L12: Drug Loading & Quantification

May 15, 2018

NANO 243/CENG 207 Course Use Only

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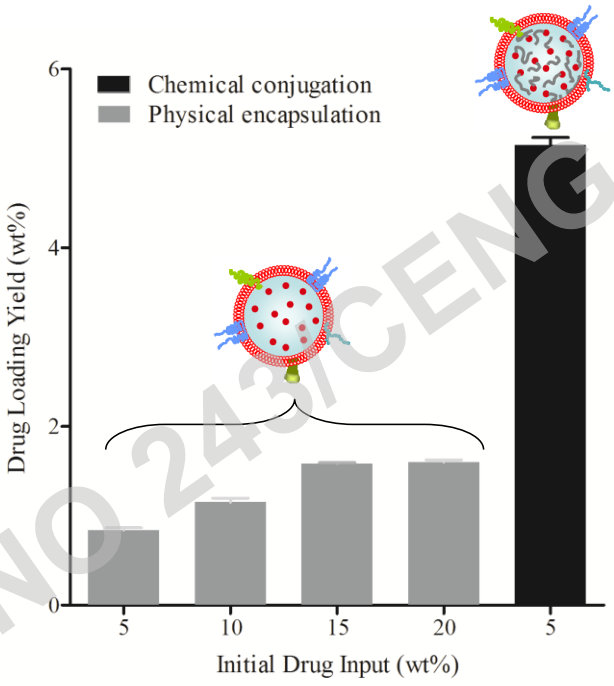
Drug Loading Techniques

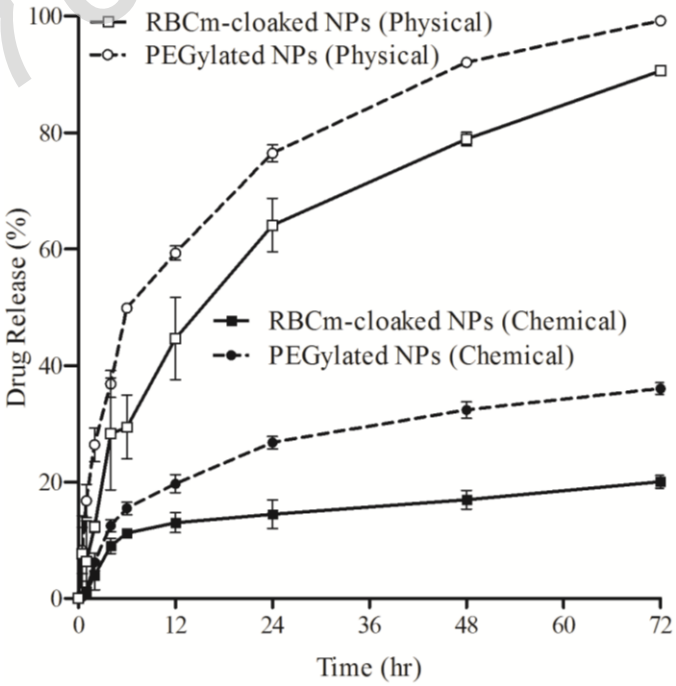
Physical approaches: Drug molecules are entrapped inside the nanocarriers or absorbed onto the surface of the carriers through non-covalent interactions.

Chemical approaches: Drug molecules are covalently linked to the nanocarrier core or surface, either before or after the nanocarrier preparation.

