاً عن امتلاكها لخواص فيزيائية مميزة. تملك الأنابيب النانوية ميزات بارزة بالمقارنة مع نظم إيتاء الدواء و النظم التشخيصية الأخرى ويعود ذلك لامتلاكها خواصاً فيزيوكيميائية مثيرة للاهتمام كالبنية المنتظمة، الوزن الخفيف جداً، والقوة الميكانيكية العالية، والناقلية العالية للكهرباء، والناقلية العالية للحرارة، والسلوك المعدني أو نصف المعدني إضافة للمساحة السطحية الكبيرة.

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٢) الجسيمات النانوية المعدنية metallic nanoparticles:

تعتبر الجسيمات المعدنية النانوية كحامل جيد لإيصال الأدوية و الحساسات البيولوجية biosensor. و رغم تصنيع جسيمات نانوية للعديد من المعادن، تبقى الجسيمات النانوية للذهب و الفضة حتى الآن الأكثر الأهمية في الاستعمال الطبي البيولوجي



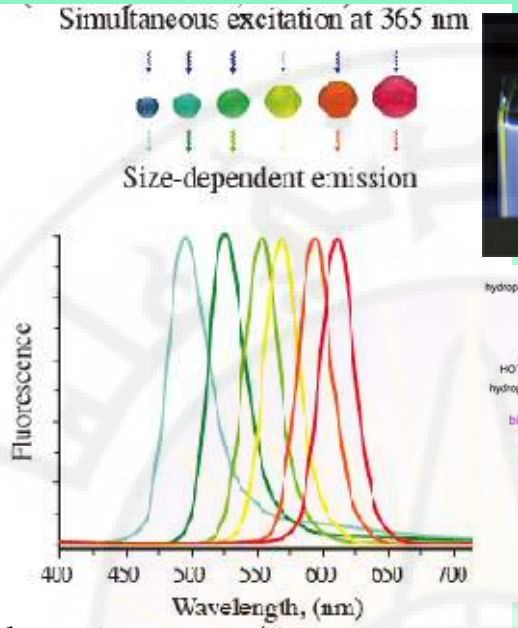
صور مأخوذة بالمجهر الإلكتروني الانتقالي لجسيمات نانوية معدنية ذات بنى متنوعة

105

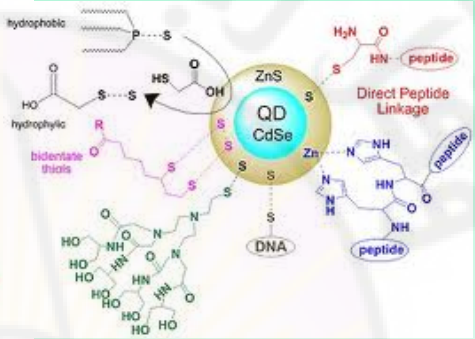
٣) النقط الكوانتومية quantum dots:

النقط الكوانتومية (المتناهية الصغر) هي جسيمات غرويدية صغيرة (عادة قطرها بين ١ و ١٢ نانومتر) من مواد نصف ناقلة (semi-conducting cdte: نواة من الكادميوم و التيلوريوم)، مغلفة بقشرة ZnSe): زنك و سيلينيوم) لتحسين الخواص البصرية، و يمكن إضافة غطاء على السطح لتحسين الذوبانية في الوقاءات المائية. تتعلق خصائص النقط الكوانتومية بحجمها الفيزيائي .

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التألق الصادر عن النقاط الكوانتومية

107

٤) جسيمات السيليكا النانوية (SN) silica nanoparticles:

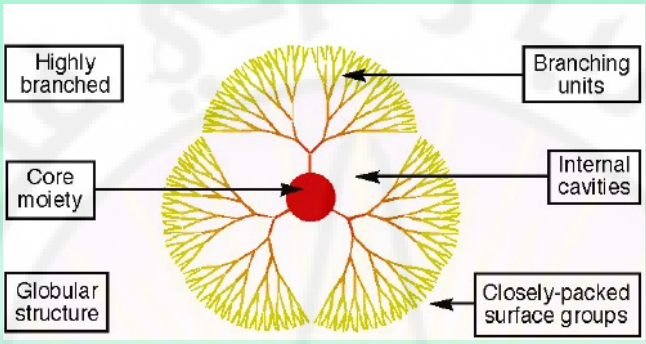
تمتلك جسيمات السيليكا النانوية تطبيقات بيولوجية طبية في إيتاء الدواء و التصوير. تسمح بنية هذه الجسيمات بأن يتم تعبئة الدواء أو الأصباغ المتألقة ضمنها و التي لا يمكن لها أن تمر من خلال الجدر الخلوية في الحالة العادية.

صور مأخوذة بالمجهر الإلكتروني الانتقالي لجسيمات السيليكا النانوية.

108

٥) الدندريميرات Dendrimers:

الدندريميرات (مشتقة من الكلمتين اليونانيتينdendron و تعني شجرة، وmaros و تعني جزء) جزيئات ضخمة ذات بنى شجرية الشكل، عالية التنظيم، متفرعة، أحادية التوزع monodisperse (متسقة من حيث الوزن الجزيئي والأبعاد). تحتوي الدندريميرات على ثلاث مناطق متمايزة: لب، و فروع، و سطح.



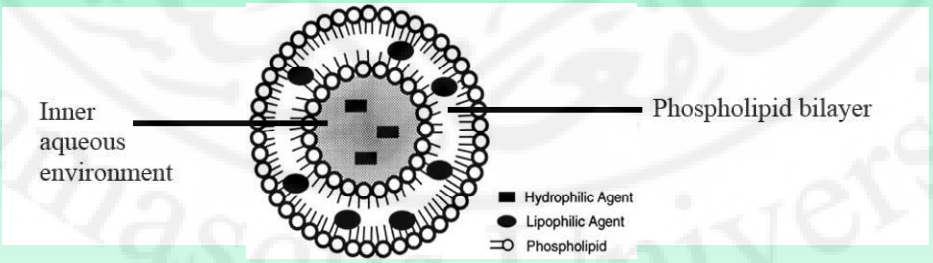
بنية الدندريمير المؤلفة من اللب، الفروع، والسطح.

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٦) الجسيمات الشحمية liposomes:

تم دراسة الجسيمات الشحمية على نحو واسع و استخدامها في تطوير حملة نانوية nanocarriers للإيتاء المهدف للأدوية.

تتشكل هذه الحويصلات المغلقة عندما يتم وضع الفوسفوليبيدات الجافة في وسط مائي



بنية الجسيمات الشحمية.

110

٧- النيوزومات niosomes أو الحويصلات vesicles الفعالة على السطح غير المتأينة

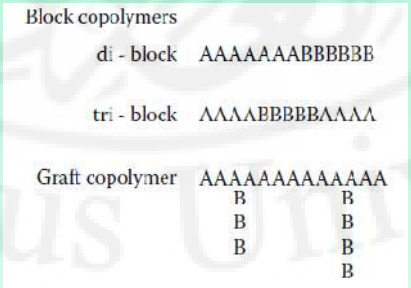
من الناحية البنيوية فإن النيوزومات مشابهة للجسيمات الشحمية من حيث أن جدارها مكون من طبقة مضاعفة أيضاً. إلا أن الطبقة المضاعفة في النيوزومات تتكون من عوامل فعالة على السطح غير متأينة بدلاً من الفوسفوليبيدات المستخدمة في تحضير الجسيمات الشحمية. تعطي معظم العوامل الفعالة على السطح عندما تغمر في الماء بنى مذيلية. في حين أن بعض العوامل السطحية يمكن أن تشكل حويصلات ذات طبقة مضاعفة، وهي ما يعرف بالنيوزومات .

111

٨- المذيلات البلمرية Polymeric micelles:

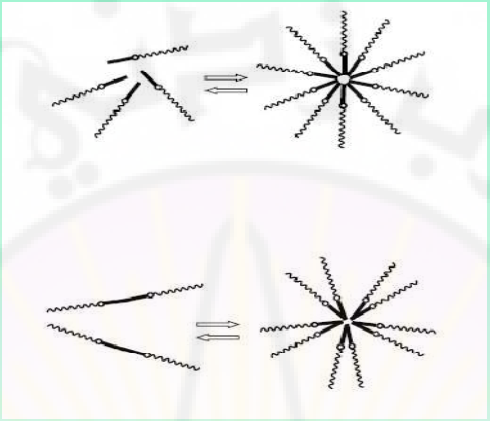
تتجمع البلمرات المشتركة الكتلية المذبذبة (ثنائية الميل) Amphiphilic block copolymers لتعطى بنى نانوية فوق جزيئية (مؤلفة من العديد من الجزئيات) مؤلفة من لب-قشرة تعرف باسم المذيلات البلمرية.

يكون قطر المذيلات البلمرية عادة أقل من ١٠٠ نانومتر، كما أن سطحها المحب للماء يحميها من القبط غير النوعي من قبل الجملة الشبكية البطانية.



بنى المكاثير المشتركة المشكلة للمذيلات A:. وحدة محبة للماء B :. وحدة كارهة للماء.

112

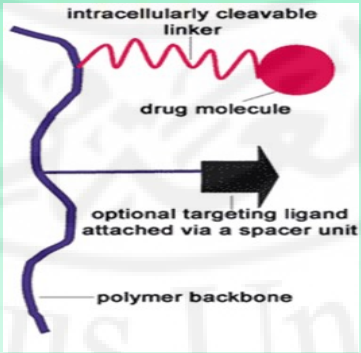


بنية المذيلات المحضرة من مكاثير مشتركة كتلية block copolymer.

113

٩- مقترن بلمر– دواء Polymer drug conjugate:

إن اقتران الأدوية منخفضة الوزن الجزيئي مع البلمرات يسبب تبدلاً جذرياً في حرائكها الدوائية في كامل الجسم و على المستوى الخلوي. ومن هنا صممت مقترنات بلمر– دواء لكي تزيد الوزن الجزيئي الكلي، و الذي يسمح باحتباسها في الخلايا السرطانية.

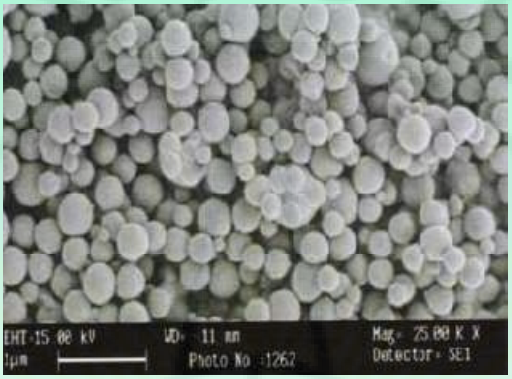


مقترن بلمر–دواء.

114

١٠- الجسيمات النانوية البلمرية Polymeric nanoparticles:

تشكل الجسيمات النانوية البلمرية أنظمة نانوية واعدة نظراً لبعض الصفات المتأصلة فيها مثل التوافق البيولوجي، عدم إحداثها للاستمناع nonimmunogenicity، عدم السمية، وقابلية التدرك البيولوجي. إن هذه الجسيمات عبارة عن جمل غرويدية تتميز بحجومها التي تتراوح بين ١٠ – ١٠٠ نانومتر مكونة من بلمرات صنعية أو طبيعية. إن الجسيمات النانوية البلمرية عبارة عن مجموعة واسعة تشمل كلاً من المحافظ النانوية و الكريات النانوية .



صورة مأخوذة بالمجهر الإلكتروني الماسح لجسيمات نانوية بلمرية .

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البلمرات المستخدمة لتحضير الجسيمات النانوية المستخدمة في إيتاء الدواء في الأحياء in vivo:

ينبغي على البلمرات المناسبة أن تحقق المتطلبات التالية:

- أن تكون قابلة للتدرك البيولوجي أو على الأقل قابلة للإطراح بشكل كامل خارج الجسم خلال فترة زمنية قصيرة مما يسمح بإعادة الاستعمال دون خطورة التراكم غير المنظم للدواء.
- أن تكون غير سامة وغير محفزة للمناعة. وأن تكون نواتج تدركها، إن وجدت، غير سامة وغير محفزة للمناعة.
- أن تعطي جسيمات نانوية بلمرية ذات خواص مناسبة فيما يتعلق بالهدف الذي صممت لأجله هذه الجسيمات.

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MANUFACTURING METHODS FOR NANOPARTICLES
مبادئ الطرق المتبعة في تحضير الجسيمات النانوية

- Emulsion polymerisation البلمرة للمستحلب
- Interfacial polymerisation البلمرة بَينَ السُّطوح
- Desolvation تحفيز ترسب البلمر عبر إزالة المذيب
- Denaturation of emulsion droplets تَمَسُّخ قطرات المستحلب
- Solvent evaporation تبخير المذيب
- Solvent deposition تَرْسيب النانومتري بالمحلات

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MATERIALS FOR NANOPARTICLES
المواد المستخدمة في الجسيمات النانومترية

- POLY(BUTYL CYANOACRYLATE)
- POLYLACTIC ACID and POLY(LACTIC-CO-GLYCOLIC) ACID
- ALBUMIN
- GELATIN
- FATS (SOLID LIPID NANOPARTICLES, SLN)
- POLY(METHYL METHACRYLATE)

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POLYMER MATERIALS FOR NANOPARTICLES

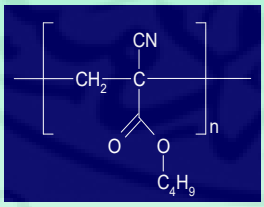
Emulsion polymerisation

- Poly(methyl methacrylate)
- Poly(alkyl cyanocrylates)
- Polyacrylate copolymers

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PREPARATION OF NANOPARTICLES
تحضير الجسيمات النانوية

- Poly(butyl cyanoacylate)



- Particle size : 230 – 250 nm – حجم الجسيمات ٢٣٠ ٢٥٠ نانو
- Monolithic particles الجسيمات متكاملة أحادية

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PREPARATION OF NANOPARTICLES
تحضير الجسيمات النانوية

- Addition of 1% butyl-cyanoacrylate to a 1% dextran solution in 0.001N HCl under constant stirring.
- After 40 min addition of doxorubicin to obtain a final doxorubicin concentration of 0.25%.
- After 2.5 h neutralization with NaOH, filtration through a glass filter.
- Lyophilisation after addition of 3% mannitol as cryoprotector.

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Emulsion Polymerisation

ADVANTAGES:

- Thermodynamically controlled process
- Easy to scale up الإنتاج على مستوى صناعي
- Poly(alkyl cyanoacrylates) are fastest artificial biodegradable material
- Stable upon freeze-drying تَجْفيد
- Drugs can be incorporated and adsorbed

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Emulsion Polymerisation

DISADVANTAGES:

- Covalent attachment of ligands to the nanoparticle surface is difficult
- Drug must have a certain solubility in the continuous aqueous phase but should not be too soluble in this phase

Therefore:

- Not every material can be incorporated into or adsorbed on to these

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POLYMER MATERIALS FOR NANOPARTICLES

Interfacial polymerisation — Poly(alkyl cyanoacrylates)

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INTERFACIAL POLYMERISATION

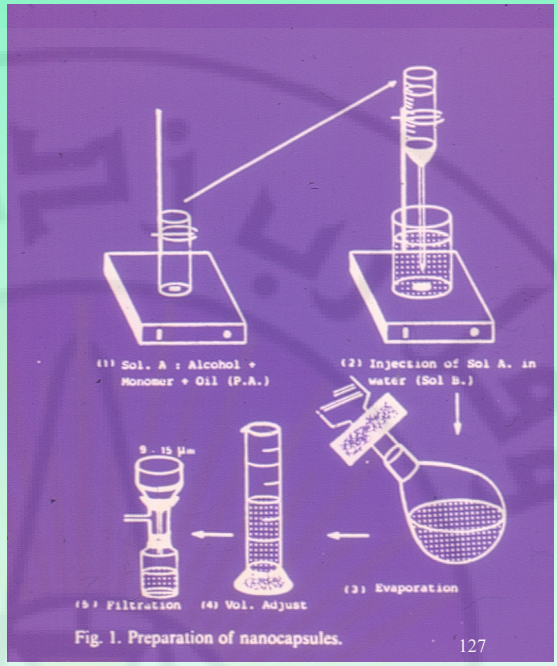
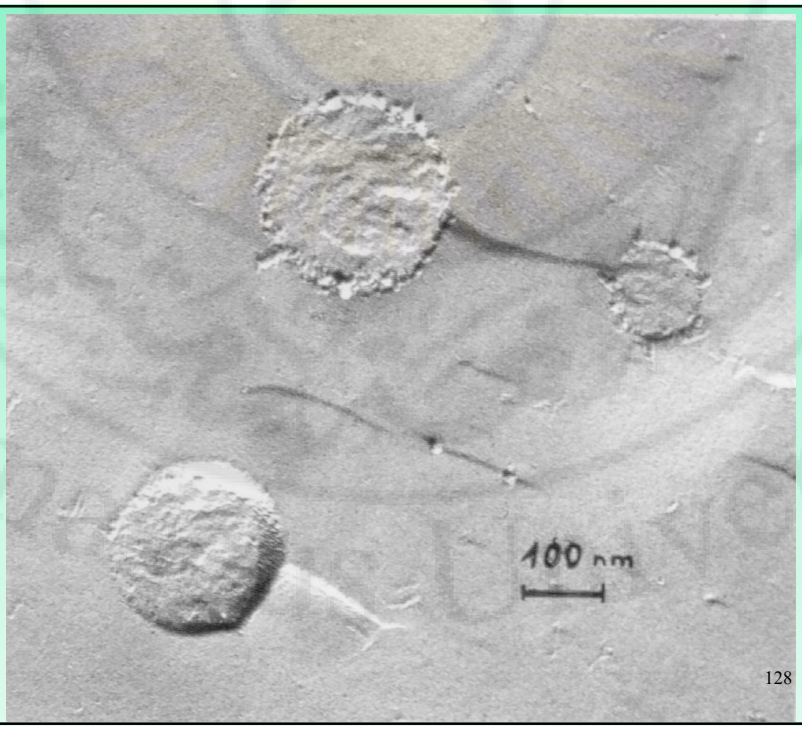


Fig. 1. Preparation of nanocapsules.

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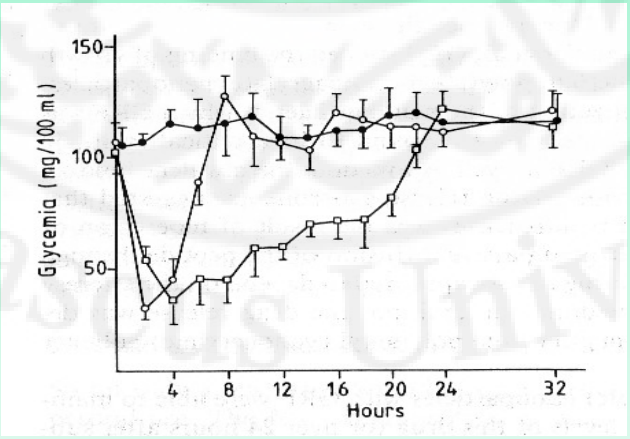
Interfacial Polymerisation

ADVANTAGES:

- **Capsules with a shell-like wall**
- **Contains a lipophilic liquid interior**
- **Poly(butyl cyanoacrylates) are fastest artificial biodegradable material**
- **Larger amounts of drugs can be incorporated (Example: insulin)**

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Glycemic effects after subcutaneous injection of
□ nanoencapsulated or O non-encapsulated insulin (25 U/kg body weight) in normal rats.
● controls



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Interfacial Polymerisation

DISADVANTAGES:

- May be difficult to scale up
- Not much experience exists with this process
- Covalent attachment of ligands to the nanoparticle surface is difficult

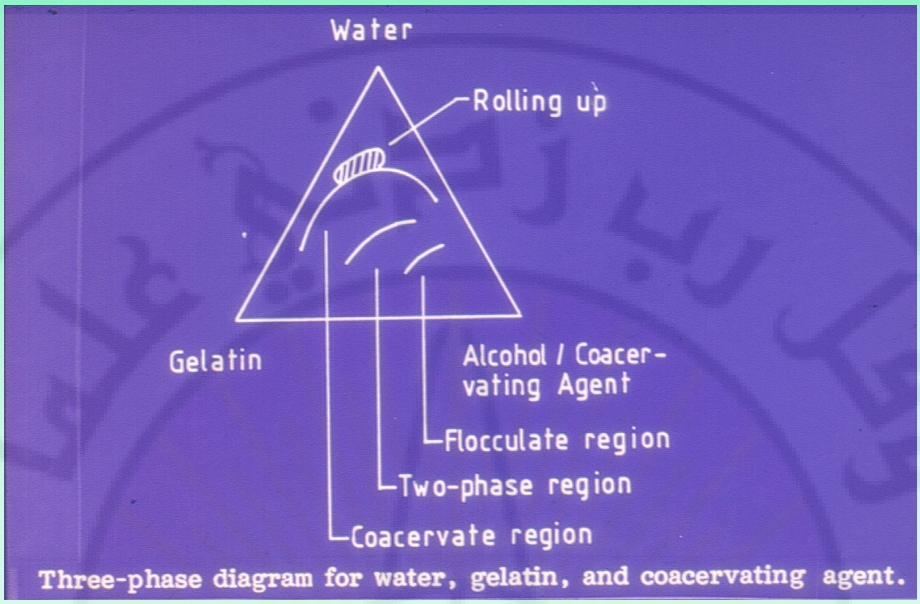
131

POLYMER MATERIALS FOR NANOPARTICLES

Desolvation

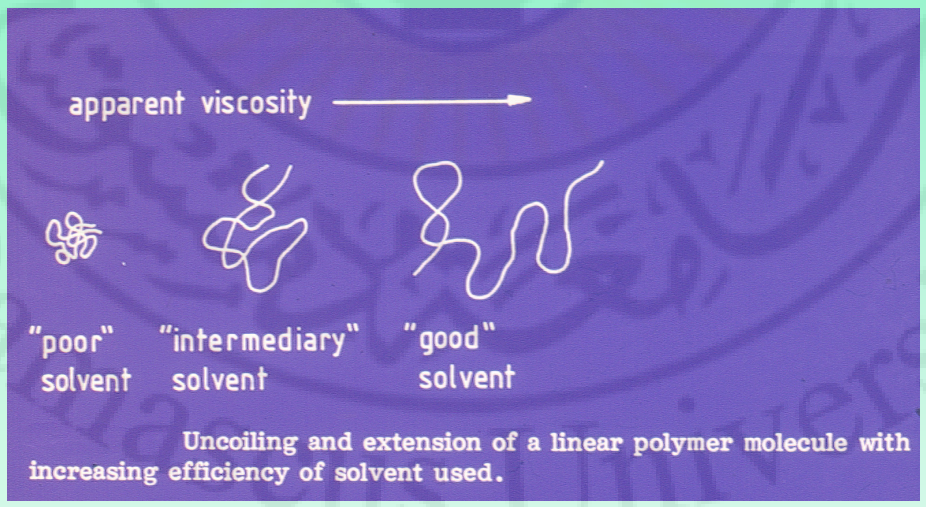
- Albumin
- Gelatine
- Cellulose derivatives

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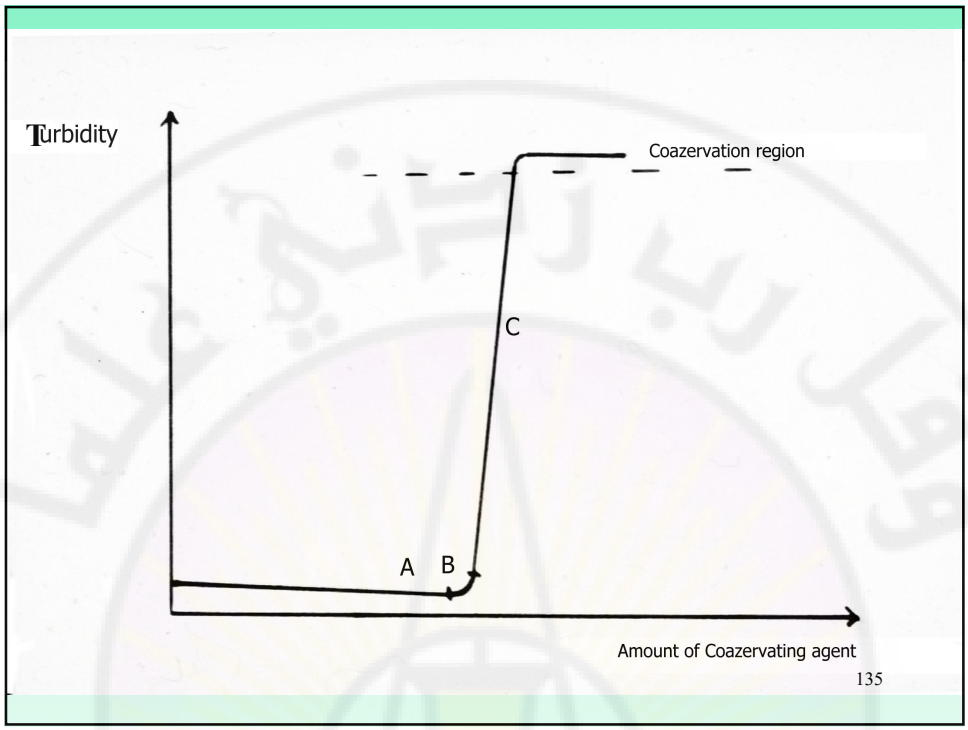
Three-phase diagram for water, gelatin, and coacervating agent.