Drug loading approaches



Drug release kinetics



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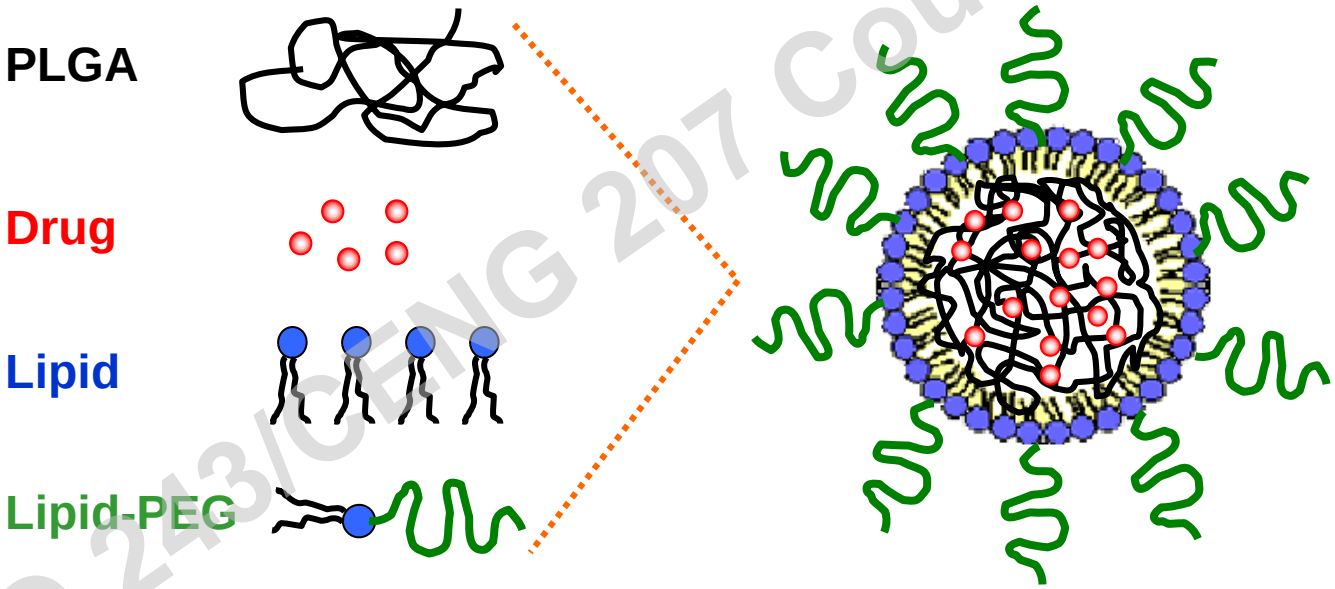
Physical Approach – Nanoprecipitation

Nanoprecipitation is the formation of nanoparticles by precipitation of a water insoluble polymer dissolved in a water miscible organic solvent upon addition to water.

e.g. Lipid-polymer hybrid nanoparticle for drug loading



PLGA: Poly lactic-co-glycolic acid
PEG: Poly ethylene glycol
Drug: Therapeutic / diagnostic agents
Lipid: Lecithin

ACS Nano, 2008, 2, 1696

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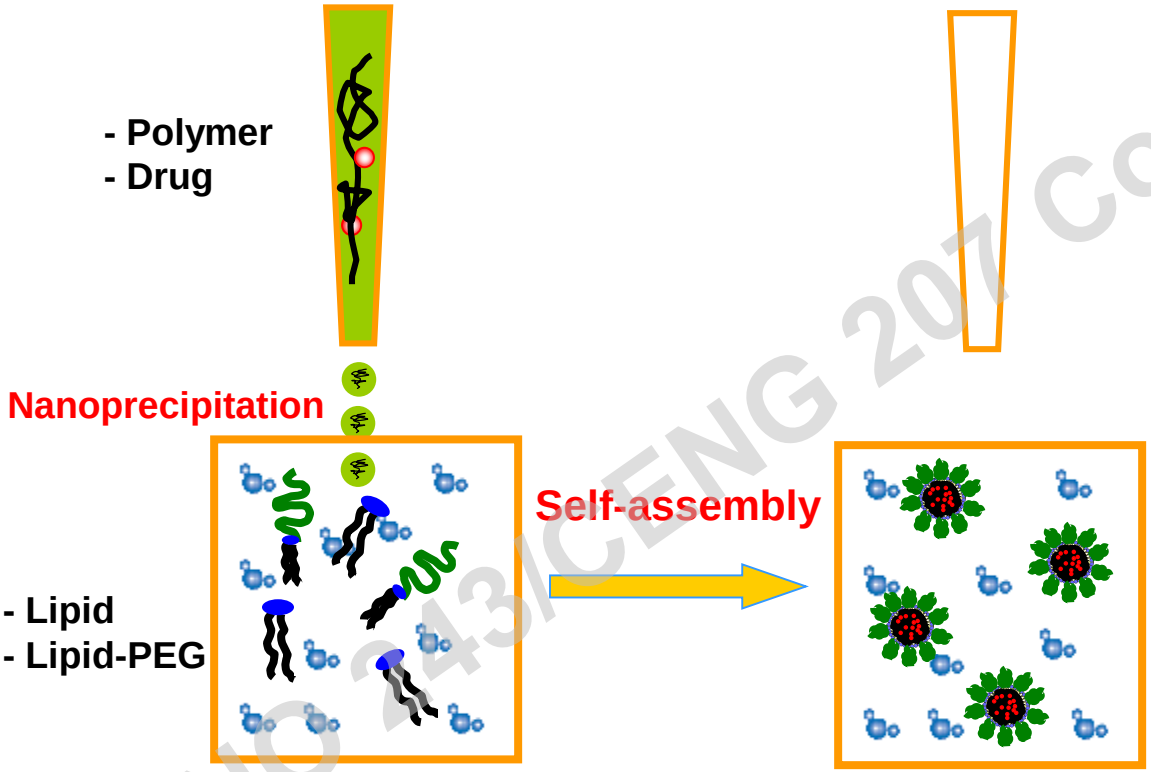
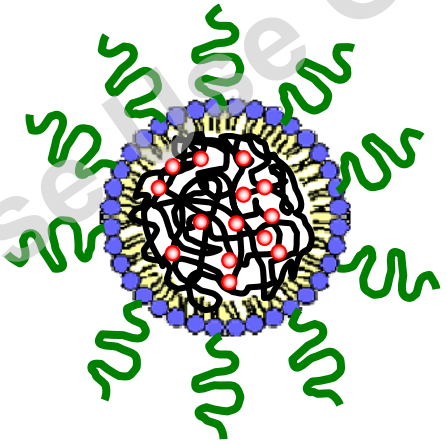
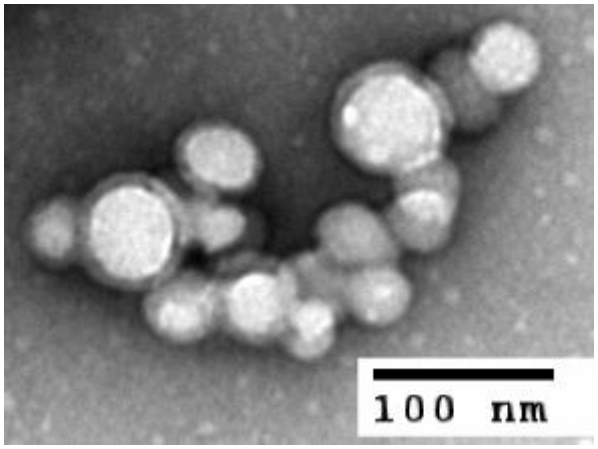
Manufacturing and Drug Loading Processes