133



Uncoiling and extension of a linear polymer molecule with increasing efficiency of solvent used.

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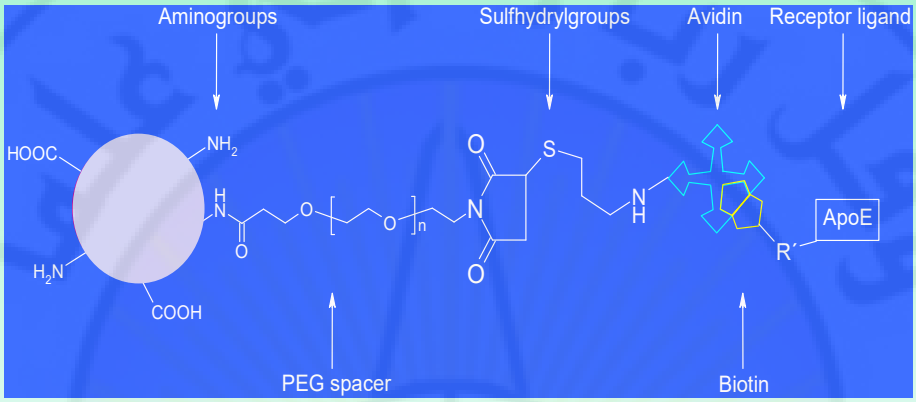
Desolvation

ADVANTAGES:

- Natural materials
- Very good and easily controllable biodegradability
- Ligands for highly specific targeting (antibodies, lipoproteins, PEG-spacers) can easily be bound covalently to the nanoparticle surface
- Drugs can be incorporated and adsorbed

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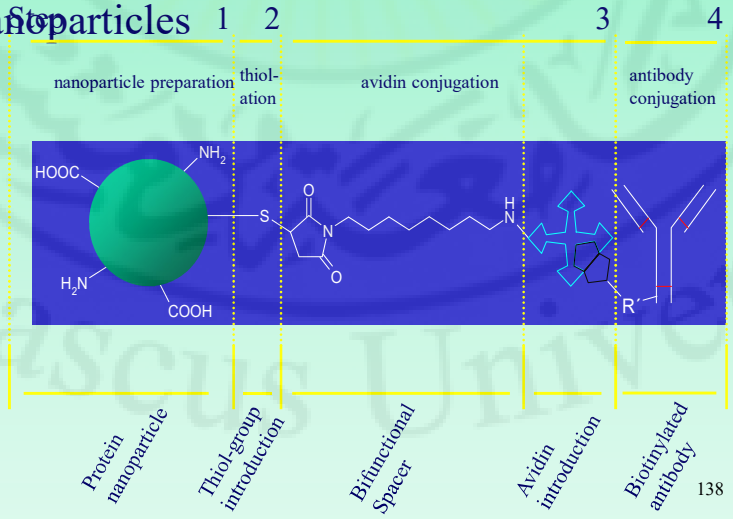
- Schematic structure of ApoE-modified NeutrAvidin-PEG-nanoparticles



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Principle of antibody-modified nanoparticles

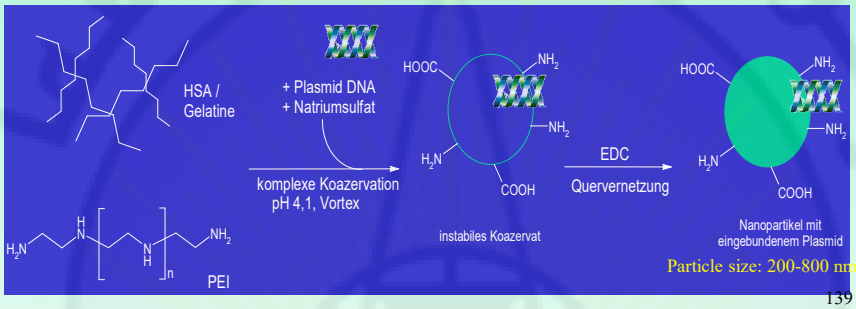
- Avidin-modified HSA / gelatin nanoparticles



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GeI-PEI Nanoparticles
HSA-PEI Nanoparticles

- Complex coaservation of plasmid-DNA with gelatin or human serum albumin (HSA)



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POLYMER MATERIALS FOR NANOPARTICLES

Denaturation of emulsion droplets

- **Albumin**
- **Gelatine**

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DENATURATION OF EMULSION DROPLETS

DRUG SOLUTION IN AN AQUEOUS (10 – 25 %) ALBUMIN SOLUTION

↓

DISPERSE IN OIL (1 PART IN 100 PARTS)

↓

HOMOGENIZE OR SONICATE

↓

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DENATURATION OF EMULSION DROPLETS

ADD DROPWISE TO HOT OIL

↓

STIRR FOR 10 MIN

↓

COOL TO ROOM TEMPERATURE

↓

PRECIPITATE WITH ORGANIC SOLVENT

↓

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DENATURATION OF EMULSION DROPLETS

CENTRIFUGE

↓

WASH WITH ORGANIC SOLVENT

↓

DRY POWDER

↓

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Denaturation of Emulsion Droplets

ADVANTAGES:

- **Natural materials**
- **Very good and easily controllable biodegradability**
- **Process yields dry material**

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Denaturation of Emulsion Droplets

DISADVANTAGES:

- Requires very large amounts of oils and organic solvents
- Not useful for heat labile drugs
- May be difficult to scale up
- More expensive than emulsion polymerisation

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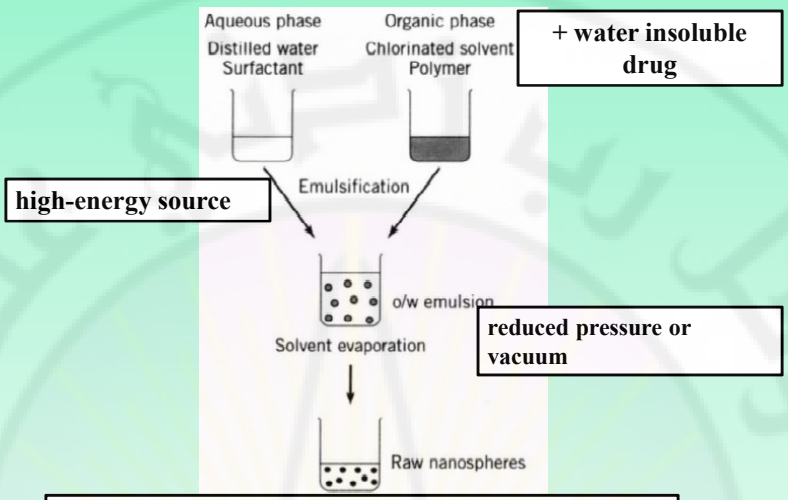
POLYMER MATERIALS FOR NANOPARTICLES

Solvent Evaporation

- Polylactic Acid
- Polylactic Acid copolymers

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Emulsion-Solvent Evaporation



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Solvent Evaporation

ADVANTAGES:

- **Large experience exists with polylactic acid and with co-glycolide copolymers**
- **PEG-chains can be attached covalently to significantly prolong blood circulaltion times after i.v. injection**

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Solvent Evaporation

DISADVANTAGES:

- Requires considerable amounts of organic solvents
- Organic solvents sometimes difficult to remove
- Some substances are not stable enough to withstand the multistep homogenisation
- Used mostly for lipophilic drugs, however hydrophilic drugs can be incorporated using the double emulsion technique

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POLYMER MATERIALS FOR NANOPARTICLES

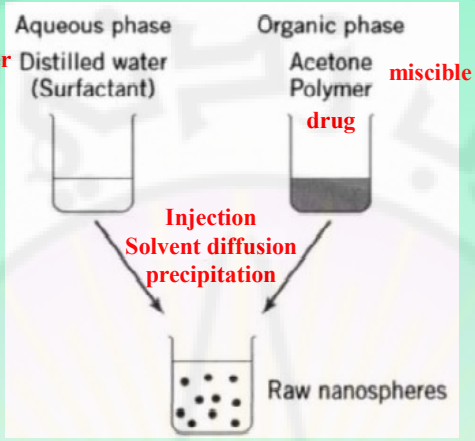
Solvent Deposition

- Polylactic acid
- Polylactic acid copolymers
- Poly(alkyl cyanocrylates)

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Solvent Diffusion (Nanoprecipitation)

non-solvent
for drug and polymer



miscible with water

❑The velocity of solvent removal is the key to obtain particles in the nanometer range instead of agglomerates

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Solvent Deposition

- Two solvents or solvent systems that are misciple are required
- Drug and polymer must be soluble in the first system (the solvent) but not in the second one (the non-solvent)

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Solvent Deposition

- Solvent deposition and nanoprecipitation occurs when the polymer solution is added to the second solvent system

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Solvent Deposition

ADVANTAGES:

- Large experience exists with polylactic acid and with co-glycolide copolymers
- Lipophilic and also some hydrophilic drugs may be bound to these nanoparticles

Bilati et al., Eur. J. Pharm. Sci. 24, 67 (2005)

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Solvent Deposition

DISADVANTAGES:

- May be difficult to scale up
- Requires considerable amounts of organic solvents
- Organic solvents sometimes difficult to remove

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PHYSICOCHEMICAL CHARACTERISATION OF NANOPARTICLES

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PHYSICOCHEMICAL CHARACTERISATION OF NANOPARTICLES

- PHOTON CORRELATION SPECTROSCOPY
- ELECTRON MICROSCOPY
- ZETA-POTENTIAL ANALYSIS
- GEL PERMEATION CHROMATOGRAPHY
- HPLC
- OTHER STANDARD ANALYTICAL METHODS
- ANALYTICAL ULTRACENTRIFUGE

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PARTICLE SIZE OF POLYACRYLIC NANOPARTICLES (MEAN ± S.D.)

Polymer type	Sizing method		
		Electron microscope	
Photon correlaton spectroscopy		Scanning	Transmission after freeze-fracturing
	(nm)	(nm)	(nm)
Poly(methyl metacrylate)	215 ± 44	-	125 ± 21
Polyacrylamide	236	66 ± 26	52 ± 2
Poly(butyl cyanoacrylate)	-	31 ± 6	51 ± 1

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ELECTROPHORETIC MOBILITY (μm • cm •s^{-1} • V^{-1}) OF NANOPARTICLES (MEAN ± S.D.)

Polymer type	Medium		
	Water	Phosphate-buffered saline, pH 7.4	Human serum
Poly(methyl methacrylate)	- 2.76 ± 0.57	- 1.30 ± 0.07	- 0.25 ± 0.051
Polyacrylamide	< - 0.35	-	-
Poly(butyl cyanoacrylate)	- 2.01 ± 0.16	- 0.87 ± 0.07	- 0.19 ± 0.03

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APPLICATION OF NANOPARTICLES

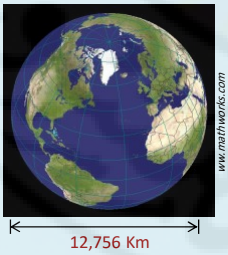
160

Most Active Areas of Application of Nanoparticles

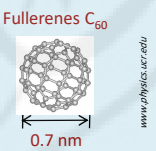
- DRUG TARGETING
- NUCLEIC ACID AND GENE DELIVERY
- ENHANCEMENT OF BIOAVAILABILITY
- ENHANCEMENT OF PHARMACOLOGICAL OR IMMUNOBIOLOGICAL EFFECTS

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What is Nanoscale







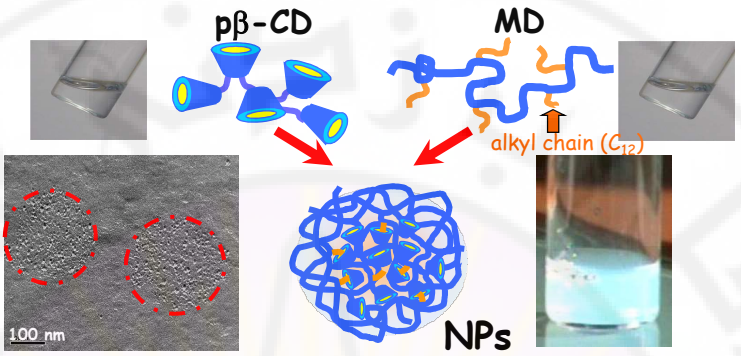
12.7×10^{6} m → 0.22 m → 0.7×10^{-9} m

57.7 millions times smaller

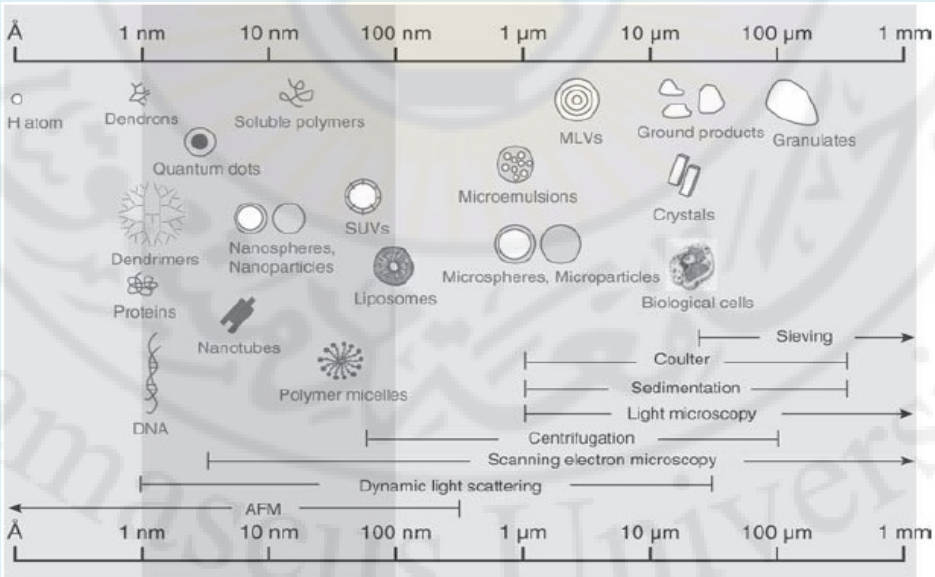
314.3 millions times smaller

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Associative water soluble polymers



- One-step method
- Organic solvent-free nanoparticles
- CD in the matrix : entrapment of molecules of interest
- sponge structures: 70% by weight is water.



The size spectrum showing **nanoparticle** and other nanosystems in the context of microparticulates and macrosystems. Also shown are the **methods employed to measure the particle size and size distribution** of this range of materials.

EQUIPMENT FOR NANOPARTICLES FORMULATION



Ultra sonicator



Homogenizer



Nanosizer
Zetasizer



Evaporator

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PHYSICOCHEMICAL CHARACTERISATION OF NANOPARTICLES

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PHYSICOCHEMICAL CHARACTERISATION OF NANOPARTICLES

- PHOTON CORRELATION SPECTROSCOPY
- ELECTRON MICROSCOPY
- ZETA-POTENTIAL ANALYSIS
- GEL PERMEATION CHROMATOGRAPHY
- HPLC
- OTHER STANDARD ANALYTICAL METHODS
- ANALYTICAL ULTRACENTIFUGE

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Characterization and Analysis of Nano-Systems

1. Particle Size Measurement

- Sedimentation or creaming tendencies can be minimized by changing the particle size of the system.
- The stability of systems can be monitored by measuring changes in particle size and size distribution.
- The biofate of some dispersed system dosage forms is dependent on their particle size distribution.

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Size and in vivo

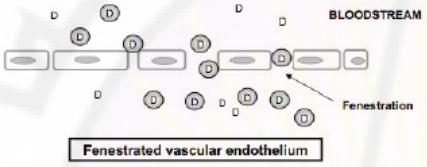
After IV administration (nanoparticles)

<20-30 nm renal excretion

>30 nm capture by the mononuclear phagocytic system cells (liver, spleen, bone marrow)

30-150 nm : bone marrow, heart, kidney, stomach

150-300 nm : liver, spleen

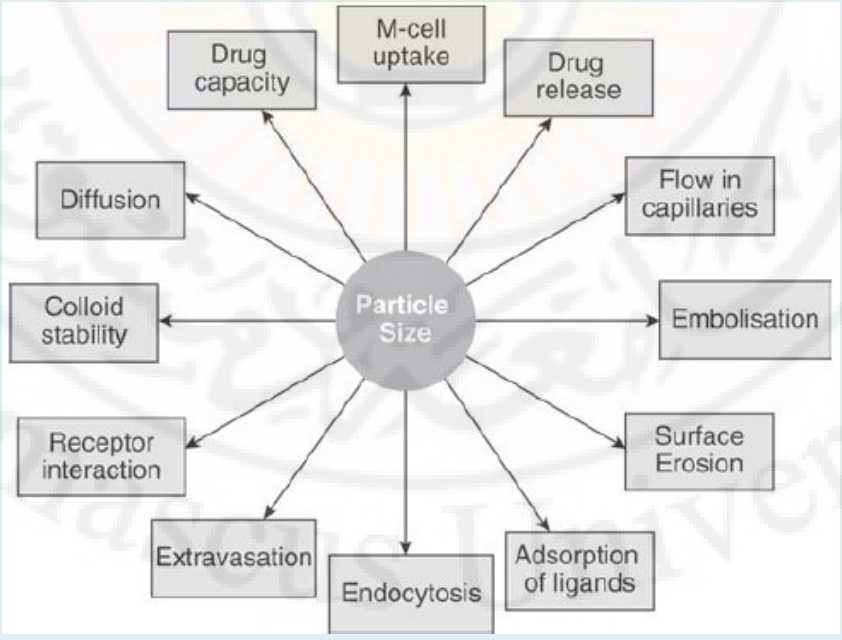


Fenestrated vascular endothelium

Organ or pathological situation	Fenestration size	Animal model
Kidney	20–30 nm	Guinea-pig, rabbit, rat
Liver	150 nm	Mice
Spleen	150 nm	Mice
Lung	1–400 nm	Dog
Bone marrow	85–150 nm	Guinea-pig, rabbit, rat
Skeletal, cardiac and smooth muscle	≤6 nm	Mice
Skin, subcutaneous and mucuous membrane	≤6 nm	Mice
Blood–brain barrier	No fenestrations	–
Tumor[a]	200–780 nm	Mice
Brain tumor[b]	100–380 nm	Rat
Inflamed organs	80 nm–1.4 μm	Hamster

Gaumet et al, EJPB, 2008

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The key position of particle size in determining directly or indirectly the behavior and fate of nanoparticles.

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Approximate Size Range of Methods for Particle Size Analysis

Particle sizing method	Size range (μm)	Comments
Optical microscopy (transmitted, reflected, polarized light, fluorescence, and confocal)	~0.5–600	Important tool for assessing particle size, shape, flocculation, aggregation, and coalescence, etc. Results are subjective and affected by sampling technique
Electron microscopy (transmission and scanning)	~0.01–10	High magnification and direct observation of particle size and shape. Samples need to be dry, coated, or frozen which can affect stability and size. Instrument is relatively expensive and difficult to operate
Electrical sensing zone (Coulter counter)	~0.5–500	Accurate but requires samples containing electrolyte to conduct current
Sedimentation	~1–500	Based on Stokes' law and applied to particles that settle in the dispersion medium by gravity without causing turbulence
Ultracentrifugation	~0.01–5	Based on Stokes' law and applied to particles that can be separated in the dispersion medium under centrifugal force
Sieving	~50–5000	No practical significance for colloidal dispersed systems
Dynamic light scattering (Malvern, Nicomp, Brookhaven)	~0.01–3	Commonly used particle size method for injectable dispersed systems. Upper size limit 3 μm
Static light scattering	~0.02–2000	Commonly used particle size method for injectable dispersed systems
X-ray and neutron scattering	~0.005–10	Similar to light scattering techniques but with better resolution
Size exclusive chromatography or field-flow fractionation	~0.05–20	Particles are separated according to size by interacting with the stationary phase or field force applied to samples (electric, magnetic, or thermal). Requires sample be stable under separating conditions
Optical sensing zone (HIAC, AccuSizer)	~0.5–500	Based on the principle of light blockage and is the commonly used method for coarse-dispersed systems

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Relation size/surface

For 100 ng spherical particles of unit density

Particle diameter (nm)	Particle number	Surface area (mm^2)
2	2.4×10^{13}	300
20	2.4×10^{10}	30
1000	190 000	9.006

x 500 | x 13×10^8 | x 50 000

The volume of a sphere is proportional to the third power of the radius ($V = 4/3\pi r^3$), while the surface area is proportional to the second power ($SA = 4\pi r^2$). Hence, the surface area to volume ratio is inversely proportional to the radius. This has numerous effects on the nature of the particles and their functioning.

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Characterization and Analysis of Nano-Systems

2. Zeta Potential

- Solids acquire a surface charge when placed in polar liquids.
- Zeta potential measurements provide information on particle surface charge.
- Charge shielding by PEG or other hydrophilic groups can be used to predict the effectiveness against opsonization in vivo.
- Zeta potential can be used to determine whether the drug is encapsulated inside the particle or simply adsorbed on the particle surface.

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Characterization and Analysis of Nano-Systems

3. Drug Loading capacity:

نسبة تحميل الدواء:كمية الدواء المحملة على الجسيمات النانومترية مقسومة على كمية الدواء الكلية.

4. Drug Release

- Depending on the polymer selected and the method of manufacture, drug release from nanoparticles usually follows the following mechanisms: desorption from surface, diffusion through matrix or wall, or erosion of the matrix.

5. stability
6. sterility

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Release mechanisms

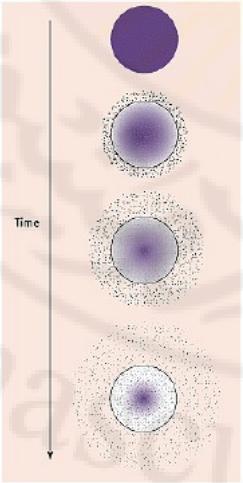
Diffusion

Degradation

Swelling followed by diffusion

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Controlled-released mechanisms. Diffusion



Initially an homogeneous system: matrix

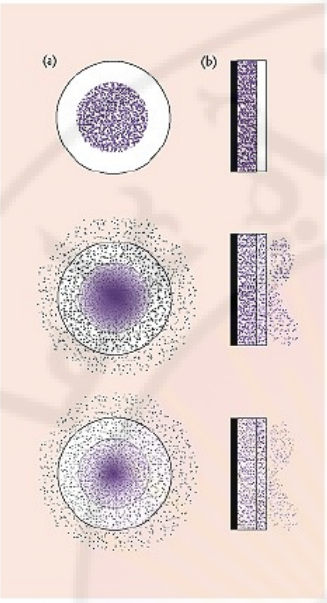
Drug passes from the polymer matrix into the external environment

Decreased release rate: active agent has a progressively longer distance to travel

Diffusion from matrix drug delivery system

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Controlled-released mechanisms. Diffusion



Drug delivery remain fairly constant.

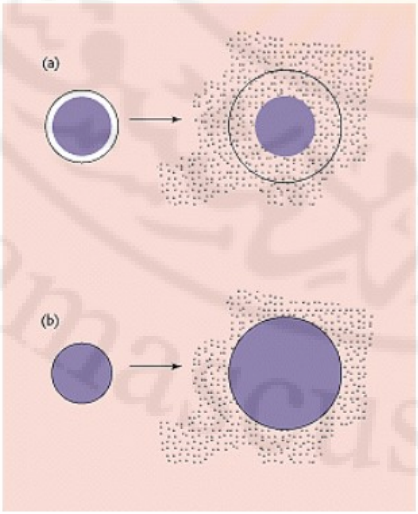
A reservoir surrounded by a film or membrane of a rate-controlling material.

Polymer layer: limits the release.

Diffusion from typical reservoir devices (a) implantable or oral systems, and (b) transdermal systems

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Controlled-released mechanisms. Swelling



Drug delivery from (a) reservoir and (b) matrix swelling-controlled release systems

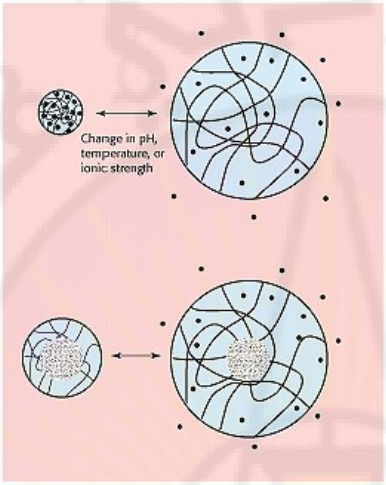
Absorbtion of water
Swelling
Diffusion of the drug through the swollen

Hydrogels

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Controlled-released mechanisms. Swelling

Environmentally sensitive release systems

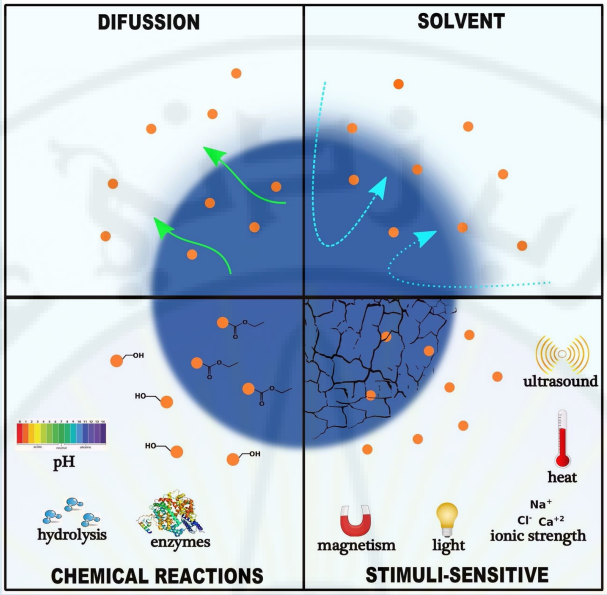


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Environmentally sensitive systems

Stimulus	Hydrogel	Mechanism
pH	Acidic or basic hydrogel	Change in pH — swelling — release of drug
Ionic strength	Ionic hydrogel	Change in ionic strength — change in concentration of ions inside gel — change in swelling — release of drug
Chemical species	Hydrogel containing electron-accepting groups	Electron-donating compounds — formation of charge/transfer complex — change in swelling — release of drug
Enzyme-substrate	Hydrogel containing immobilized enzymes	Substrate present — enzymatic conversion — product changes swelling of gel — release of drug
Magnetic	Magnetic particles dispersed in alginate microshperes	Applied magnetic field — change in pores in gel — change in swelling — release of drug
Thermal	Thermoresponsive hydrogel poly(N-isopro-pylacrylamide)	Change in temperature — change in polymer-polymer and water-polymer interactions — change in swelling — release of drug
Electrical	Polyelectrolyte hydrogel	Applied electric field — membrane charging — electrophoresis of charged drug — change in swelling — release of drug
Ultrasound irradiation	Ethylene-vinyl alcohol hydrogel	Ultrasound irradiation — temperature increase — release of drug

180

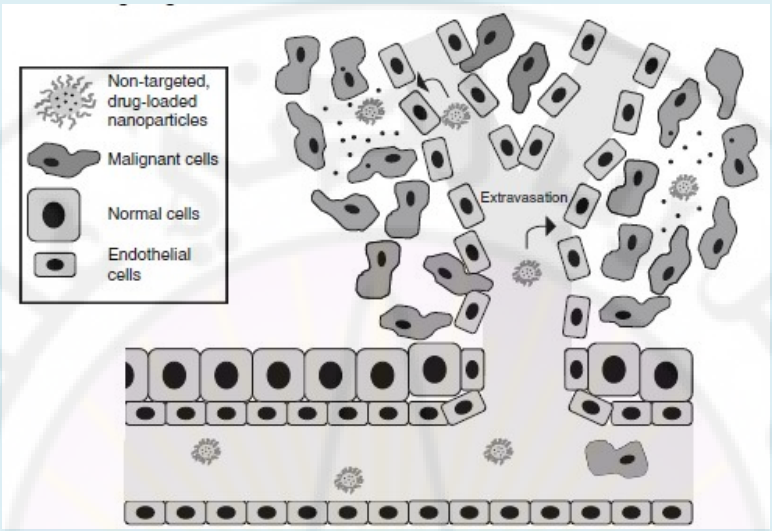


Mechanisms for controlled release of drugs using different types of nanocarriers
atra *et al. J Nanobiotechnol (2018) 16:71*

Nano-targeting التهديف النانوي

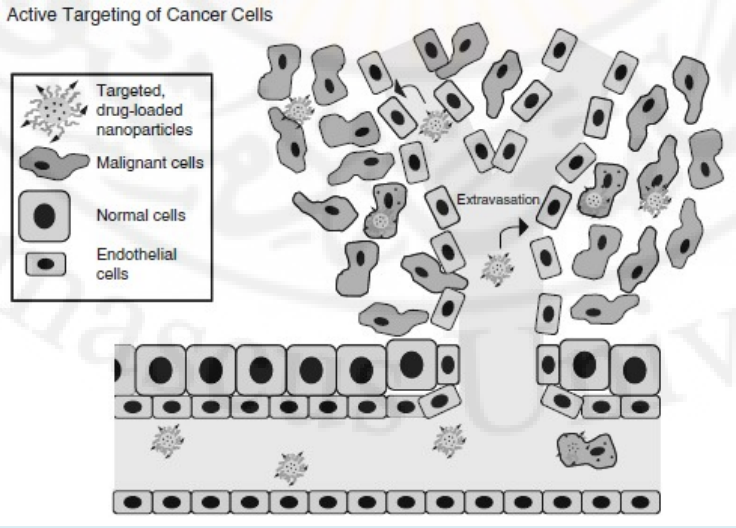
182

Schematic of "passive targeting" via enhanced permeability and retention effect (EPR)

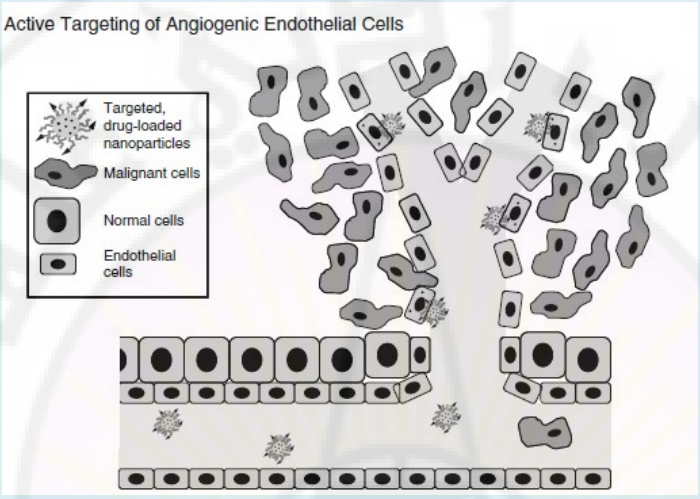


183

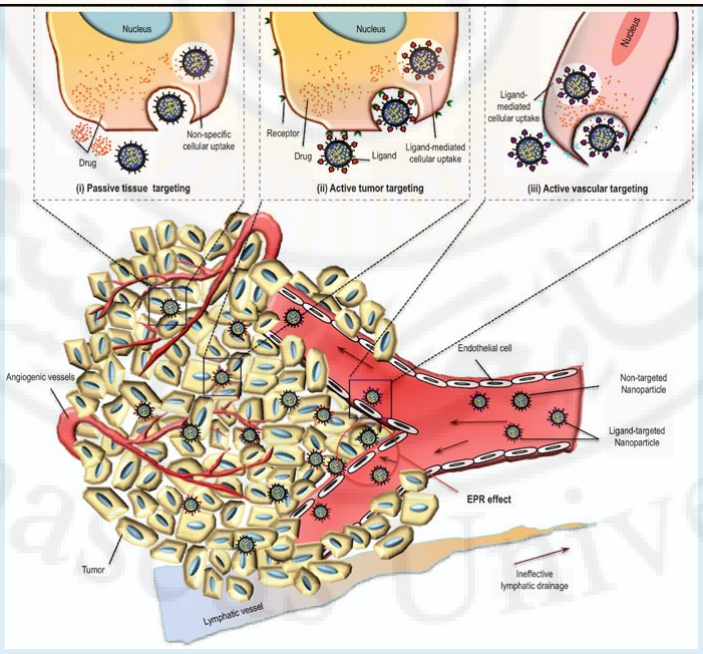
Schematic of "active targeting" of functionalized nanoparticles to cancer cells.



Schematic of "active targeting" of functionalized nanoparticles to endothelial wall.

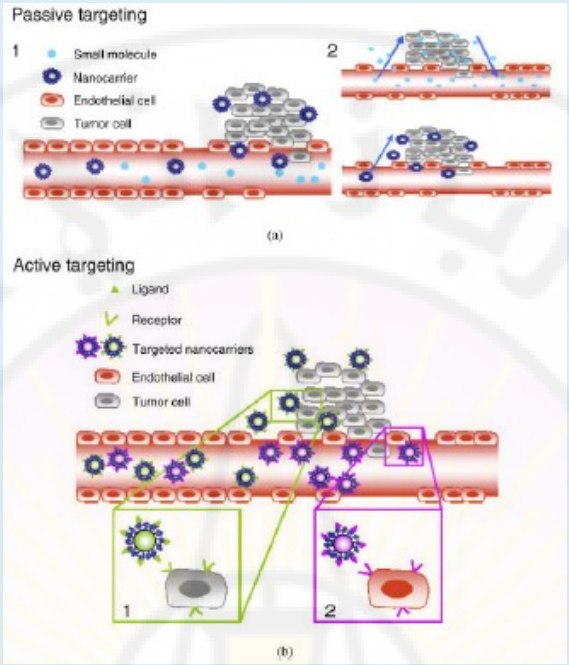


185



Schematic presentation of passive *vs . active targeting of nanoparticles.*

Targeting strategies for macromolecules and nanosystems.



187

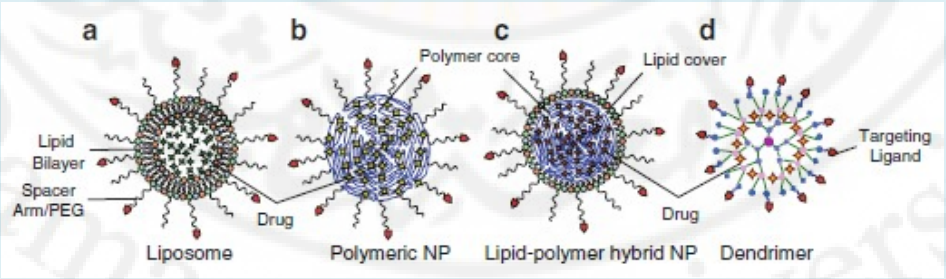
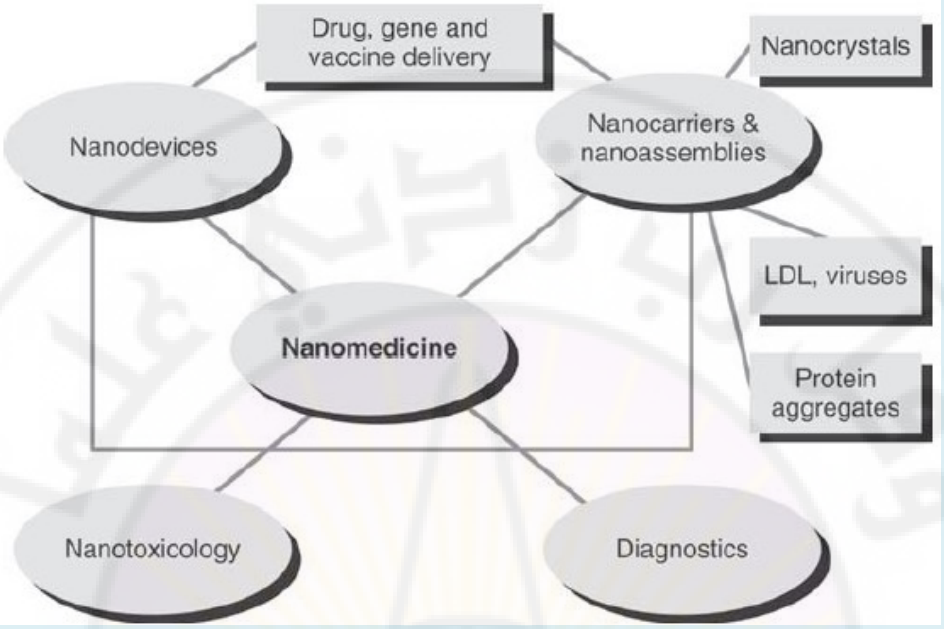


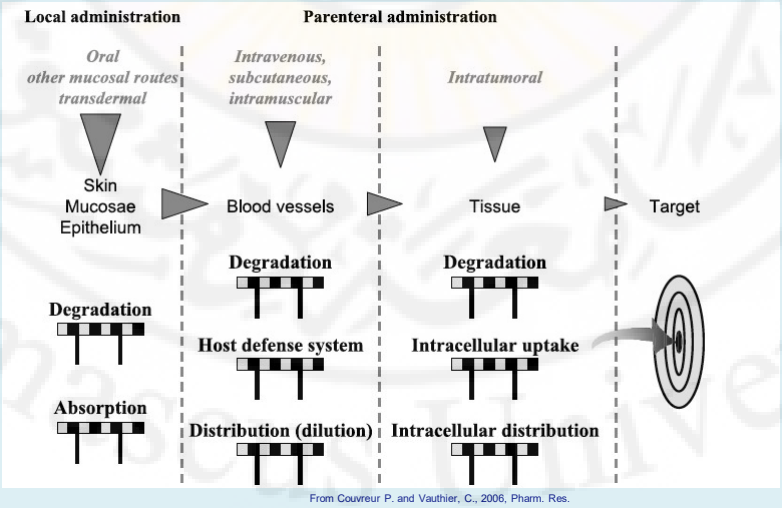
Fig. -Schematic representation of several major therapeutic nanoparticle platforms for the targeted and/or temporally controlled delivery of drug



The domains of nanomedicine including aspects of pharmaceutical nanotechnology.

189

Barriers



From Couvreur P. and Vauthier, C., 2006, Pharm. Res.

190

Drug Delivery Systems (DDS)

PURPOSE

These systems are exploited for therapeutic purpose to carry the drug in the body in a controlled manner from the site of administration to the therapeutic target (P.Couvreur, 2006)

191

Definition

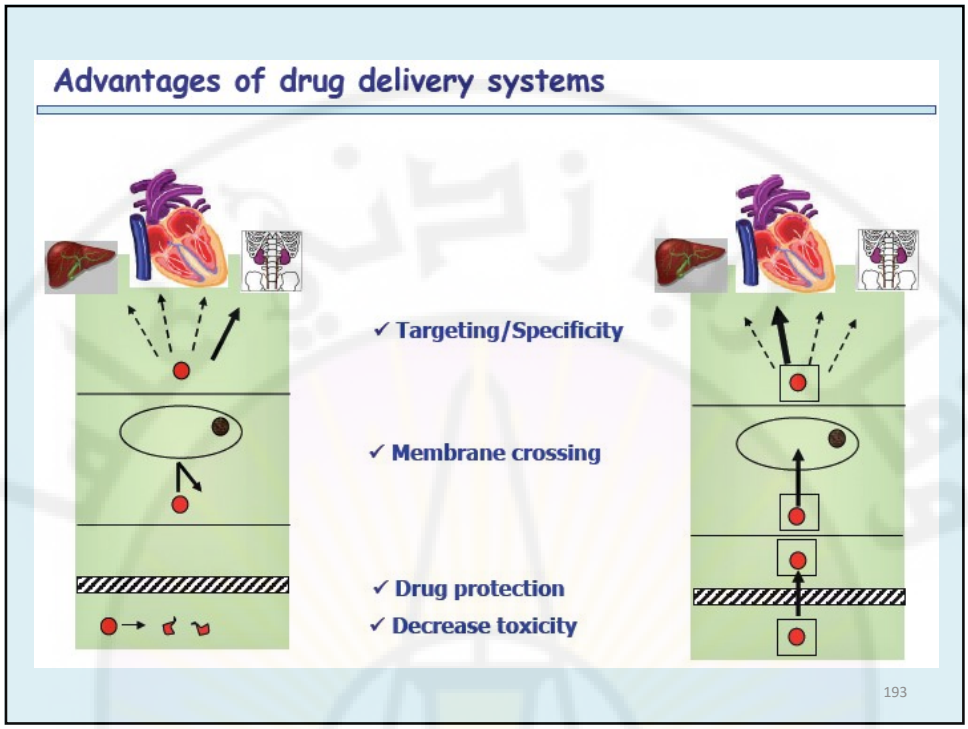
Any pharmaceutical formulation is « drug delivery system » (creams, eye drops, tablets….)

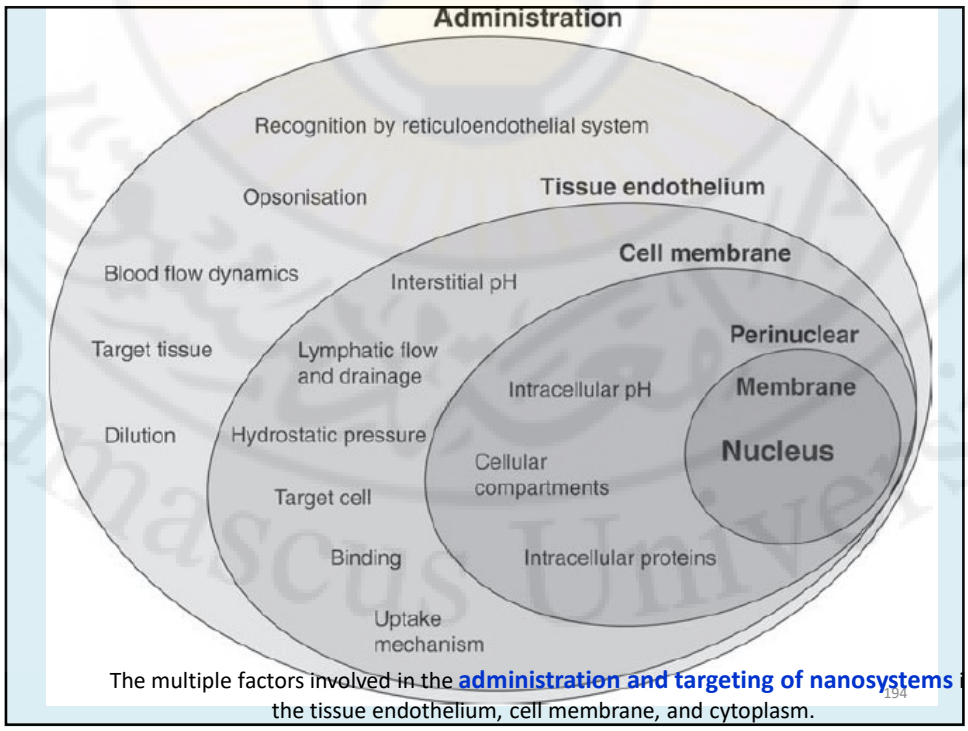


However, most would be considered to be « conventional » as no specific new technology needs to be used in their preparation or use.