TEM images of the NPs with negative staining (uranyl acetate)

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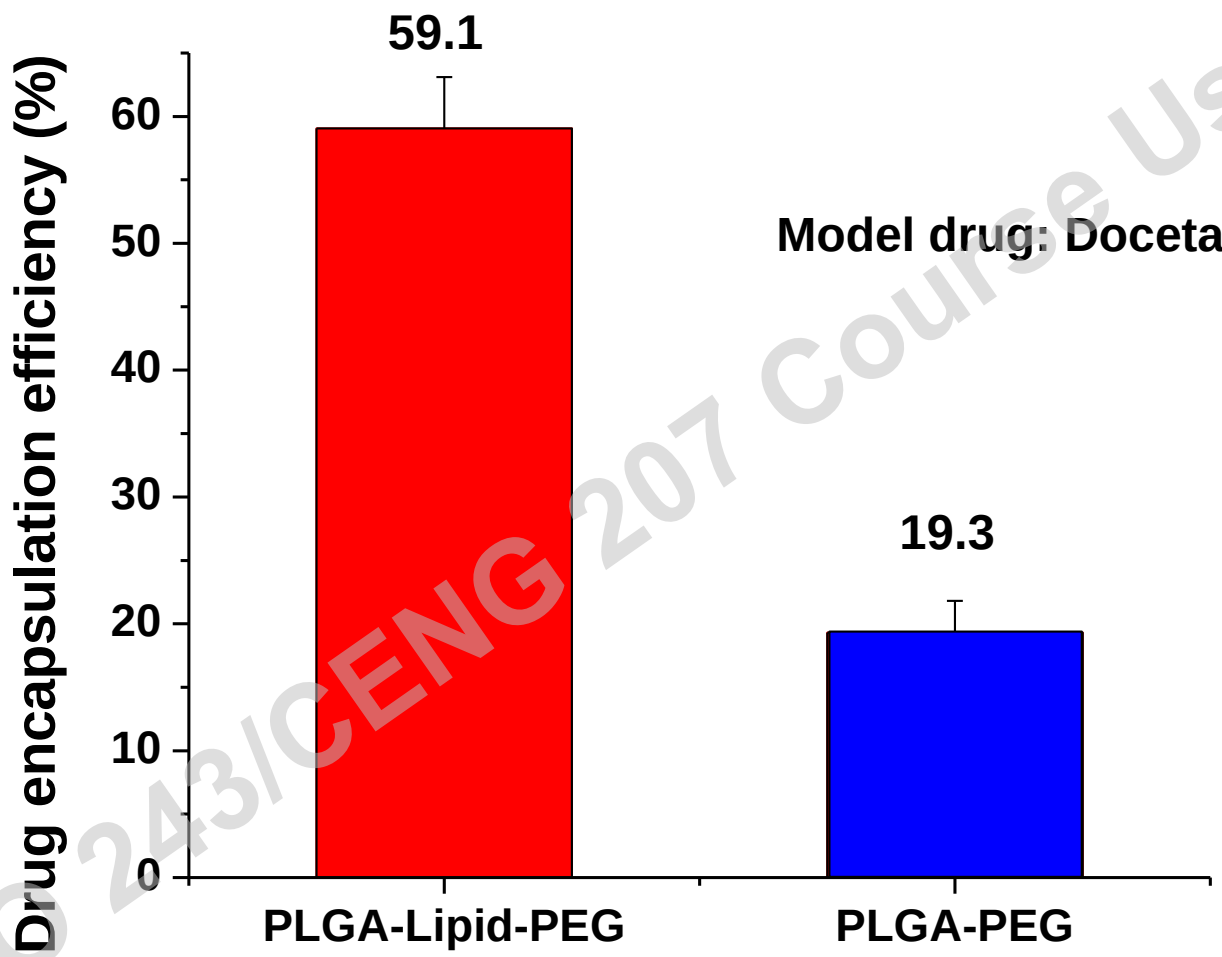
High Drug Encapsulation Efficiency