More specialized systems to overcome delivery problems

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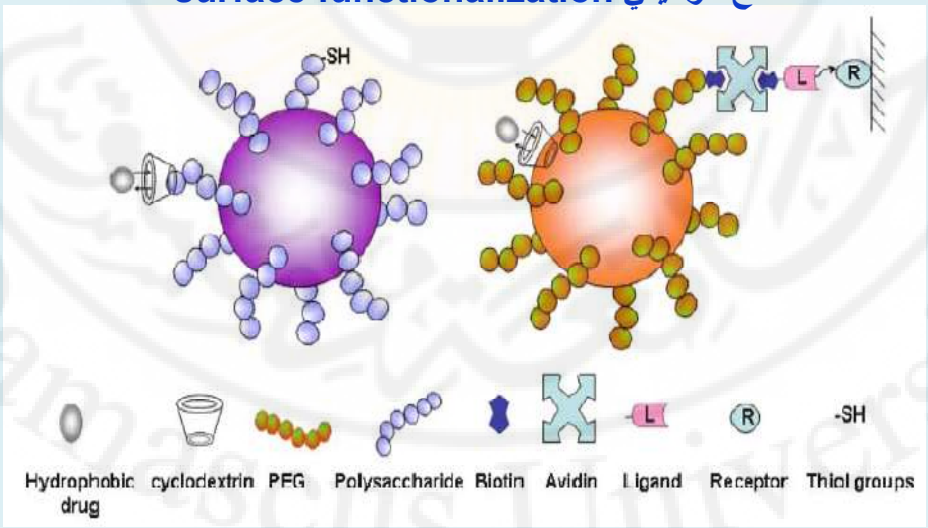
The multiple factors involved in the **administration and targeting of nanosystems** i the tissue endothelium, cell membrane, and cytoplasm.

Table 1 Nanoparticles with Medical Uses

System	Uses
Dendrimers	Targeting of cancer cells, drug delivery, imaging.
Ceramic nanoparticles	Passive targeting of cancer cells
Lipid-encapsulated perfluorocarbon nanoemulsions	Passive targeting of cancer cells
Magnetic nanoparticles	Specific targeting of cancer cells, tissue imaging
LH-RH-targeted silica-coated lipid micelles	Specific targeting of cancer cells
Thiamine-targeted nanoparticles	Directed transfer across Caco-2 cells
Liposomes	Specific targeting of cancer cells, gene therapy, drug delivery
Nanoparticle-aptamer bioconjugate	Targeting of prostate cancer cells
Anti-Flk antibody-coated ^{90}Y nanoparticles	Anti-angiogenesis therapy
Gold nanoshells	Tissue imaging, thermal ablative cancer therapy
Anti-HER2 antibody-targeted gold/silicon nanoparticles	Breast cancer therapy
CLIO paramagnetic nanoparticles	Imaging of migrating cells
Quantum dots	Tissue imaging
Silicon-based nanowires	Real-time detection and titration of antibodies, virus detection, chip-based biosensors
CNTs	Electronic biosensors
Transfersomes	Noninvasive vaccine delivery, drug delivery

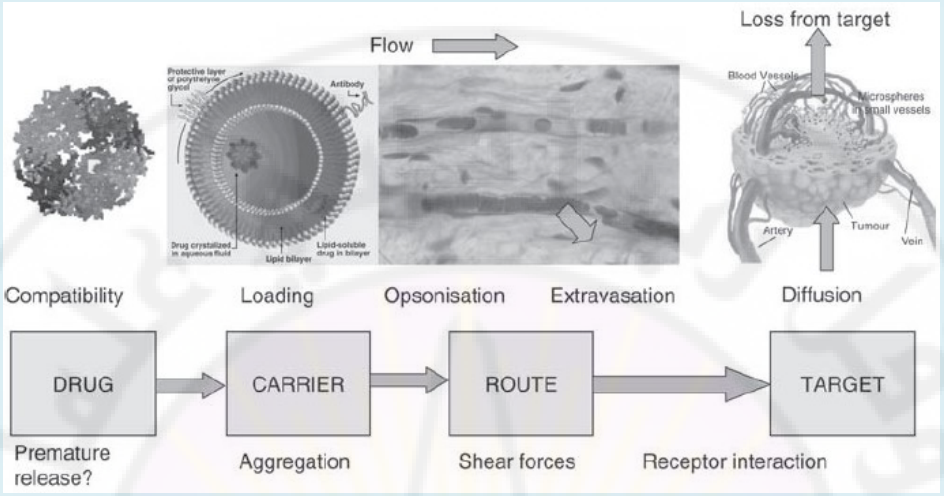
195

surface functionalization السطح الوظيفي



رسم يبين عدة مجموعات وظيفية يمكن ربطها إلى الأنظمة النانوية.

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Diagrammatic illustration of the issues associated with the **presentation of drugs in carrier systems designed for targeting**: compatibility, loading, opsonization, extravasation, and diffusion are the main parameters that determine arrival at the target site. Premature release of drug, aggregation of the particles, and shear forces in capillary flow affect receptor interaction and uptake.

Barriers to drug targeting الحواجز أمام تهديف الدواء

إن أغلب النظم النانوية ليست فعالة بشكل كافٍ لدى استخدامها في التطبيقات الطبية البيولوجية و الصيدلانية، و يعود ذلك إلى القبط غير النوعي الذي تمارسه الجملة الشبكية البطانية reticulo endothelial system (RES)، والطهاية opsonization، والتكدس aggregation، والتوافق البيولوجي الضعيف لهذه النظم.

إن التعديل في حجم هذه النظم وسطحها، و ذلك باستخدام بلمرات متوافقة بيولوجياً، و بلمرات محبة للماء و لجائن ligands تهديف نوعية يجعل من هذه النظم حوامل إيتاء فعالة للعديد من الأدوية ويسمح باستعمالها في تطبيقات طبية بيولوجية متنوعة.

إن التعديل في خواص السطح باستخدام مواد مثل البلمرات و/أو اللجائن وذلك بإدخالها في سطح النظم النانوية، أو ادمصاصها على هذا السطح أو الاقتران التساهمي مع هذا السطح يعرف بما يسمى "السطح الوظيفي surface functionalization".

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Barriers to drug targeting الحواجز أمام تهديف الدواء

بالنسبة للنظم النانوية التي تعطى بالحقن الوريدي، فإن أول حاجز يعترضها هو ***الجملة الشبكة البطانية***. يتعلق مرور جزيئات الدواء أو نظام إيتاء الدواء عبر البطانة بالوزن الجزيئي للنظام وحجمه على التوالي. إن التغير في بنية البطانة في حالات الأورام يسمح بتعزيز نفوذية الجزيئات الضخمة macromolecules، ونظم إيتاء الدواء الجسيمائية particulate drug delivery systems. كما تملك بروتينات البلازما القدرة على التأثير على التوزع البيولوجي للنظم الحاملة للدواء عند دخولها إلى مجرى الدم.

يتحكم حجم النظم النانوية و صفاتها السطحية بتوزعها البيولوجي داخل الجسم in vivo و بتأثير الطهاية في الدوران الدموي.

طرق تهديف الدواء Approaches to drug targeting:

إن التهديف المثالي للدواء لن يؤدي إلى زيادة الفعالية العلاجية للدواء فقط بل سينقص أيضاً من السمية المترافقة مع الدواء مما يسمح بتخفيض الجرعات الدوائية المستخدمة في المعالجة.

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Active targeting Active targeting involves attaching to the drug delivery system something like an antibody, or carrier protein or a ligand which will allow it to go and meet and complex with the cell having the receptor for the attachment. Active targeting is also called ligand based targeting. We employ active targeting in the treatment of cancer. In **passive targeting**, we make use of and modify the physicochemical properties of the drug carrier complex, so that it escapes body defence systems and accumulates in the target tissue.

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طرق تهديف الدواء

التهديف الفعال	التهديف المنفعل
١. الأهداف البيولوجية	١. العوامل المرضية الفيزيولوجية
الأعضاء	الالتهاب / العدوى
الخلايا	تأثير EPR
العضيات	٢. العوامل الفيزيو كيميائية
الأهداف داخل الخلوية	الحجم
٢. التنبيه الفيزيائي / الخارجي	الوزن الجزيئي
الأمواج فوق الصوتية	٣. استغلال الخصائص التشريحية
الحقل المغناطيسي	القثطرة
٣. التهديف المسبق أو التهديف الشطائري	الحقن المباشر
٤. استهداف انتساخ الدنا	٤. الطرق الكيميائية
Iontophoresis	طلائع الأدوية
	نظم الإيتاء الكيميائية

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. طرق التهديف المنفعل Passive Targeting Approaches:

يقصد بـ "التهديف المنفعل" تراكم الدوائي أو النظام الحامل للدواء في موقع معين نتيجة للعوامل الفيزيوكيميائية أو الفارماكولوجية. يمكن أن تكون النظم النانوية الدوائية أو الحاملة للدواء مهدفة بشكل منفعل باستغلال الخصائص الفيزيولوجية المرضية و التشريحية.

1. الخصائص المرضية الفيزيولوجية:

2. استغلال الخصائص التشريحية Anatomical Opportunities:

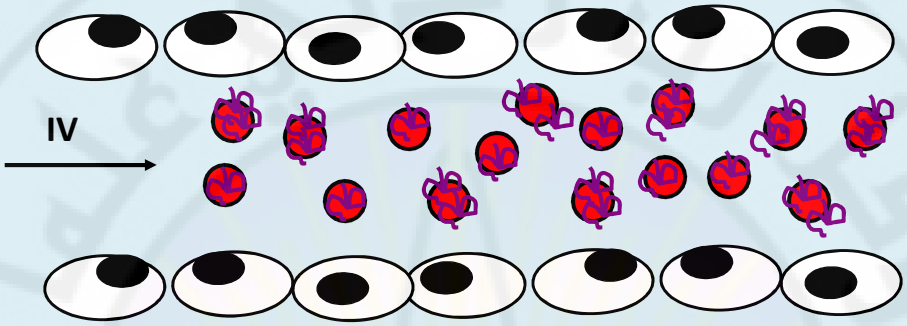
٣. العوامل الفيزيوكيميائية Physicochemical Factors:

إن هذا الميل الطبيعي للجسيمات النانوية للتوضع ضمن RES يسمح بالتهديف المنفعل للدواء إلى البلاعم الموجودة في الكبد والطحال.

ولقد تم تسخير هذه الميزة لتهديف المضادات الحيوية والعوامل المضادة للفيروسات في الإنتانات داخل الخلوية (مثال: الأدوية المضادة للطفيليات المستخدمة لعلاج داء الليشمانيات leishmaniasis ، وفي إيتاء الأدوية المضادة للإيدز).

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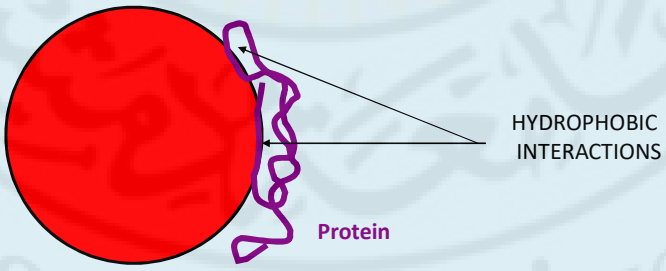
OPSONISATION OF NANOCARRIERS AFTER INTRAVENOUS ADMINISTRATION



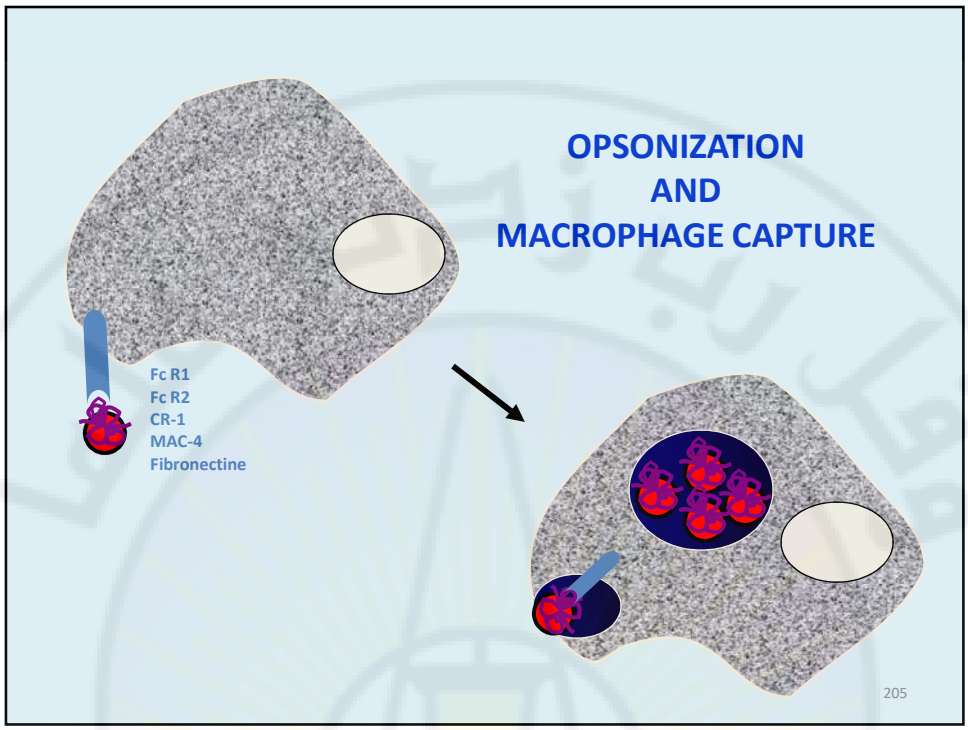
OPSONINES
Fragment Fc des IgG,
Fibronectine,
elements of complement

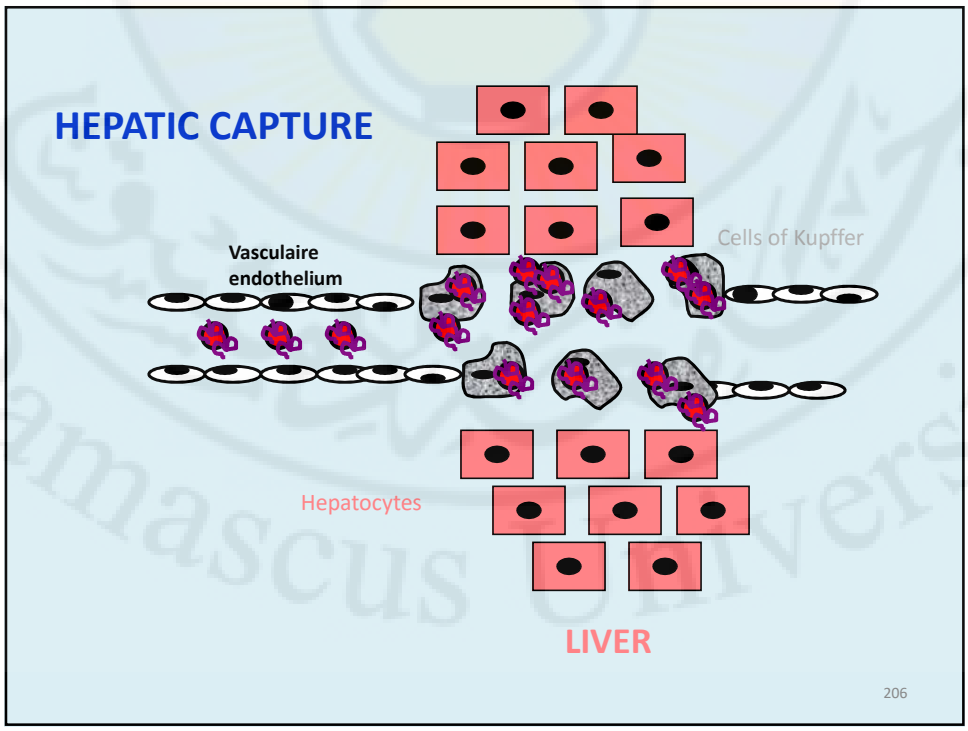
203

OPSONISATION BY HYDROPHOBIC INTERACTIONS

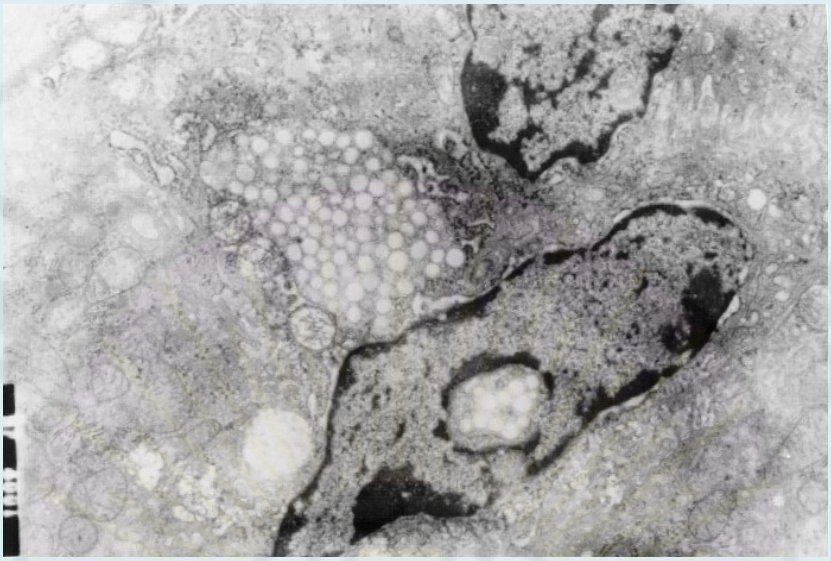


204


OPSONIZATION
AND
MACROPHAGE CAPTURE
Fc R1
Fc R2
CR-1
MAC-4
Fibronectine
205


HEPATIC CAPTURE
Vasculaire
endothelium
Cells of Kupffer
Hepatocytes
LIVER
206

CAPTURE OF NANOPARTICLES BY CELLS OF KUPFFER



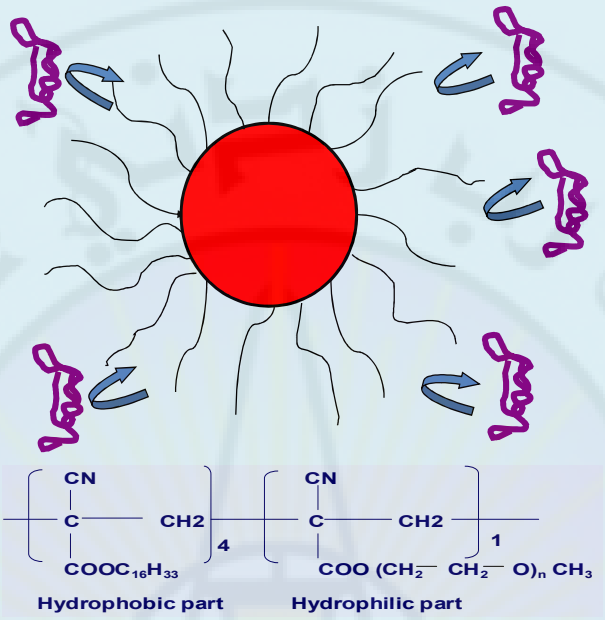
207

DISTRIBUTION OF NANOPARTICLES IN A RAT PORTER OF A SARCOMA OF SOFT TISSUES



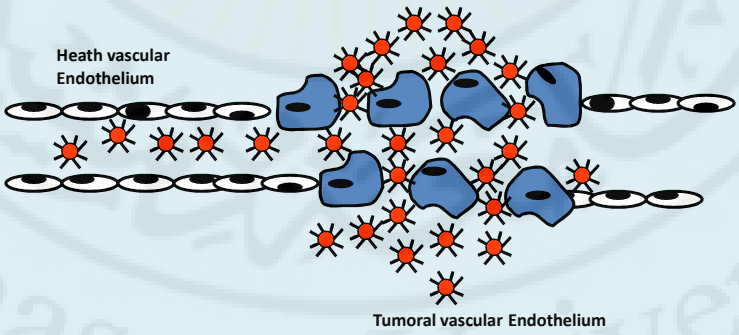
208

STERIC REPULSION AND DYSOPSONIZATION



209

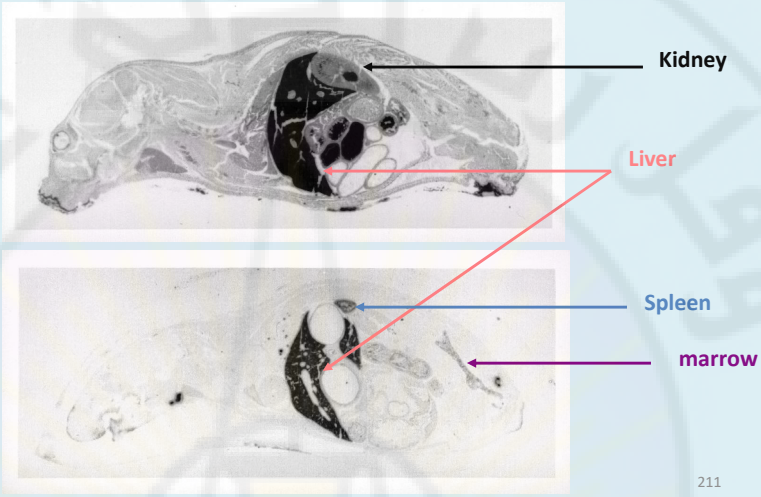
SELECTIVE TUMORALE EXTRAVASATION



210

INTRAVENOUS ADMINISTRATION ROUTE: TARGETING HEPATO-SPLENIC

L. Grislain, P. Couvreur, V. Lenaerts, M. Roland, Int. J Pharm., 1983



211

٣. ٢. طرق التهديف الفعال Active Targeting Approaches:

يتطلب التهديف الفعال للدواء إجراء تعديلات نوعية في الأدوية أو النظم النانوية الحاملة للدواء باستخدام عوامل "فعالة" ذات ألفة انتقائية وقادرة على التعرف على خلية محددة أو نسيج محدد أو عضو محدد في الجسم و التآثر معها.

إن طرق التهديف المنفعل للدواء محدودة بمجالات تطبيقها ولهذا يتم توظيف جهود حثيثة بغية تطوير طرق فعالة active approaches لتهديف الدواء.