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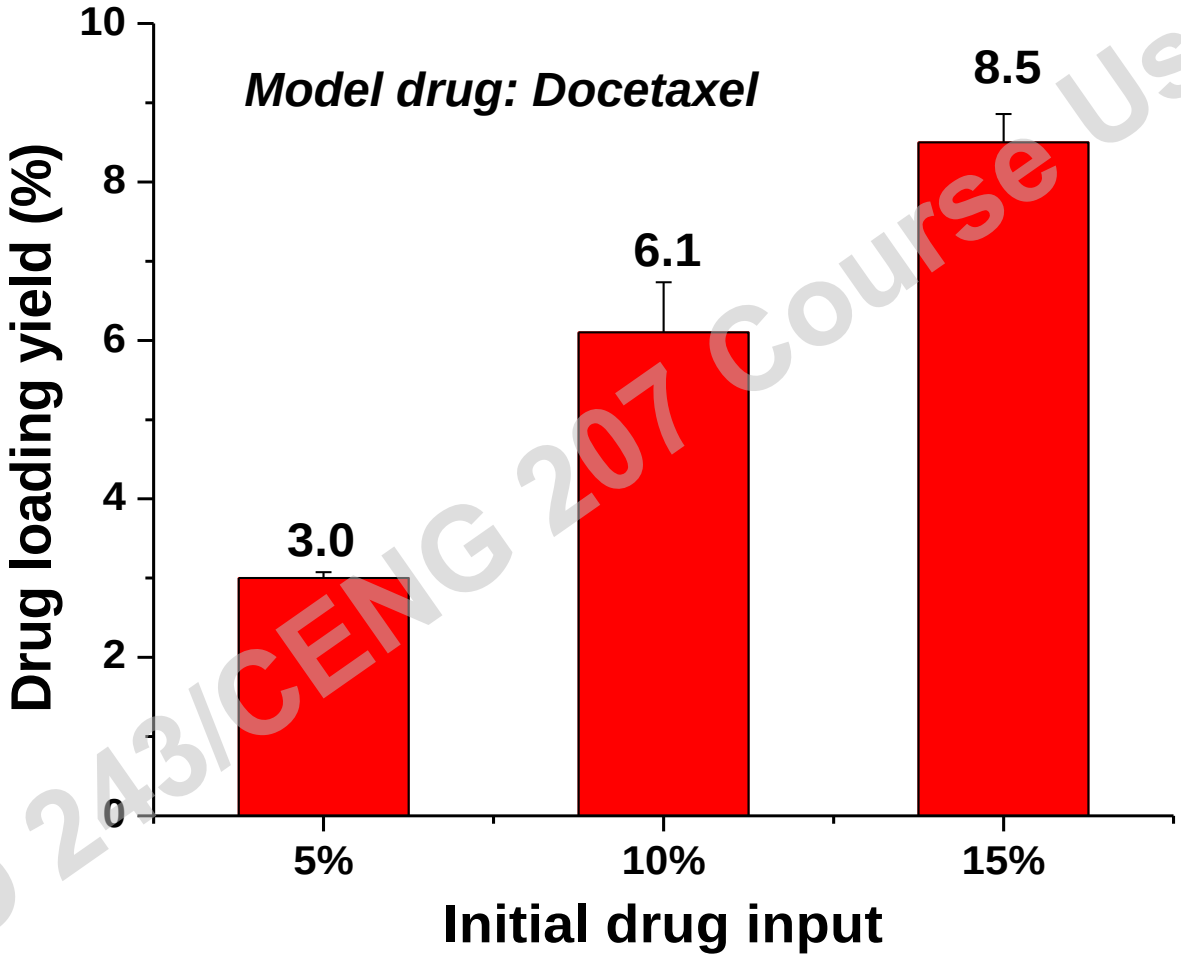
By adding 10 wt % lipid to the interface of PLGA and PEG, the lipid-polymer NP has a drug encapsulation efficiency improved by ~ 300%.