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Active Targeting

١- الأهداف

(١)المستقبلات

(أ) مستقبل حمض الفوليك

(ب) مستقبلات LDL

(ج) المستقبلات الببتيدية

(٢) المكونات الدسمة لأغشية الخلية

(٣) المستضدات/ البروتينات السطحية

اللجائن المستهدفة

أ- حمض الفوليك:

ب- السكاكر:

ج- اللاكتين:

د- الألبومين المعدل:

هـ- الببتيدات:

و- الأضداد:

- الاستراتيجيات :

١- التهديف الوعائي Vascular Targeting:

٢- التهديف داخل الخلوي Intracellular Targeting:

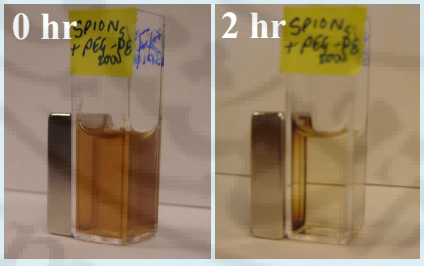
٣-٥- التهديف الفيزيائي Physical Targeting:

٥-٣-١- الحقل المغناطيسي Magnetic Field:

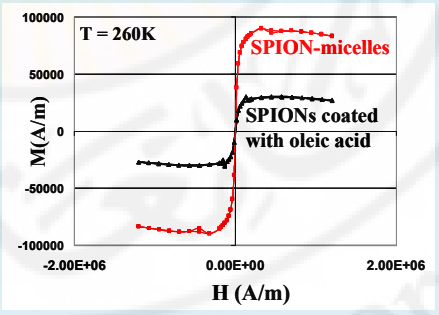
٥-٣-٢- الأمواج فوق الصوتية Ultrasound:

213

Magnetic susceptibility of SPION-micelles



SQUID measurements

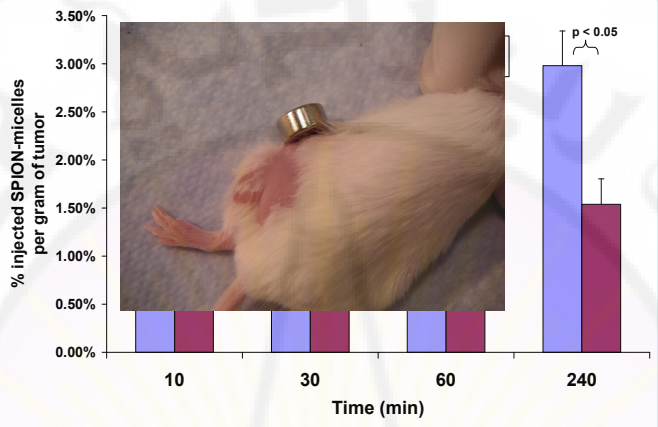


No coercivity is seen indicating the super paramagnetic behavior of SPIONs.

Entrapment of the SPIONs within the PEG-PE micelles does not affect the magnetic properties of the SPIONs

214

Tumor Accumulation of SPION-micelles in Presence of External Magnets



215

APPLICATION OF NANOPARTICLES

216

Most Active Areas of Application of Nanoparticles

- DRUG TARGETING
- NUCLEIC ACID AND GENE DELIVERY
- ENHANCEMENT OF BIOAVAILABILITY
- ENHANCEMENT OF PHARMACOLOGICAL OR IMMUNOBIOLOGICAL EFFECTS

217

الجسيمات النانوية المستخدمة كعوامل تصوير و حوامل للأدوية

الاستخدام	الوضع الحالي	المركب	المجال
			عوامل تصوير
عامل للتصوير بالرنين المغناطيسي	في الأسوق	superparamagnetic iron oxide nanoparticle	Endorem®
عامل للتصوير بالرنين المغناطيسي – قلبي وعائي	في الطور الثالث من التجارب السريرية	Dendrimer-based MRI agents	Gadomer®
			إيتاء الدواء
سرطان الثدي	في الأسوق	Albumin nanoparticle containing paclitaxel	Abraxane®

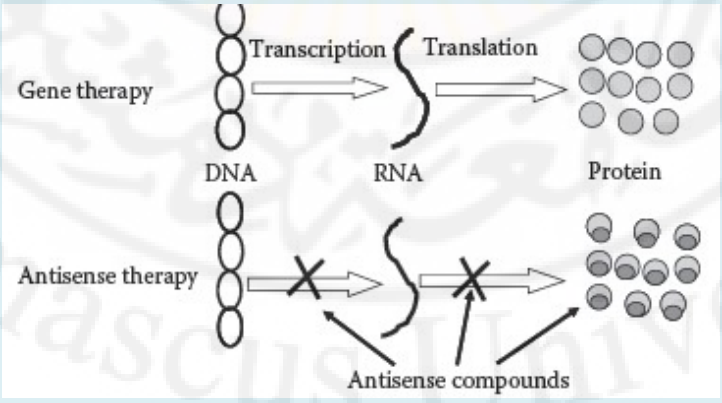
218

TABLE 2.4
Nominal pH Values of Some Body Fluids and Sites

Sites	Nominal pH
Aqueous humor	7.21
Blood	7.40
Cerebrospinal fluid	7.35
Duodenum	7.35
Ileum	8.00
Colon	5.5–7
Lacrimal fluid (tears)	7.4
Saliva	6.4
Semen	7.2
Stomach	1–3
Urine	5.7–5.8
Vaginal secretions, pre-menopause	4.5
Vaginal secretions, post-menopause	7.0

219

Mode of action of nucleic acids. Gene therapy aims at producing therapeutic proteins, while antisense therapy aims at blocking the production of aberrant proteins.



220

Chitosan

CS, derived from deacytalation of chitin, is a copolymer of *β-(–14)-linked* D-glucosamine and N-acetyl-D-glucosamine linked through glycosidic bonds. It has basic amine groups with a *pK_a around 6.5, which results in various* pH-dependent behaviors regarding solubility and polymer conformation.

Table 1. Relevant examples of polysaccharides as biomaterials and their main characteristics.

Name	Origin	Composition	Structure	Charge
Alginate	Algae	Mannuronate Guluronate	Linear	Negative
Dextran	Plant	Glucose	Branched	Neutral
Hyalu			Linear	Negative
Chitos		samine -acetyl	Linear	Positive

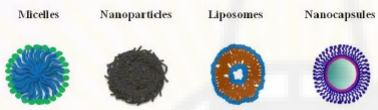


Fig. 4. Schematic representation of main CS and HA-based nanocarriers in the literature.

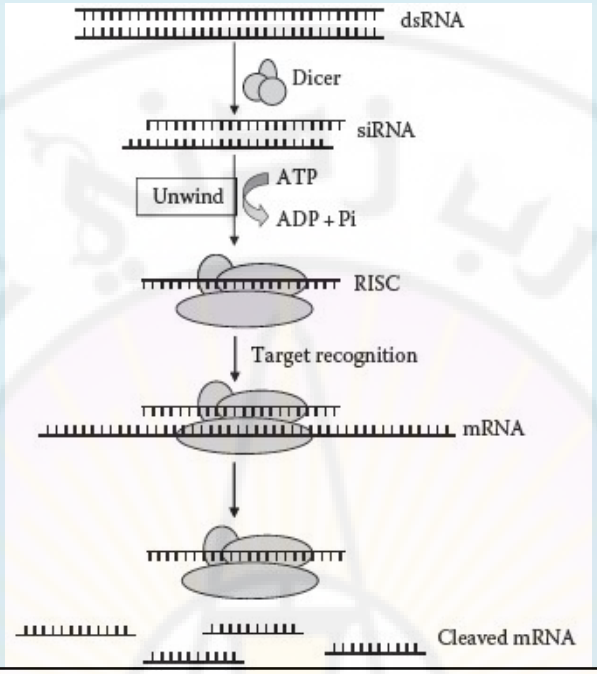
221

Interference RNAi

is a phenomenon, in which double-stranded RNA (dsRNA) molecules efficiently and specifically inhibit gene expression at a posttranscriptional level. The cell has a specific enzyme called Dicer which recognizes the dsRNA and chops it up into small fragments between 21 and 25 base pairs in length. These short dsRNA fragments are called small interfering RNA (siRNA), which bind to the RNA-induced silencing complex (RISC). The RISC gets activated when the siRNA unwinds and the activated complex binds to the corresponding mRNA using the antisense RNA. siRNA silences a target gene by binding to its complementary mRNA and triggering its elimination. Potent knockdown of the target gene with high sequence specificity makes siRNA a promising therapeutic strategy. Three different ways are commonly used for producing siRNA: chemical synthesis, plasmid DNA, and viral vectors encoding small hairpin RNA or short hairpin RNA (shRNA) expression cassette.

222

The mechanisms of RNAi are illustrated

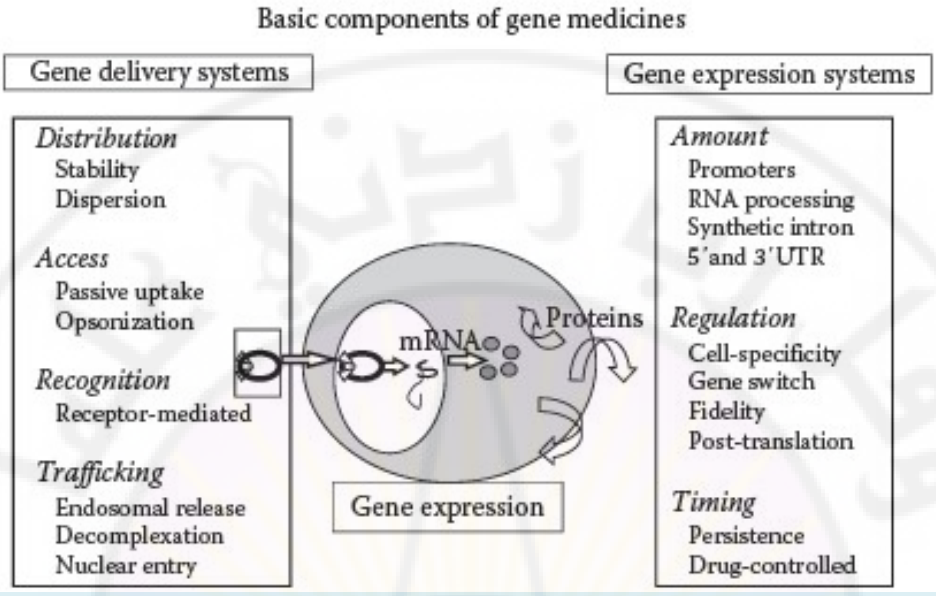


223

TABLE 23.1
Characteristics of Viral Vectors

Viral Vector Classification	DNA/RNA	Insertion Size (kb)	Expression	Advantages	Disadvantages
Retrovirus (DNA) (MMLV)	RNA	9.0	Stable	Integrates, no immune response	Low viral titer, transduces only dividing cells, insertional mutations
Retrovirus (lentivirus)	RNA	9.0	Stable	Transduces nondividing cells	Low viral titer, transduces only dividing cells, insertional mutations
Adenovirus	dsDNA	7.5	Transient	High viral titer, transfects both dividing and nondividing cells	Immunogenic, transient expression
AAV	ssDNA	4.5	Stable	Little immunogenicity, integrates	Low transfection efficiency
HSV	dsDNA	30	Stable	Can target neuronal tissues	Immunogenic

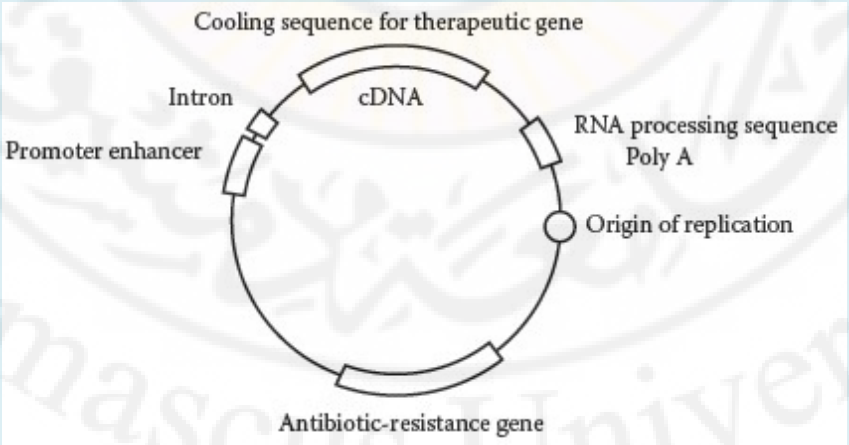
224



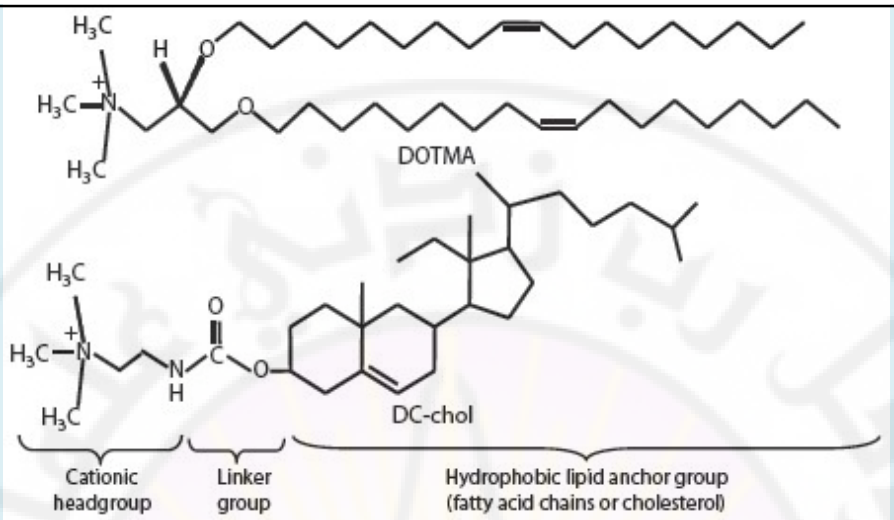
Basic components of a nonviral gene medicine. Therapeutic gene, gene delivery system, and gene expression plasmid are the three basic components of a nonviral gene medicine.

225

Basic components of a gene expression plasmid.



226

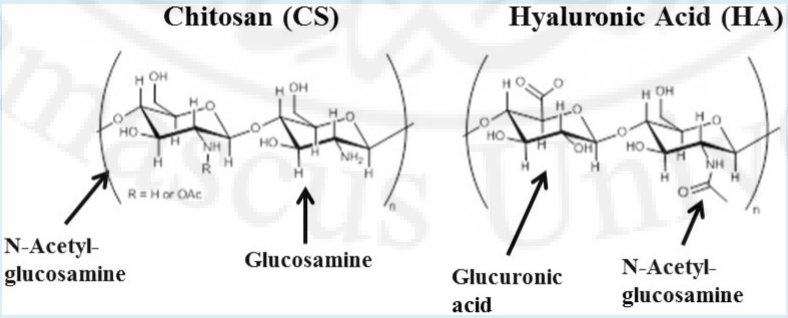


Basic components of a cationic lipid: hydrophobic lipid group, linker group, and cationic head group.

227

Hyaluronic acid

HA is an endogenous biomolecule found in all tissues and body fluids of vertebrates. It is a structural component of the extracellular matrix, sinovial fluids, and soft tissue, which connects and support structures and organs of the body.
As in the case of CS, HA is composed by D-glucuronic acid and N-acetylglucosamine linked through alternating *β(1 → 4) and β(1 → 3)* glycosidic bonds (Fig. 2). Native HA has a molecular weight (Mw) of 10000–100000 kDa, however, it can be obtained from commercial sources in a large range of Mws. Within this broad range, HA of Mw up to 10 kDa is normally named as oligomeric HA19 and HA of Mw up to 45 kDa20,21 is generally considered as LMw–HA.



228

$$P = \frac{\text{(Concentration of drug in octanol or nonpolar phase)}}{[(1-\alpha)(\text{Conc. of drug in water or polar phase})]}$$

If log *P = 0, equal drug distribution in both phases.*
If log *P > 0, the drug is lipid soluble.*
If log *P < 0, the drug is water soluble.*

229

TABLE 2.5
pK_a Values of Typical Acidic and Basic Drugs

Drugs	pK_a
Acidic drugs	
Acetylsalicylic acid	3.5
Barbital	7.9
Phenobarbital	7.4
Penicillin G	2.8
Phenytoin	8.3
Theophylline	8.6
Tolbutamide	5.3
Basic drugs	
Amphetamine	9.8
Atropine	9.7
Quinine	4.2, 8.8
Codeine	7.9
Morphine	7.9
Procaine	9.0
Verapamil	8.8

Source: Martindale, W. and Reynolds, J.E.F., *Martindale: The Extra Pharmacopoeia*, 30th edn., The Pharmaceutical Press, London, U.K., 1993.

230

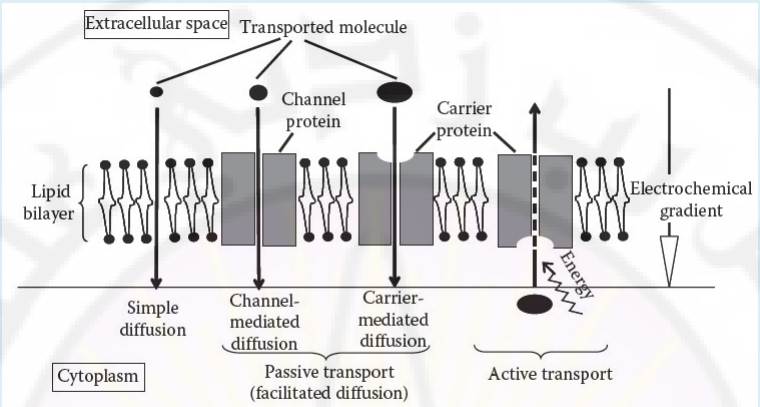


FIGURE 3.4 An illustration of main transport processes across cellular membranes.

231

Temperature nterconversions

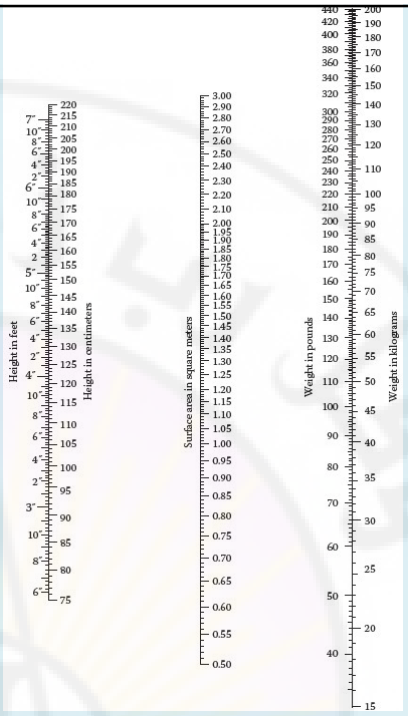
$$\frac{C}{5} = \frac{F - 32}{9} \quad (4.1)$$

$$K = C + 273.15 \quad (4.2)$$

Interestingly, −40°C = −40°F.
At temperatures >−40°C, °F < °C.
At temperatures <−40°C, °F > °C.

232

Example of a typical adult nomogram for the calculation of BSA for patients over 65 lb weight or 3 ft tall. (From Braafladt, K. et al., Science Museum of Minnesota, Saint Paul, MN, http://www.smm.org/heart/lessons/nomogram_child.htm [last accessed September 2011].)



$$\text{Child's dose} = \text{Adult dose} \times \frac{\text{Child's BSA in m}^2}{1.73\ \text{m}^2}$$

a child's dose is also calculated using the age of child in months (Fried's rule) or years (Young's rule), or using the weight of the child in pounds (Clark's rule).

$$\text{Child's dose} = \text{Adult dose} \times \frac{\text{Child's age in months}}{150\ \text{months}}$$

$$\text{Child's dose} = \text{Adult dose} \times \frac{\text{Child's age in years}}{\text{Child's age in years} + 12\ \text{years}}$$

$$\text{Child's dose} = \text{Adult dose} \times \frac{\text{Child's weight in pounds}}{150\ \text{lb}}$$

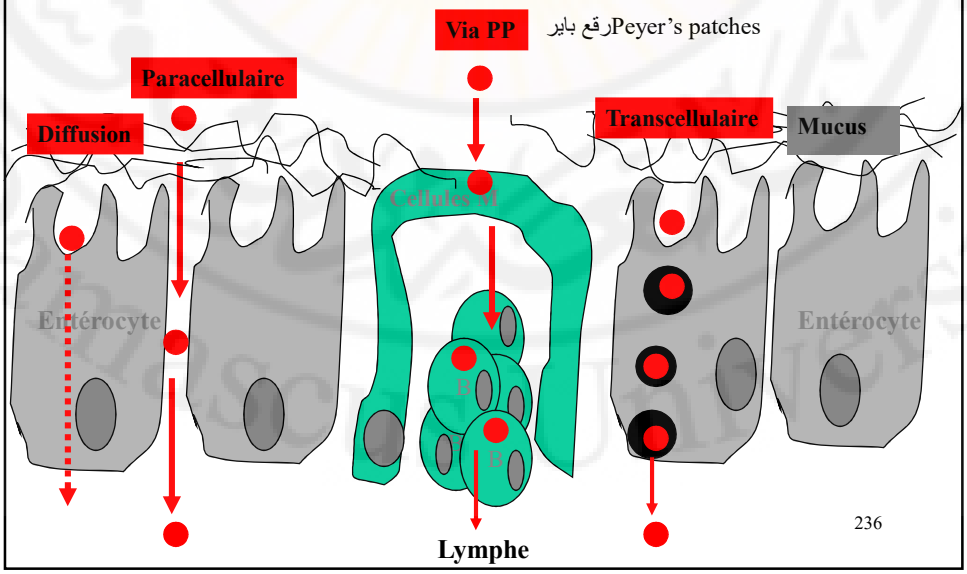
234

Therapeutic applications of Nanoparticles

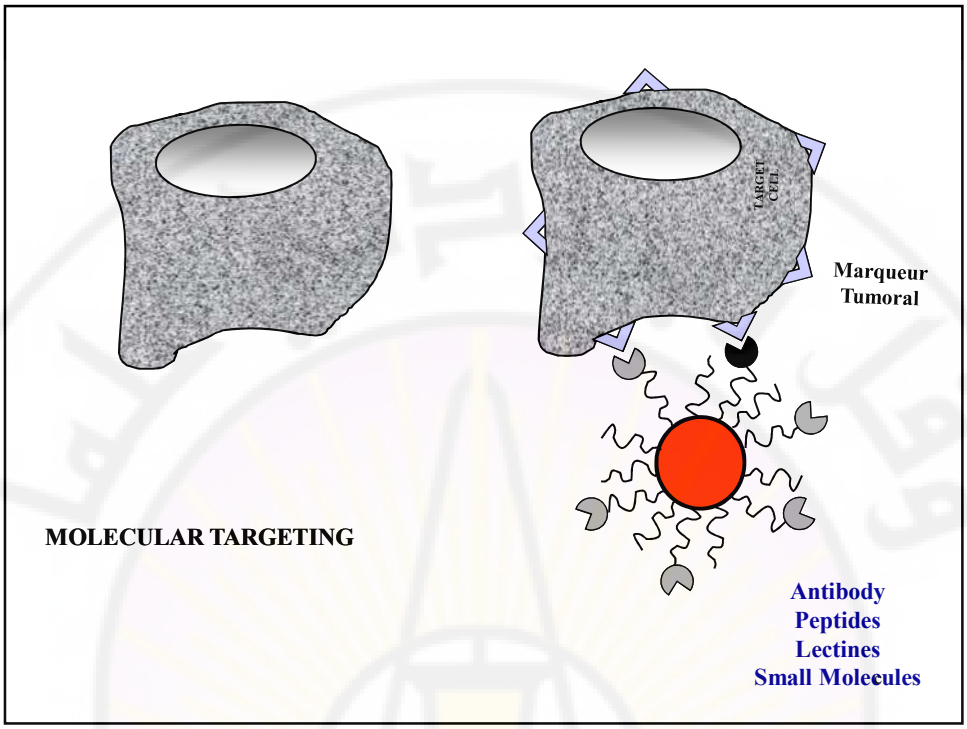
- ❖Treatment of cancer
  - Anticancer drugs
  - Cytokines
  - Nucleic acids
- ❖Radiodiagnostic
- ❖Targeting inflammed tissue (infections)