High Drug Loading Yield



High drug encapsulation efficiency retains for a broad range of initial drug loading

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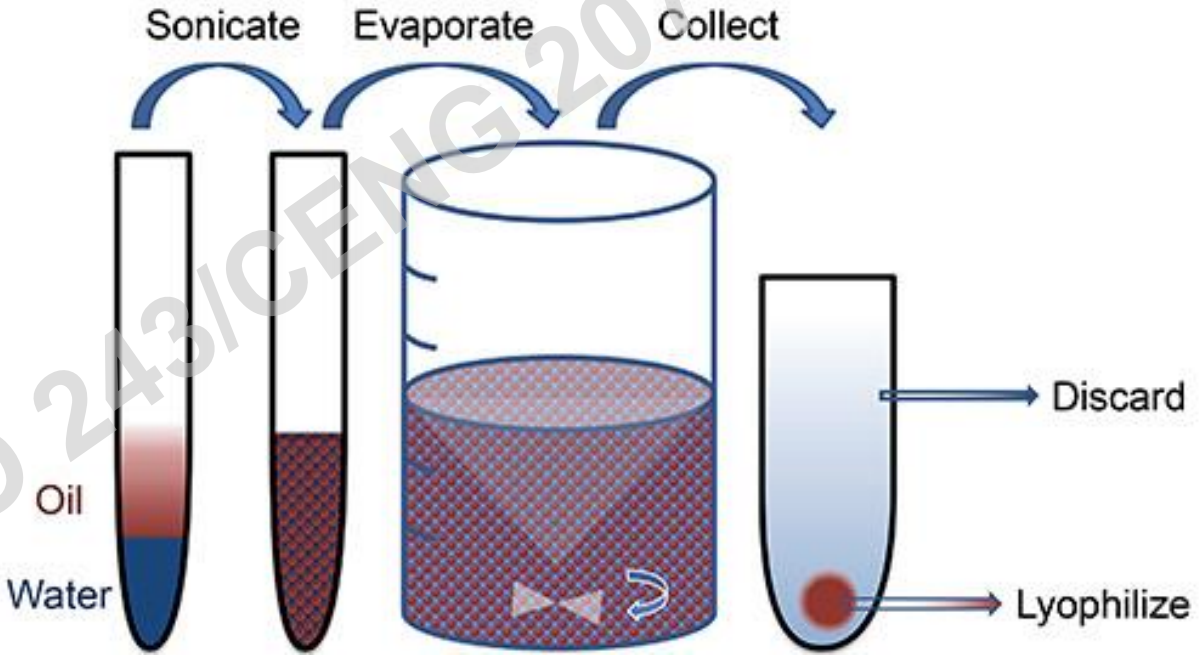
Physical Approach – Single Emulsion

Single emulsion (Oil-water) is one method by which polymers (e.g. PLGA) can be used to encapsulate hydrophobic drugs in micro- or nano-scale form. Briefly, PLGA is dissolved into an organic phase (oil) that is emulsified with a surfactant or stabilizer (water). Hydrophobic drugs are added directly to the oil phase. High intensity sonication bursts facilitate the formation of small polymer droplets. The resulting emulsion is added to a larger aqueous phase and stirred for several hours, which allows the solvent to evaporate. Hardened nanoparticles are collected and washed by centrifugation.