235

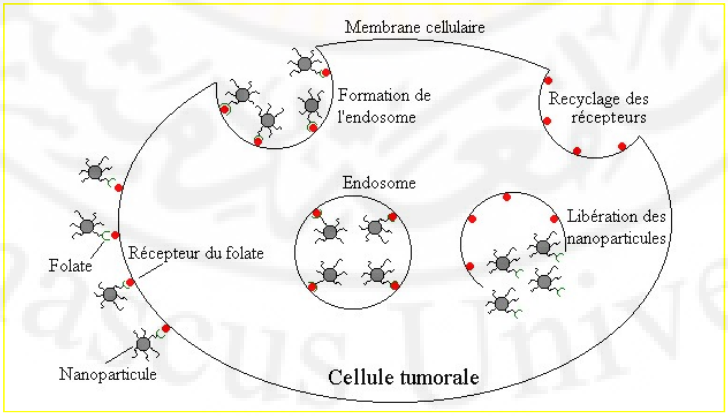
ROUTE OF TRANSLOCATION OF NANOPARTICLES in GIT



236



TARGETING OF TUMORAL RECEPTORS

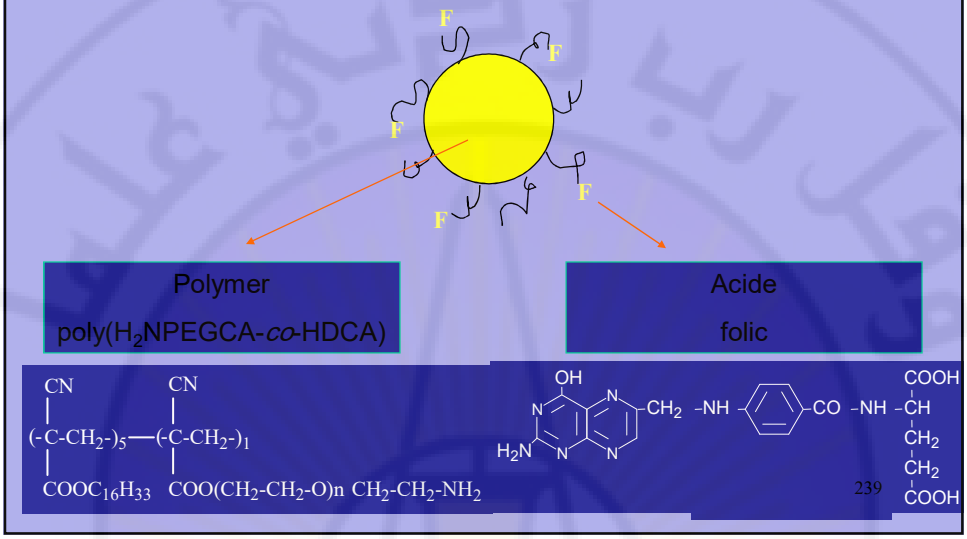


Stella et al., J. Pharm. Sci., 89, 1452-1464, 2000

238

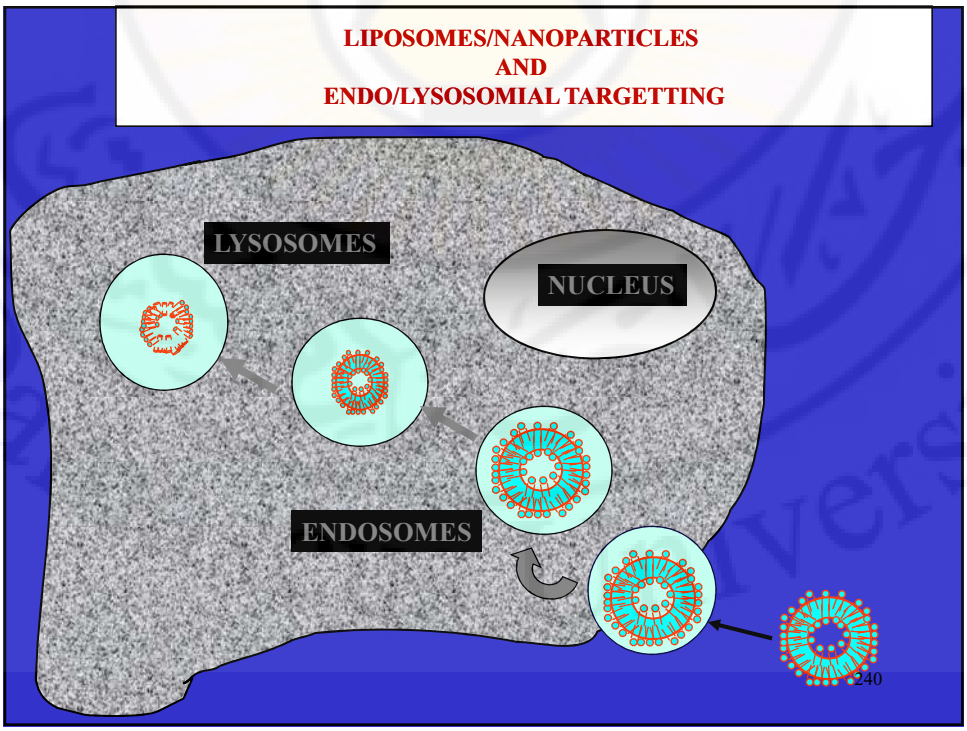
DECORATED NANOPARTICLES WITH FOLIC ACID

Stella et al., J. Pharm. Sci., 89, 1452-1464, 2000



239

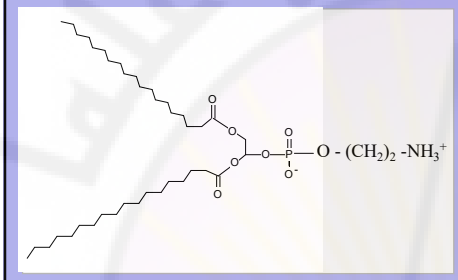
LIPOSOMES/NANOPARTICLES AND ENDO/LYSOSOMIAL TARGETTING



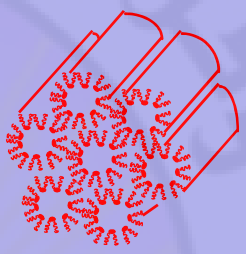
240

Concept of pH sensitive liposomes

- Lipids forming non lamellar phases: Phosphatidyl ethanolamine (DOPE)



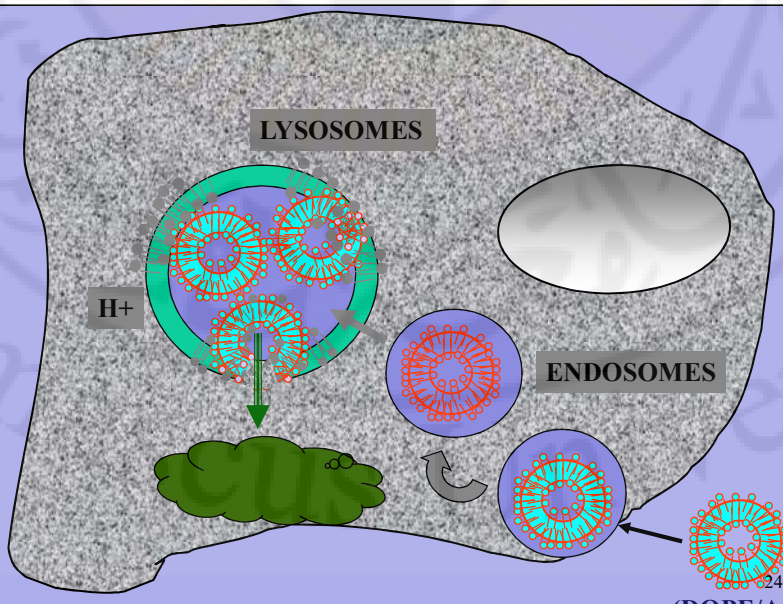




LIPIDS SHAPE PHASE

241

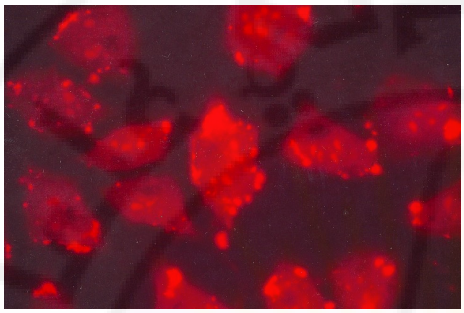
pH SENSITIVE LIPOSOMES AND CYTOPLASMIC DELIVERY



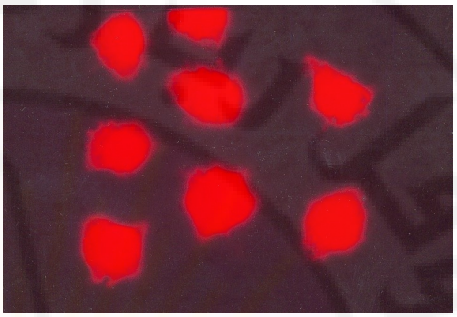
242

(DOPE/AO/Chol)

SUBCELLULAR DISTRIBUTION OF pH-SENSITIVE LIPOSOMES AND NON pH-SENSITIVES



non pH-sensitive Liposomes
(DOPC/OA/Chol)
= lysosomiale delivery

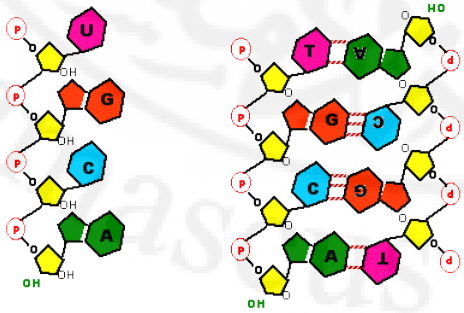


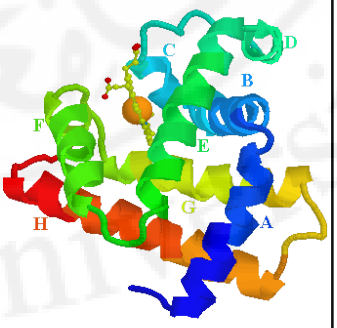
pH-sensitive Liposomes
(DOPE/OA/Chol)
= cytoplasmic delivery

243

Poorly stable molecules

- Nucleic Acids
- Peptides





Highly water soluble

244

DNA

DNA of varying molecular weights (base pairs) is used in gene therapy.

❑ **As a large hydrophilic, polyanionic and sensitive macromolecule, successful delivery to target cells and the nucleus within these cells is an issue.**

❑ **Shearing of high-molecular-weight DNA while stirring in solution can lead to breakage of the molecule.**

❑ **One approach to delivery is to complex the DNA with polymers or particles of opposite charge to produce, by condensation, more compact species.**

❑ **DNA can be condensed to nanoparticles with cationic polymers (e.g. polylysine and chitosan), cationic liposomes and dendrimers. These retain an overall positive charge and are able to transfect cells more readily than native or naked DNA.**

245

Oligonucleotides

❑ Antisense oligonucleotides (used for the sequence-specific inhibition of gene expression) are polyanionic molecules
with between 10 and 25 nucleotides, which resemble single stranded
DNA or RNA.

❑ They have a molecular weight from 3000 to 8000 and are hydrophilic, having a log *P of approximately –3.5.*

❑ Like DNA, they clearly do not have the appropriate properties for transfer across biological membranes.

❑ They are also sensitive to nucleases and non-specific adsorption to biological surfaces.

246

Gene therapy can be defined as the insertion, alteration, or removal of genes within an individual's cells and biological tissues to treat disease. The most common form of gene therapy involves the insertion of functional genes which may interfere with a gene's function, restore a lost function or initiate a new function .

Genetic disorders caused by protein over-expression may be treated by one of two approaches: antisense and RNA interference (RNAi).

Antisense RNA is a single-stranded* (طاق) RNA that is complementary to a messenger RNA (mRNA) strand transcribed within a cell. Antisense RNA may be introduced into a cell to inhibit translation of a complementary mRNA by attaching to it and physically obstructing the translation machinery.

RNA interference (RNAi) is a system within living cells that takes part in controlling which genes are active and how active they are. RNAi molecules, known as small interfering RNAs (siRNAs), can bind to specific other RNAs and work through the cleavage of mRNA leading to its destruction and ultimately the prevention of protein synthesis.

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**stranded = (طاق)*



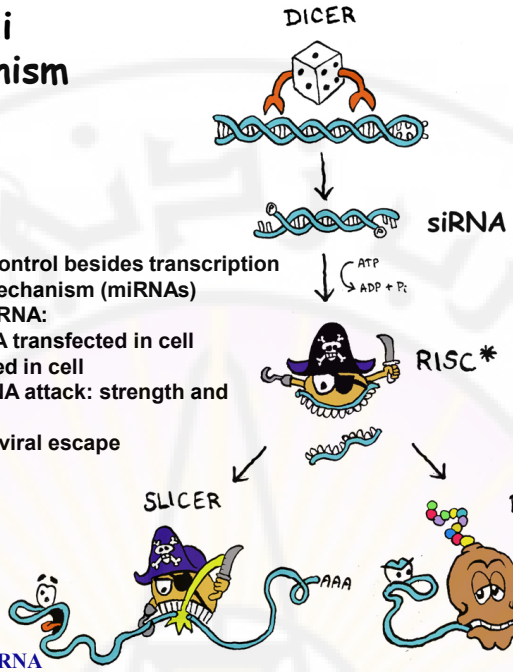


Two US scientists win the Nobel prize in Medicine 2006:
Craig Mello (UMass) & Andrew Fire (Stanford)
For the discovery of RNA interference (RNAi)
Gene regulation mechanism that controls cell development
via double-stranded RNA (microRNAs, miRNA)
Biological relevance obvious, clinical relevance in the works



248

RNAi mechanism



- Another layer of gene control besides transcription
- Endogenous cellular mechanism (miRNAs)
- 20 bp double-stranded RNA:
  - synthetic siRNA transfected in cell
  - shRNA produced in cell
- Sequence-specific mRNA attack: strength and weakness
  - specificity, but viral escape

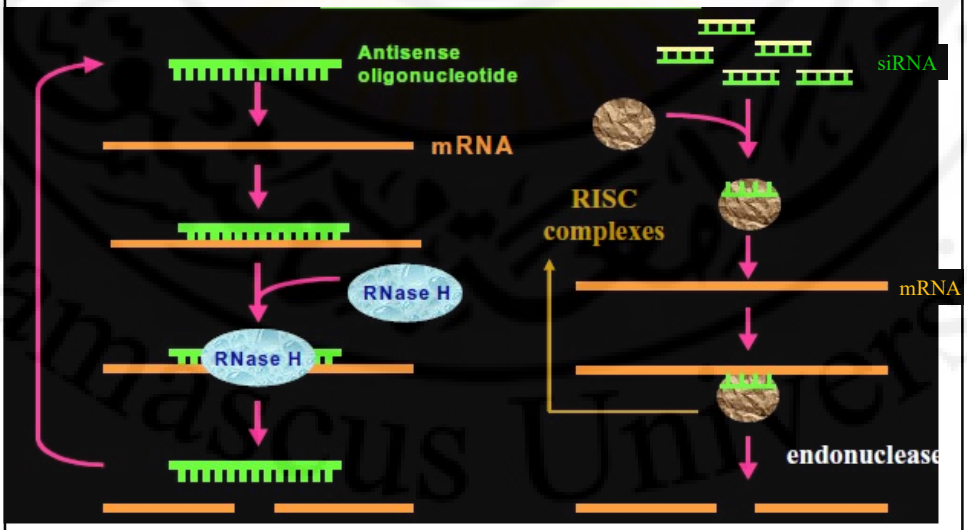
shRNA = Small hairpin RNA

bp = base pair(s)-one bp corresponds to circa 3.4 Å of length along the strand

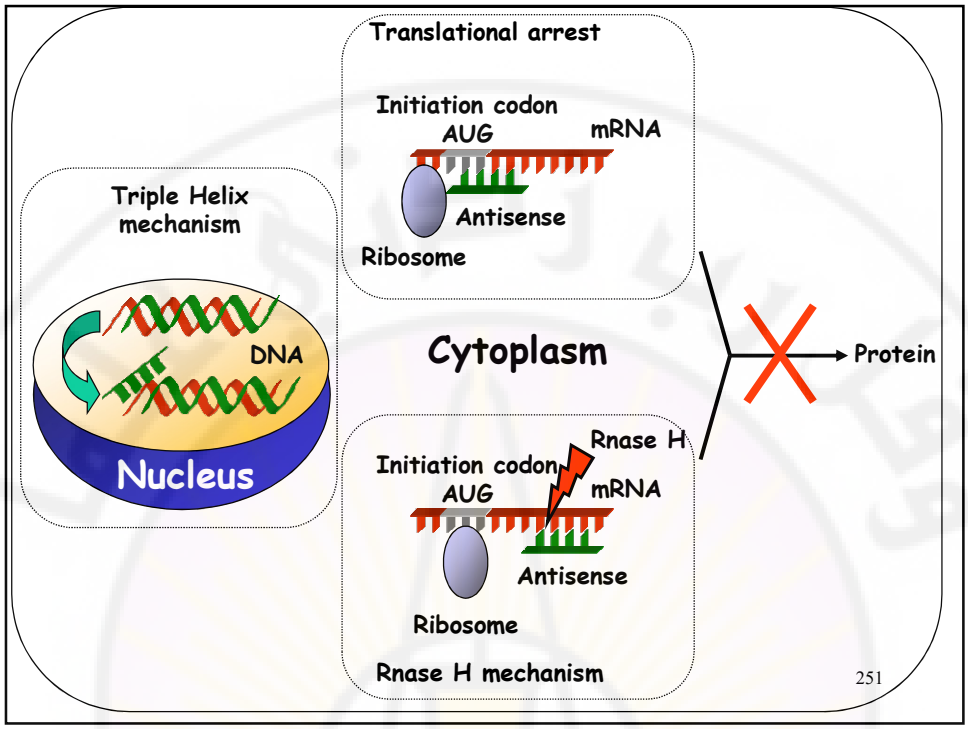
249

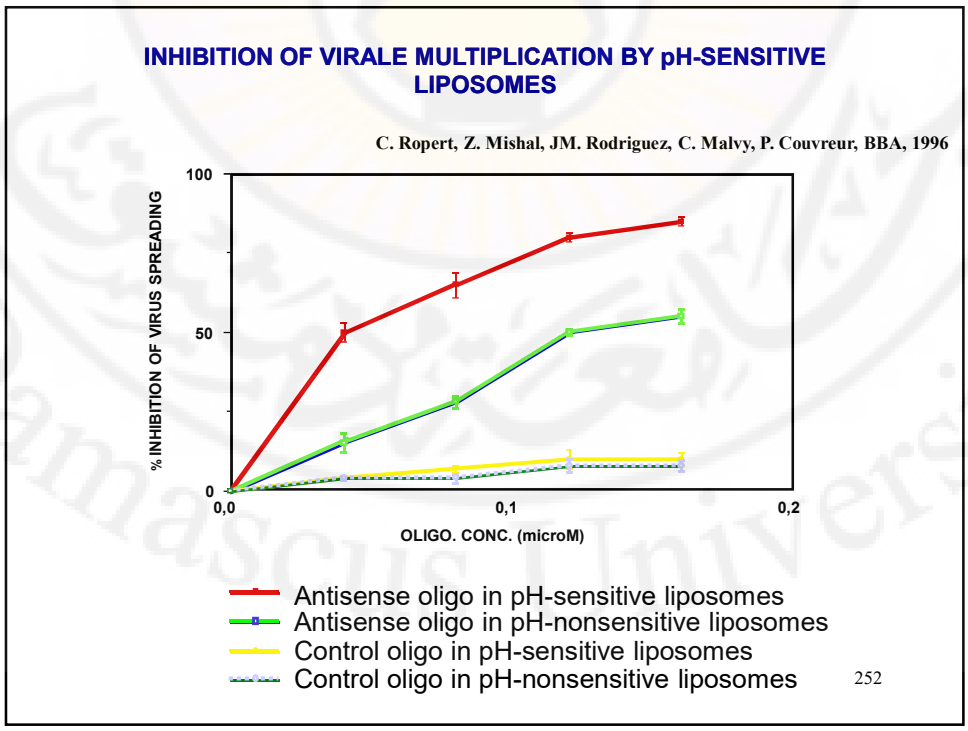
RNA antisense mechanism

siRNA mechanism

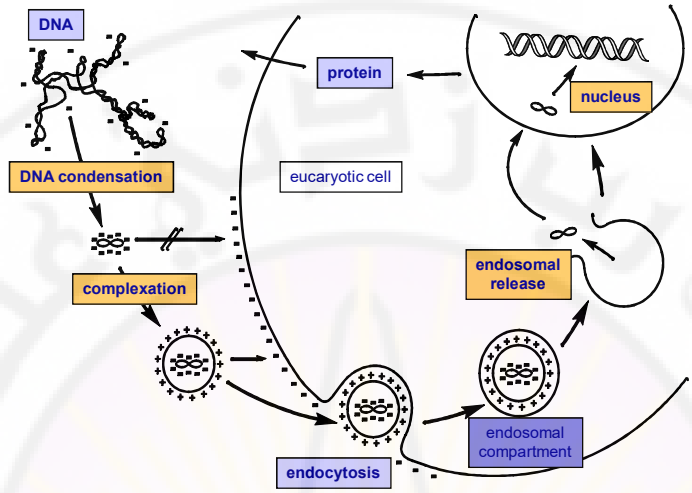


250


Translational arrest
Initiation codon
AUG
mRNA
Antisense
Ribosome
Triple Helix
mechanism
DNA
Nucleus
Cytoplasm
Protein
Rnase H
Initiation codon
AUG
mRNA
Antisense
Ribosome
Rnase H mechanism
251


INHIBITION OF VIRALE MULTIPLICATION BY pH-SENSITIVE LIPOSOMES
C. Ropert, Z. Mishal, JM. Rodriguez, C. Malvy, P. Couvreur, BBA, 1996
% INHIBITION OF VIRUS SPREADING
100
50
0
0,0
0,1
0,2
OLIGO. CONC. (microM)
Antisense oligo in pH-sensitive liposomes
Antisense oligo in pH-nonsensitive liposomes
Control oligo in pH-sensitive liposomes
Control oligo in pH-nonsensitive liposomes
252

Cellular Uptake and Intracellular Processing of DNA



متطلبات تصميم نظم إيتاء الجينات وتتضمن:
(I) القدرة على تكثيف الجينات، (II) القبط من قبل الخلية المستهدفة، (III) الهروب من الجسيم الداخلي، (IV) تحرر الدنا من المعقد، (V) تجاوز الهيولى وصولاً إلى النواة، (VI) الحصول على التعبير الجيني

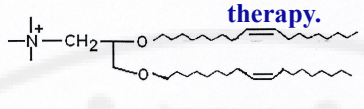
253

- **٢ـ أنواع النواقل غير الفيروسية:**
- لم يتم حتى تاريخه التوصل إلى نظام ناقل للجينات مثالي من حيث الفعالية والمأمونية. إلا أن تخليق مثل هذا النظام يتطلب توفير مجموعة من الصفات المرغوبة وهي:

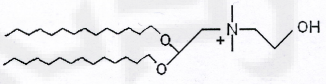
ـ تكثيف الدنا، وهذا يمكن إنجازه بالتأثر الكهربائي الساكن مع مكثور إيجابي الشحنة.
ـ أن يحتوي النظام على تلبيس مسهّل للانسلال، لمنع التعرف على المعقد الحاوي على الدنا من قبل البالعات في الجملة الشبكية البطانية.
ـ أن يحافظ النظام على ثباته الغرويدي في الدم وتجنب الارتصاص في الشعريات الدموية الرئوية.
ـ أن يحتوي النظام على مجموعات كيميائية (لجائن) تيسّر الدخول النوعي للخلية الهدف، مع امتلاك سطح معقد النظام الناقل لشحنة إيجابية لتسهيل دخول الخلية.
ـ أن يكون قادراً على الهروب من الجسيم الداخلي.

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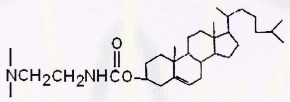
Chemical structure of some cationic lipids commonly used in gene therapy.



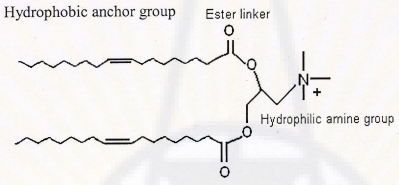
DOTMA



DMRIE



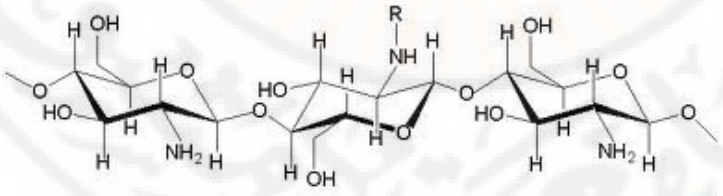
DC-Chol



DOTAP

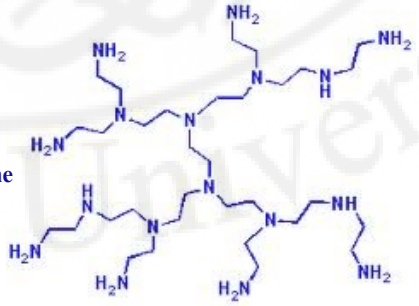
255

Chemical structure of some cationic polymers commonly used in gene targeting.



R = H or $COCH_3$

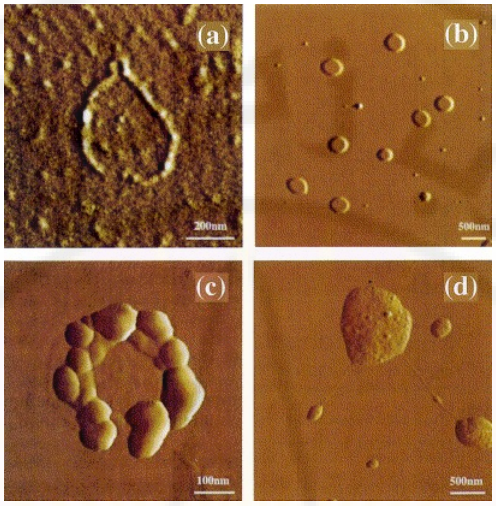
Chitosan



poly ethylen imine

CATIONIC LIPOSOMES FOR GENE DELIVERY

DNA

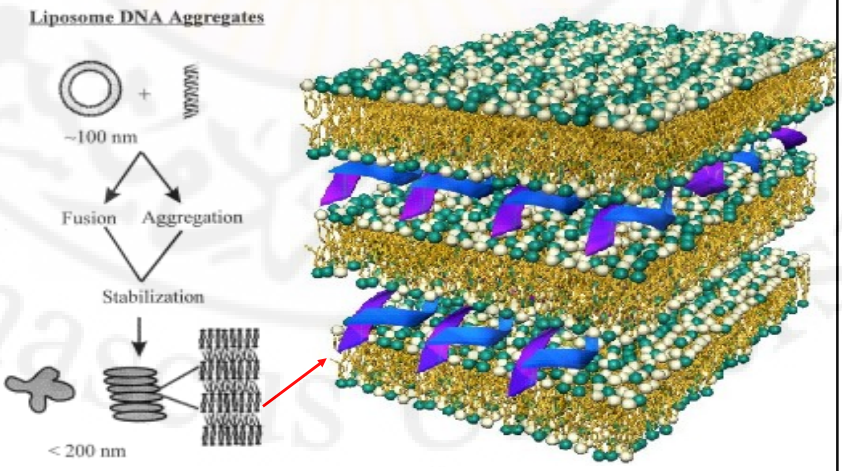


liposome

complexes

257

Cationic lipids formulation of lipoplexes



Wong et al. Science 2000 16;288(5473):2035-2039

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•الحواجز داخل الخلوية Intracellular Barriers

- **١- الهروب من الجسيم الداخلي Endosomal Escape**
- يمكن لبعض معقدات البولي بلكس أن تساعد في عملية الهروب من الجسيم الداخلي حيث أن شحنته الإيجابية، و الناجمة عن العدد الكبير من مجموعات الأمين المتواجدة في المكاثير الإيجابية المكونة للبولي بلكس مثل البولي إيثيلين إيمين، تعمل كدارئ (وقاء) وتقوم بسحب البروتونات الحمضية المتواجدة في حويصلات الجسيمات الداخلية مما يؤدي إلى ارتفاع باهاء الوسط داخل الجسيم الداخلي و إعاقة عمل إنزيمات التدرك، و في النهاية ينفجر الجسيم الداخلي بفعل ازدياد المحتوى الأيوني و الذي يؤدي إلى ارتفاع في الضغط الحلولي مسبباً ازدياد المحتوى المائي داخله.

تسمّى هذه الآلية "فرضية إسفنجة البروتونات proton sponge hypothesis".

٢- الثبات في الهيولى (السيتوبلاسما)

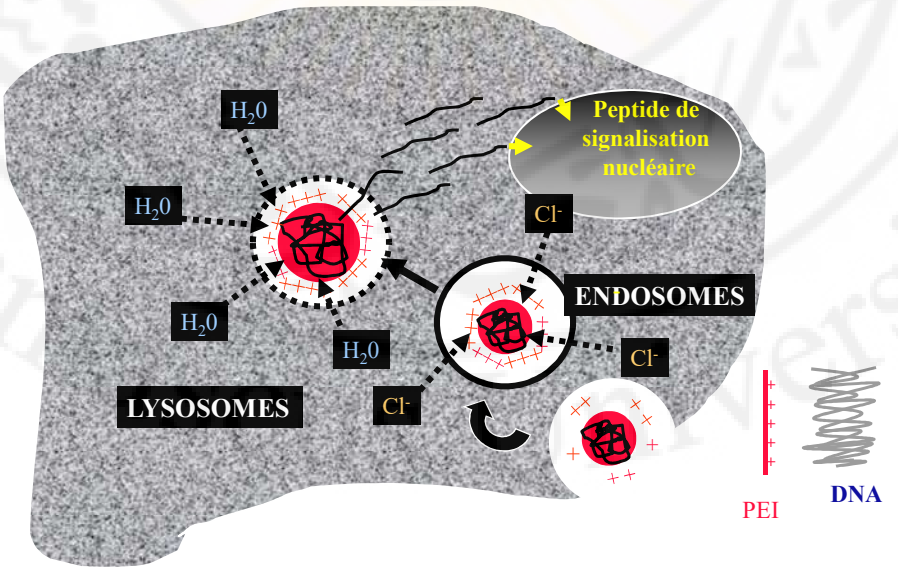
بعد أن ينجو الدنا من الجسيمات الداخلية قد يتعرض لهجوم إنزيمات النيوكلياز في الهيولى. فعند حقن بلازميدة DNA في الهيولى تقوم النيوكلياز الهيولية بتخريبه بشكل سريع حيث يتراوح عمر النصف ما بين ٥٠-٩٠ دقيقة.

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PROTON SPONGE HYPOTHESIS LIBERATION CYTOPLASMIC AND NUCLEAR OF DNA

O. Boussif, F Lezoualc'h, M. Zanta, M. Mergny, D. Scherman, B. Demeneix, J-P Behr, PNAS, 1995

M. Zanta, P. Belguise-Valladier, J-P Behr, PNAS, 1999



• **المرور النووي**

خلال انقسام الخلايا حقيقية النوى يحدث نقل الدنا بين الهيولى و النواة عندما تتحطم المحفظة النووية خلال مرحلة الانقسام الفتيلي mitosis.

Problems of siRNA therapy and strategies

Problems:

- Rapid cleavage by nucleases
- Poor cellular uptake

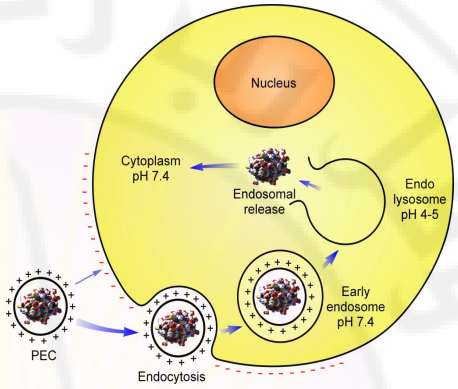
Strategies

- Chemical modification
- Viral vectors (Recombinant virus)
- Non-viral carrier system: synthetic vectors
  - Cationic lipids
  - ✓ Cationic polymers

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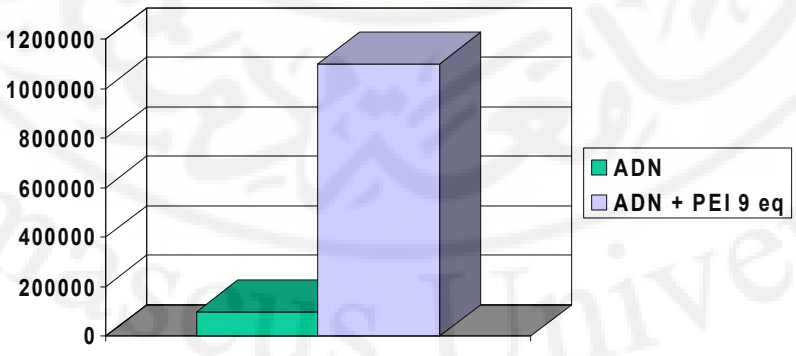
Advantages of cationic polymers

- To promote the cell membrane adhesion of siRNA.
- To compact the siRNA for a better cellular penetration and protection from nucleases.
- To promote siRNA transfer through the plasmatic or endosomal membrane.



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TRANSFECTION OF LUCIFERASE GENE TO THE CEREBRUM OF A MOUSE



O. Boussif, F Lezoualc'h, M. Zanta, M. Mergny, D. Scherman, B. Demeneix, J-P Behr, PNAS, 1995

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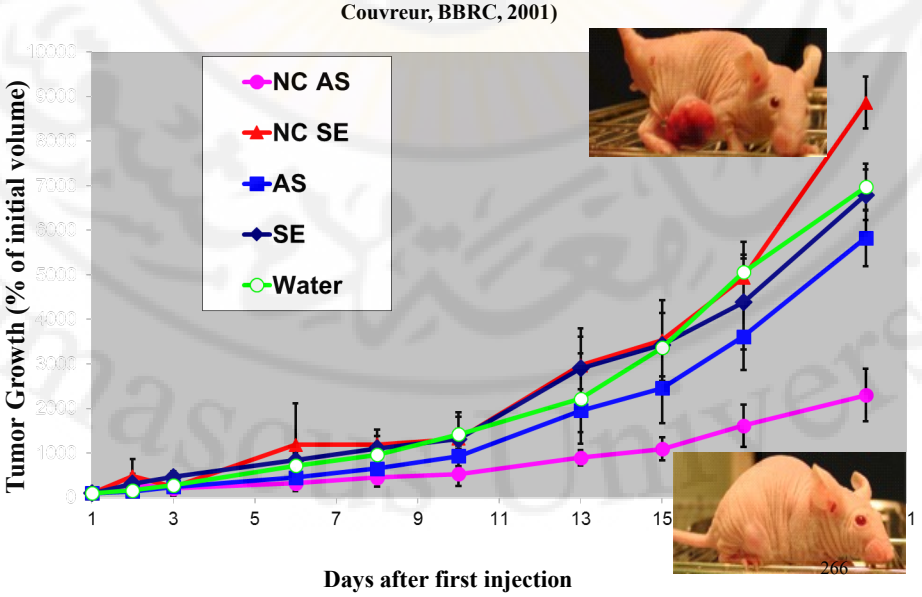
Limits of the strategy

- Efficacy weaker than viral vectors
- Problems to achieve a nuclear targeting
- Short time of expression of the gene
- Cytotoxicity and interactions with plasma proteins

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EFFECT OF ODN NANOCAPSULES ON SUBCUTANEOUSLY GRAFTED EWING SARCOMA

(G. Lambert, J.R. Bertrand, E. Fattal, F. Subra, H. Pinto-Alphandary, C. Malvy,C. Auclair, P. Couvreur, BBRC, 2001)



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Peptides and proteins

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Structure of peptides and proteins

Proteins have an increasing order of Complexity :

Primary structure – the order in which the individual amino acids are arranged.

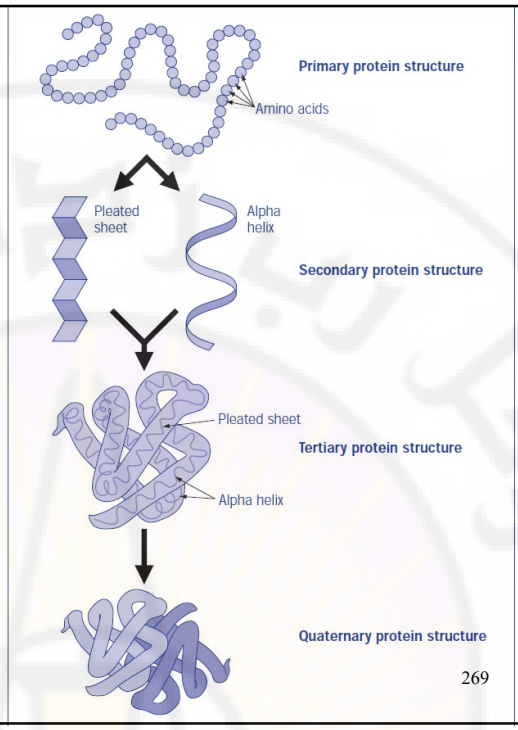
Secondary structures – including coiled α-helix and pleated sheets.

Tertiary structure – the three-dimensional arrangement of helices and coils.

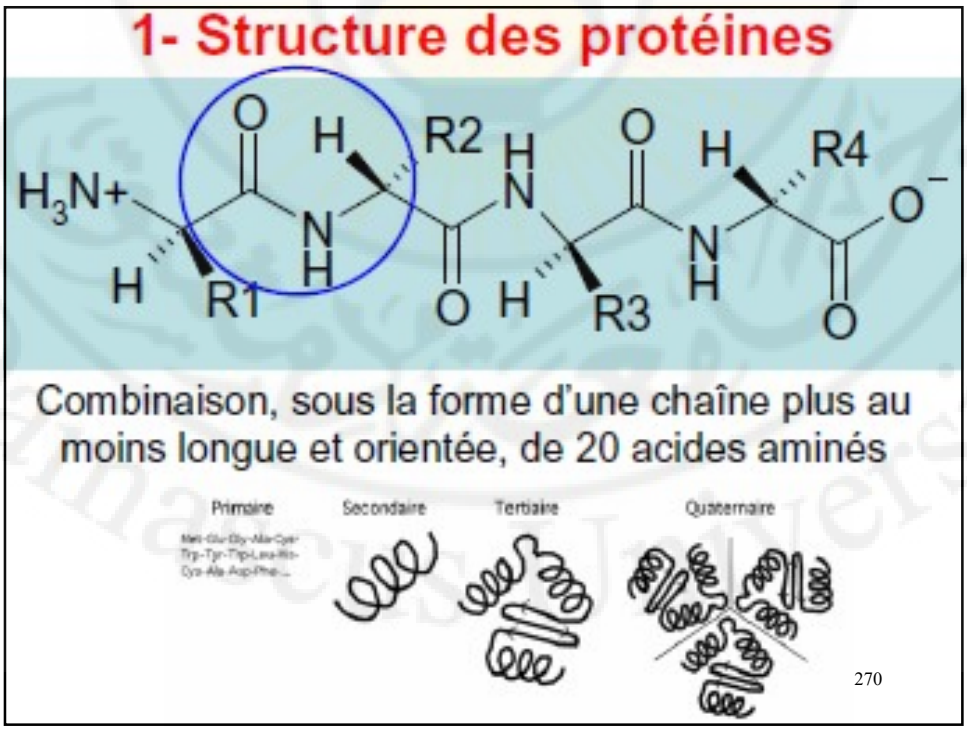
Quaternary forms – the association of ternary ثُلاثِيّ *forms (e.g. the* hexameric form of insulin).

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Diagrammatic representation of protein structure.



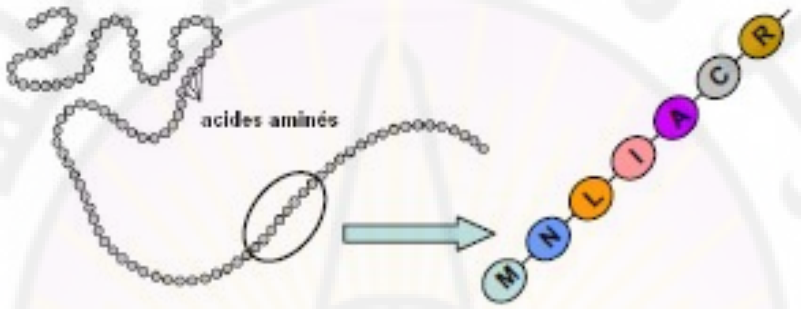
269



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1- Structure des protéines

La séquence des acides aminés d'une protéine constitue la structure primaire de la protéine.

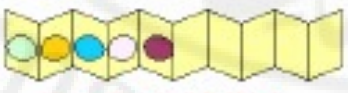


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1- Structure des protéines

Des régions de la protéines peuvent adopter 2 formes particulières : hélices ou feuillets. On parle de **structure secondaire**.
La structure tridimensionnelle finale qu'adopte la chaîne d'acides aminés, constitue la **structure tertiaire** de la protéine.







Structures secondaires

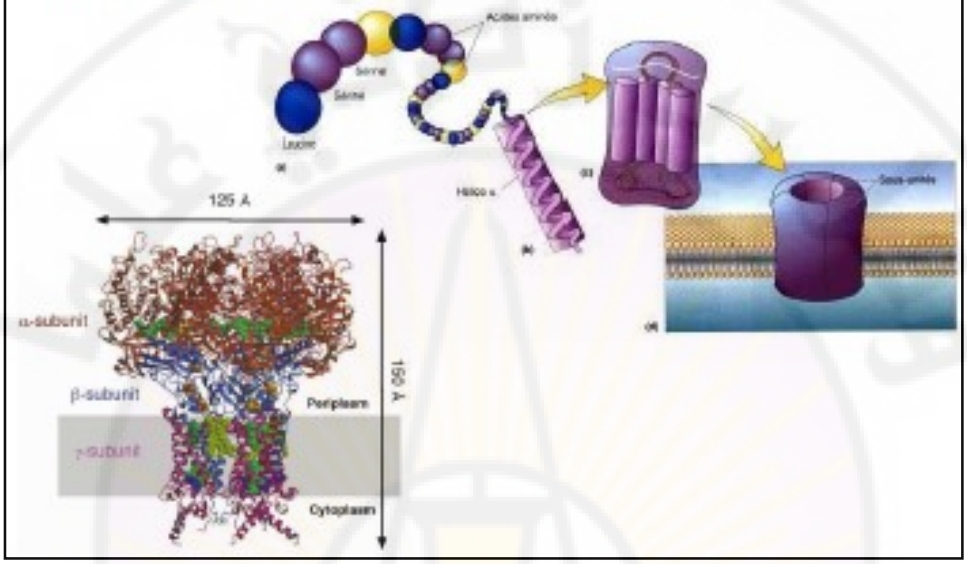


Structure tertiaire d'une protéine

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1- Structure des protéines

structure quaternaire: Plusieurs protéines peuvent s'associer pour former des ensembles complexes



2- Problèmes de stabilité des protéines

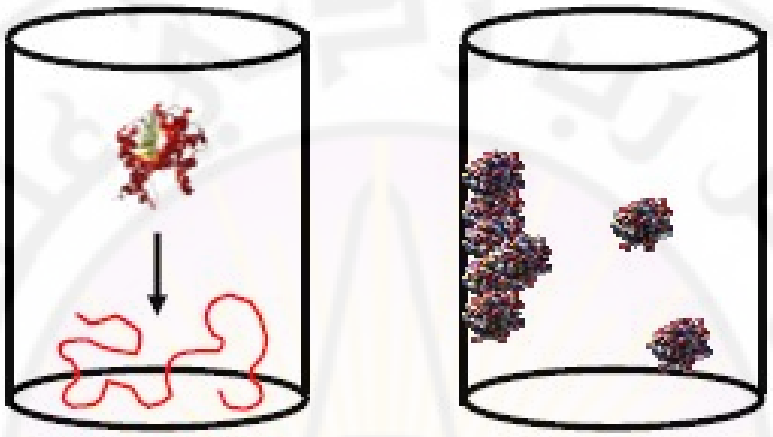
Sans modification chimique

- Dénaturation
- Agrégation
- Précipitation
- Adsorption

Avec modification chimique

- Déamidation
- Oxydation
- Échange de pont disulfures
- Protéolyse

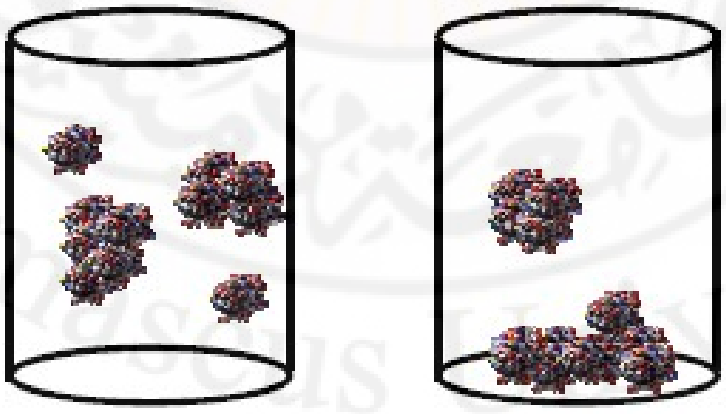
2-1 Sans modification chimique



Dénaturation Adsorption

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2-1 Sans modification chimique



Agrégation Précipitation

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List of Some Commercial Products of Therapeutic Proteins

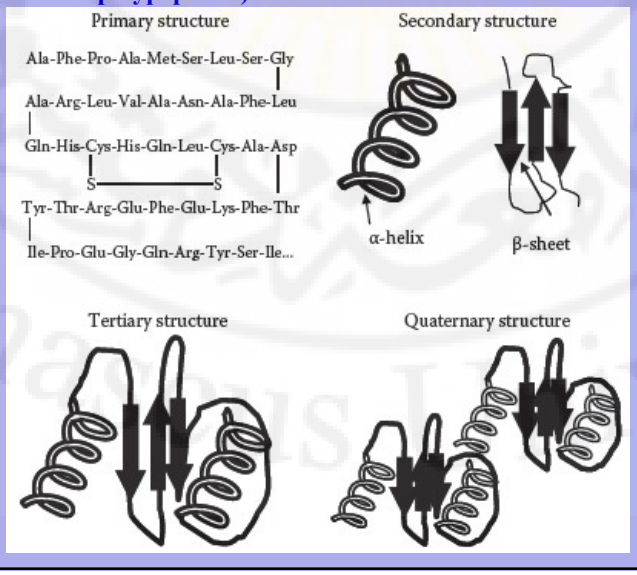
Protein Type	Protein Names	Description	Indication
Polyclonal antibodies (lyophilized)	Sandoglobulin	Human immune globulin for IV administration. It is a polyvalent antibody product that contains all IgG antibodies which regularly occur in the donor population in a concentrated form. It is prepared by fractionation of the plasma of volunteer donors It is a lyophilized preparation	Primary immune deficiencies such as severe combined immunodeficiency (SCID), common variable immunodeficiency, X-linked agammaglobulinemia, and immune thrombocytic purpura (ITP)
Polyclonal antibodies (solution)	Gammagard	Concentrated human IgG antibodies similar to that of normal plasma. It is manufactured from pooled human plasma from donors It is available as a 10% ready-to-use sterile liquid formulation	Primary immunodeficiencies
Monoclonal antibodies	Rituximab	Monoclonal antibody that recognizes specific proteins on the surface of some lymphoma cells and triggers body's immune system	Combination therapy for tumors such as non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL), and autoimmune diseases such as rheumatoid arthritis
Radioactively tagged antibodies	Ibritumomab tiuxetan	Monoclonal antibody radioimmunotherapy. It is prepared from monoclonal mouse IgG1 antibody ibritumomab and uses the chelator tiuxetan, which has a radioactive isotope (yttrium-90 or indium-111)	B-cell NHL
Mose-antibodies	Tositumomab	IgG2 anti-CD20 monoclonal antibody of murine origin. Also available as radioactively labeled ^{131}I-tositumomab, which has covalently bound iodine-131	Follicular lymphoma
Chimeric antibodies	Infliximab	Monoclonal antibody against TNFα	Psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, and ulcerative colitis

List of Some Commercial Products of Therapeutic Proteins

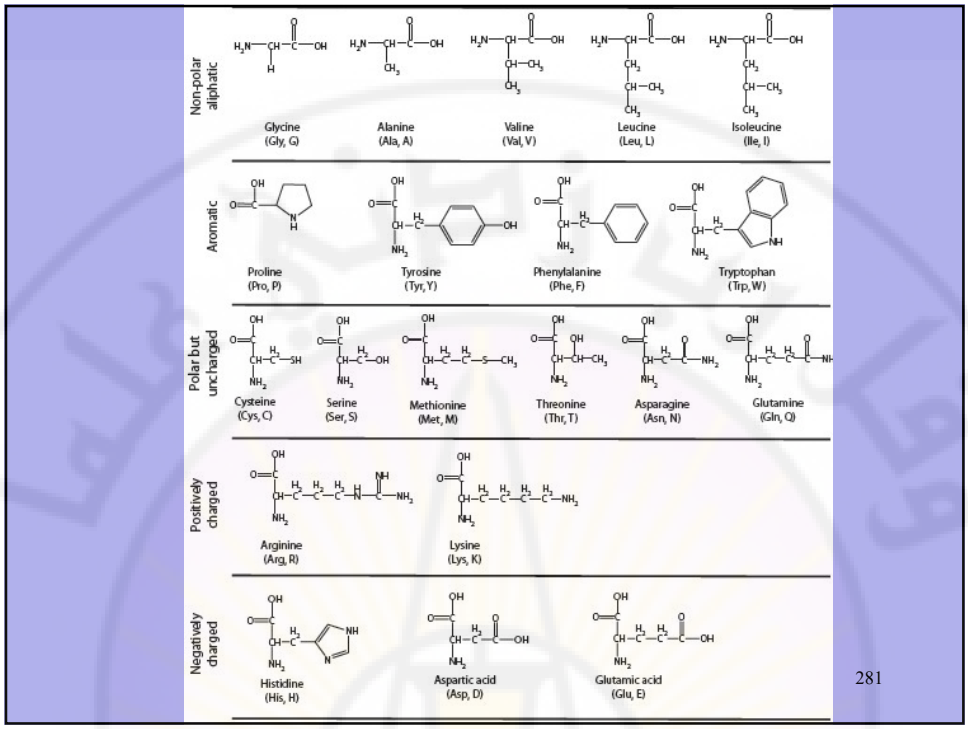
Protein Type	Protein Names	Description	Indication
Humanized antibodies	Daclizumab	Monoclonal antibody against the α subunit of IL-2 receptor on T-cells	To prevent the rejection in organ transplantation, especially in kidney transplantation
Fusion proteins	Abatacept	Fusion protein that is composed of human Ig fused to the extracellular domain of CTLA-4, a molecule involved in T-cell stimulation	Rheumatoid arthritis
Physiological proteins	Erythropoietin	Glycoprotein hormone that controls erythropoiesis (red blood cell production). It is available as a lyophilized preparation	Kidney diseases, anemia, and cancer

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Illustration of protein structures: primary structure (amino acid sequence), secondary structure (α-helix and β-sheets), tertiary structure (further folding of the secondary structurally folded protein), and quaternary structure (combination of polypeptides).



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Hydrophobicity and Acidity of Amino Acids

Amino Acid	Three-Letter Abbreviation	One-Letter Designation	Log *P* Value	pK_a Value of Carboxylate Group	pK_a Value of Amino Group	pK_a Value of Side Chain
Alanine	Ala	A	−2.83	2.35	9.87	—
Arginine	Arg	R	−1.43	2.01	9.04	12.48
Asparagine	Asn	N	−2.33	2.02	8.80	—
Aspartic acid	Asp	D	−1.67	2.10	9.82	3.86
Cysteine	Cys	C	−0.92	2.05	10.25	8.00
Glutamic acid	Glu	E	−1.39	2.10	9.47	4.07
Glutamine	Gln	Q	−2.05	2.17	9.13	—
Glycine	Gly	G	−1.39	2.35	9.78	—
Histidine	His	H	−1.67	1.77	9.18	6.10
Isoleucine	Ile	I	0.41	2.32	9.76	—
Leucine	Leu	L	−1.62	2.33	9.74	—
Lysine	Lys	K	−1.15	2.18	8.95	10.53
Methionine	Met	M	−0.56	2.28	9.21	—
Phenylalanine	Phe	F	−1.49	2.58	9.24	—
Proline	Pro	P	−0.40	2.20	10.60	—
Serine	Ser	S	−1.75	2.21	9.15	—
Threonine	Thr	T	−1.43	2.09	9.10	—
Tryptophan	Trp	W	−1.07	2.38	9.39	—
Tyrosine	Tyr	Y	−2.15	2.20	9.11	10.07
Valine	Val	V	−0.01	2.29	9.72	—

An amino acid backbone can involve the ionization of the acid and/or base:

$$\text{R-COOH} \rightleftharpoons \text{R-COO}^- + \text{H}^+$$

$$\text{R-NH}_2 + \text{H}^+ \rightleftharpoons \text{R-NH}_3^+$$

$$k_a = \frac{[\text{R-COO}^-][\text{H}^+]}{[\text{R-COOH}]} \text{ (ionization constant for the acid functional group)}$$

$$k_b = \frac{[\text{R-NH}_3^+]}{[\text{R-NH}_2][\text{H}^+]} \text{ (ionization constant for the base functional group)}$$

$$pk_a = -\log k_a$$

$$pk_b = -\log k_b$$

$pk_a + pk_b = pk_w = 14$, where pk_w is the ionization constant of water

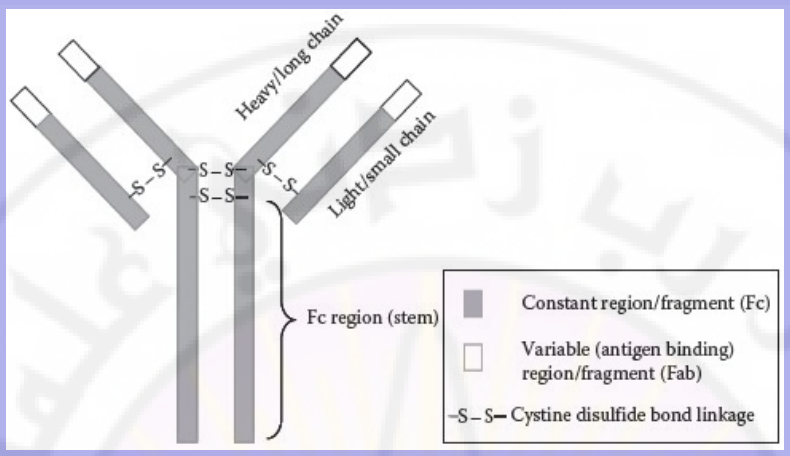
and

$$\log P = \log\left(\frac{[\text{solute}]_{\text{Octanol}}}{[\text{solute}]_{\text{Water}}}\right)$$

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TABLE 22.3
Types of Antibodies

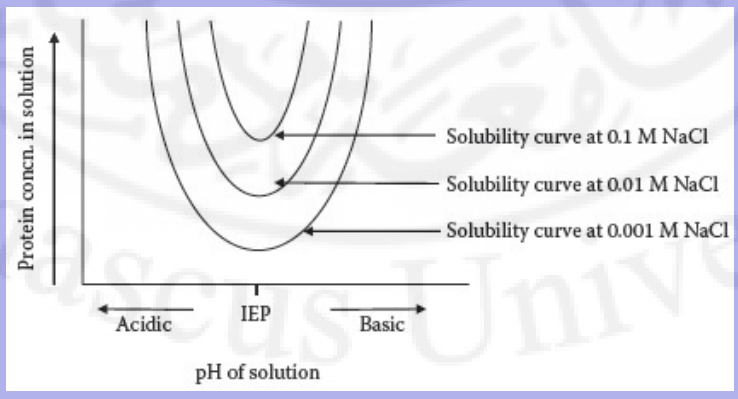
Antibody	Proportion of Total Antibodies	Where Found in the Body	Function	Size
IgA	10%–15%	Nose, breathing passages, digestive tract, ears, eyes, saliva, vagina, tears, and blood	Protection on the mucosal surfaces of the body exposed to the outside environment	
IgG	75%–80%	All body fluids. Smallest and the most common	Fighting bacterial and viral infections. Only type of antibody that can cross placenta	Smallest
IgM	5%–10%	Blood and lymph	First type of antibody made in response to infection. Stimulate other immune cells	Largest
IgE	Small amounts	Lungs, skin, and mucous membranes	React to pollen, fungal spores, and animal dander. May be involved in allergic reactions	
IgD	Small amounts	Tissue that lines belly or chest	Not clear	



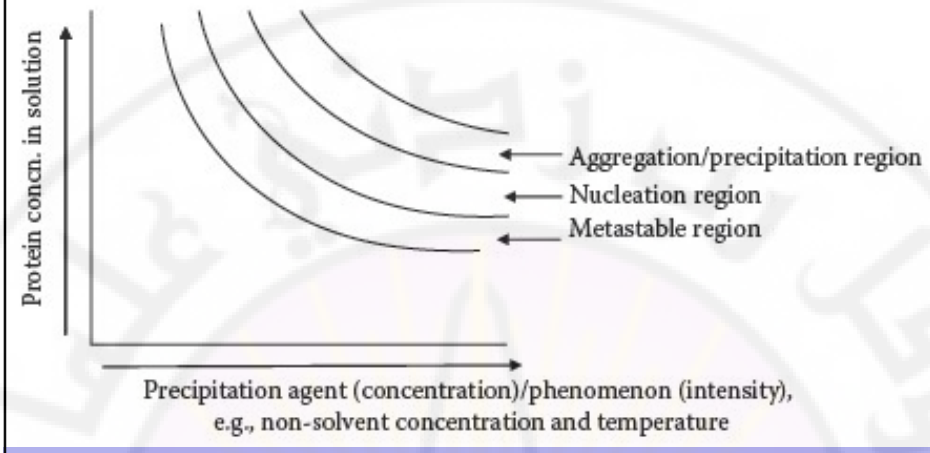
Typical structure of an antibody

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A typical profile of protein solubility in solution as a function of solution pH and salt concentration.

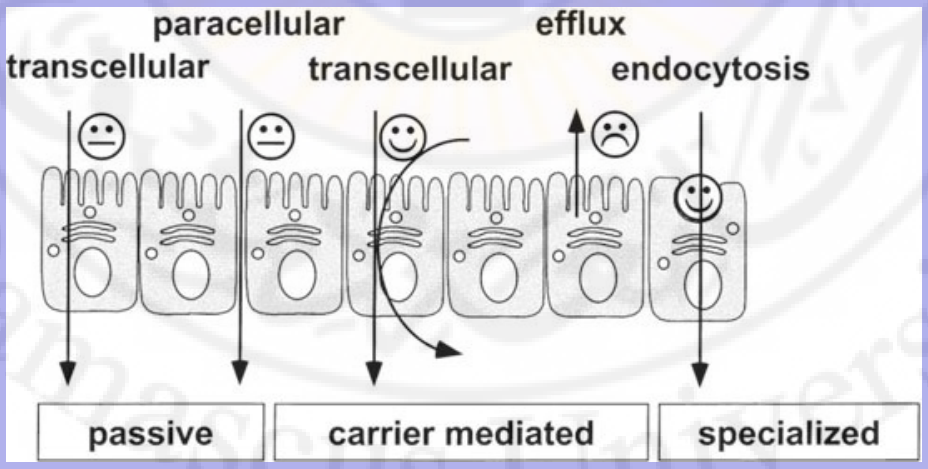


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Phase behavior of proteins in solution formulation. Typical phases of physical instability of protein in solution with the addition of a precipitating agent (such as salt) or change of a precipitation inducing phenomenon (such as temperature).

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GI membrane transport for drug absorption (Lobenberg and Amidon 2000)

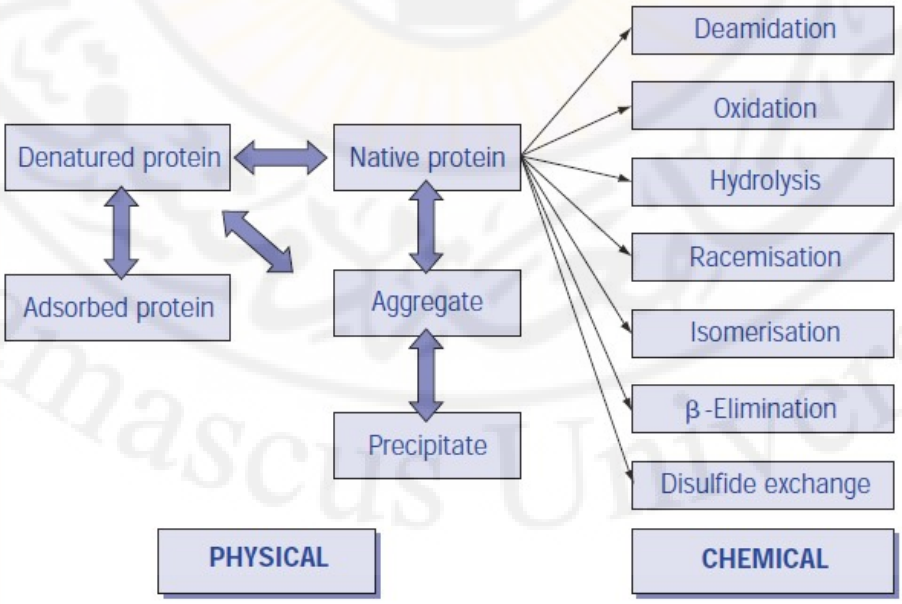
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•- العواقب الفيزيولوجية الناجمة عن تغيرات في بنية البروتينات:

•إن البنى المذكورة سابقاً مسؤولة عن شكل البروتين ووظيفته، وأي تغير في هذه البنى يمكن أن يؤدي إلى فقدان الفاعلية الوظيفية أو حدوث تأثيرات جانبية سمية وعواقب خطرة. فعلى سبيل المثال، إن استبدال حمض أميني بآخر في جزء معين من السلسلة الببتيدية، يمكن أن يؤدي إلى نقصان الفعالية البيولوجية أو إلغائها، كما هو الحال في فقر الدم المنجلي Sickle cell Disease، الذي ينشأ عن تغير في حمض أميني واحد في جزيء الهيموغلوبين، فعوضاً عن ثمالة حمض الغلوتاميك توجد ثمالة الفالين (الحمض الأميني السادس في السلسلة الببتيدية) ويؤدي هذا إلى تغير في بنية البروتين وتشكل بروتين غير طبيعي مما يؤثر على وظيفته في نقل الأوكسجين.

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Physical and chemical pathways of protein degradation.



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تعاني البروتينات، سواء على مستوى التصنيع أو المعالجة أو التخزين أو الاستخدام، من مشاكل متعددة. من أهمها:

- الحجم الكبير يجعل من الجزيئة أقل ثباتاً وامتصاصاً عبر الأغشية الخلوية.
- قوى الربط داخل الجزيئة ضعيفة.
- تتخرب بسهولة في شروط التخزين العادية.
- من الصعب استحصالها بكميات كبيرة.
- اطراح البروتينات ذات الحجم الصغير بسرعة من الكلية.
- حدوث تفاعلات سمية غير مرغوبة.

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بغية تقليل الضياع في البروتينات العلاجية يمكن إضافة سواغات عديدة لإطالة فترة الثباتية وبالتالي إنقاص الادمصاص على السطح. إن إضافة الألبومين المصلي لصيغة المستحضر البروتيني يمنع ضياع البروتين وذلك عن طريق التنافس على الارتباط بالسطح. كما أن العوامل الفاعلة بالسطح مثل البلورونيك Pluronic و التوين (بولي سوربات) والمذيبات غير القطبية (مثل الكحول) أثبتت فعاليتها في منع الادمصاص.

٣-١- المواد المضافة:

إن إضافة بعض المواد يمكن أن يزيد من ثباتية الأشكال الصيدلانية بعدة آليات وكأمثلة نذكر :

- إضافة بعض الأملاح أوالشوارد المعدنية مثل الزنك إلى الإنسولين يؤدي إلى زيادة ثباتيته.
- إضافة عديدات الكحول الـ Polyols (غليسيرول أو بولي إيثيلن غليكول) تزيد من الذوبانية.
- إضافة السكاكر تساعد على امتصاص الرطوبة وبالتالي تقلل من النمو الجرثومي.

إضافة عوامل فاعلة بالسطح وذلك للتقليل من الادمصاصات وبالتالي التكدسات والترسب.

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-- إضافة البولي إيثيلين غليكول PEGylation:

•يؤدي البولي إيثيلين غليكول إلى:
•زيادة العمر النصفي للمادة الدوائية (٤- ٤٠٠) مرة.
•يقلل من التفاعلات المناعية المخربة للبروتين.
•يزيد من المقاومة لأنزيمات البروتياز.
•يزيد من الذوبانية والثباتية.

الاعطاء المتشارك مع معززات الامتصاص:

يعتقد أن آلية عملها كالآتي:
•زيادة الأضطراب في الطبقة الدسمة الكارهه للماء لخلايا ظهارة الأمعاء مما يسمح بزيادة النقل العابر للخلايا.
•توسيع قطر المسامات في الارتباطات المحكمة مما يسمح بزيادة النقل المجاور للخلايا.
•تخفيض لزوجة الطبقة المخاطية وبالتالي زيادة الامتصاص.
• الشحنة الموجبة لمعزز الامتصاص تعدل الشحنة السالبة لغشاء خلايا جدر المعي مما يزيد الامتصاص

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البروتينات وبسبب وزنها الجزيئي الكبير وشحنتها لا تعبر بسهولة عبر الجلد والنقل الجيد يتطلب آليات معززة مثل تعزيز اختراق الببتيدات والبروتينات للجلد بوسائل كهربائية والذي يلقى اهتماماً بالغاً حالياً مثل الرحلان الأيوني **Ionotrophesis** وهجرة الجزئيات المشحونة تحت تطبيق جهد كهربائي وتيار مستمر.

إن مبدأ الرحلان الأيوني يعتمد على أن جزيئات الدواء البروتيني المشحونة يمكن أن تنتقل عند تطبيق جهد كهربائي عبر الجلد وذلك بتطبيق مسريين كهربائيين على الجلد في محلول كهرلي واحد منهما فيه الدواء.

من الطرق الأخرى المستخدمة لإيتاء البروتينات عبر الجلد هي استخدام الأمواج فوق الصوتية **Ultrasound (phonophoresis)** إن الطاقة فوق الصوتية تؤثر من خلال الحرارة، الضغط، الإشعاع.

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Some therapeutic proteins and peptides, their molecular weights and actions

Protein/peptide	Size (kDa)	Use/action
Oxytocin	1.0	Uterine contraction
Vasopressin	1.1	Diuresis
Leuprolide acetate	1.3	Prostatic carcinoma therapy
LHRH analogues	~1.5	Prostatic carcinoma therapy
Somatostatin	3.1	Growth inhibition
Calcitonin	3.4	Ca^{2+} regulation
Glucagon	3.5	Diabetes therapy
Parathyroid hormone (1–34)	4.3	Ca^{2+} regulation
Insulin	6	Diabetes therapy
Parathyroid hormone (1–84)	9.4	Ca^{2+} regulation
Interferon-gamma	16 (dimer)	Antiviral agent
TNF-α	17.5 (trimer)	Antitumour agent
Interferon α-2	19	Leukaemia, hepatitis therapy

Some therapeutic proteins and peptides, their molecular weights and actions

Protein/peptide	Size (kDa)	Use/action
Interferon β-1	20	Lung cancer therapy
Growth hormone	22	Growth acceleration
DNase	~32	Cystic fibrosis therapy
α_1 -Antitrypsin	45	Cystic fibrosis therapy
Albumin	68	Plasma volume expander
Bovine IgG	150	Immunisation
Catalase	230	Treatment of wounds and ulcers
Cationic ferritin	400+	Anaemias

LHRH, luteinising hormone-releasing hormone; TNF-, tumour necrosis factor-; IgG, immunoglobin G.

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