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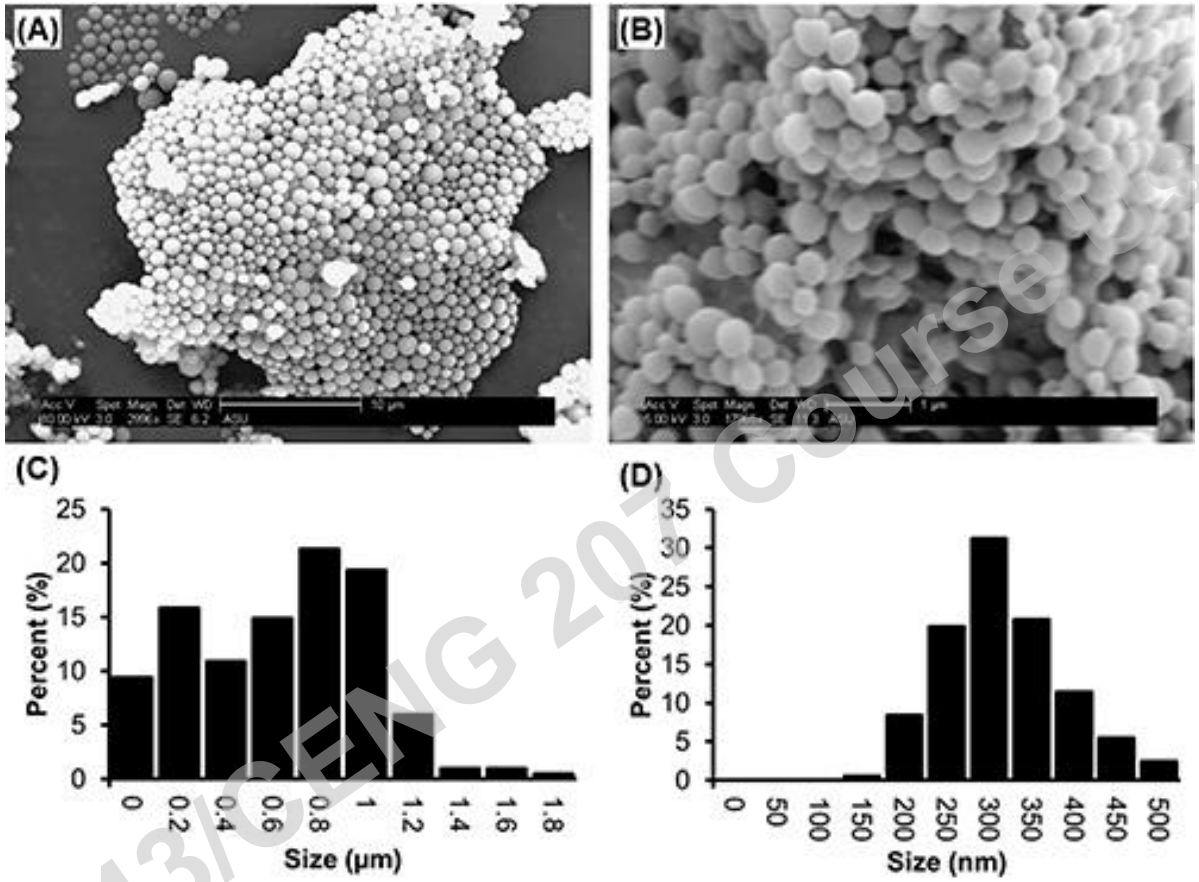
JVOE, 2013, Issue 82; doi: 10.3791/51015

Controllable Particle Size



SEM images and corresponding size distributions of PLGA microparticles and nanoparticles produced by the single emulsion method. In (**A**), particles were formed by emulsifying 100 mg PLGA dissolved in 1ml ethyl acetate into 2 ml of 0.05% Vitamin E-TPGS, and hardened in 45 ml 0.01% Vitamin E-TPGS. The corresponding size distribution is shown in (**C**). In (**B**), particles were formed by emulsifying 200 mg PLGA and 40mg of camptothecin dissolved in 4mL ethyl acetate into 4 ml 0.3% Vitamin E-TPGS, and hardened in 90 ml 0.3% Vitamin E-TPGS. The corresponding size distribution is shown in (**D**).

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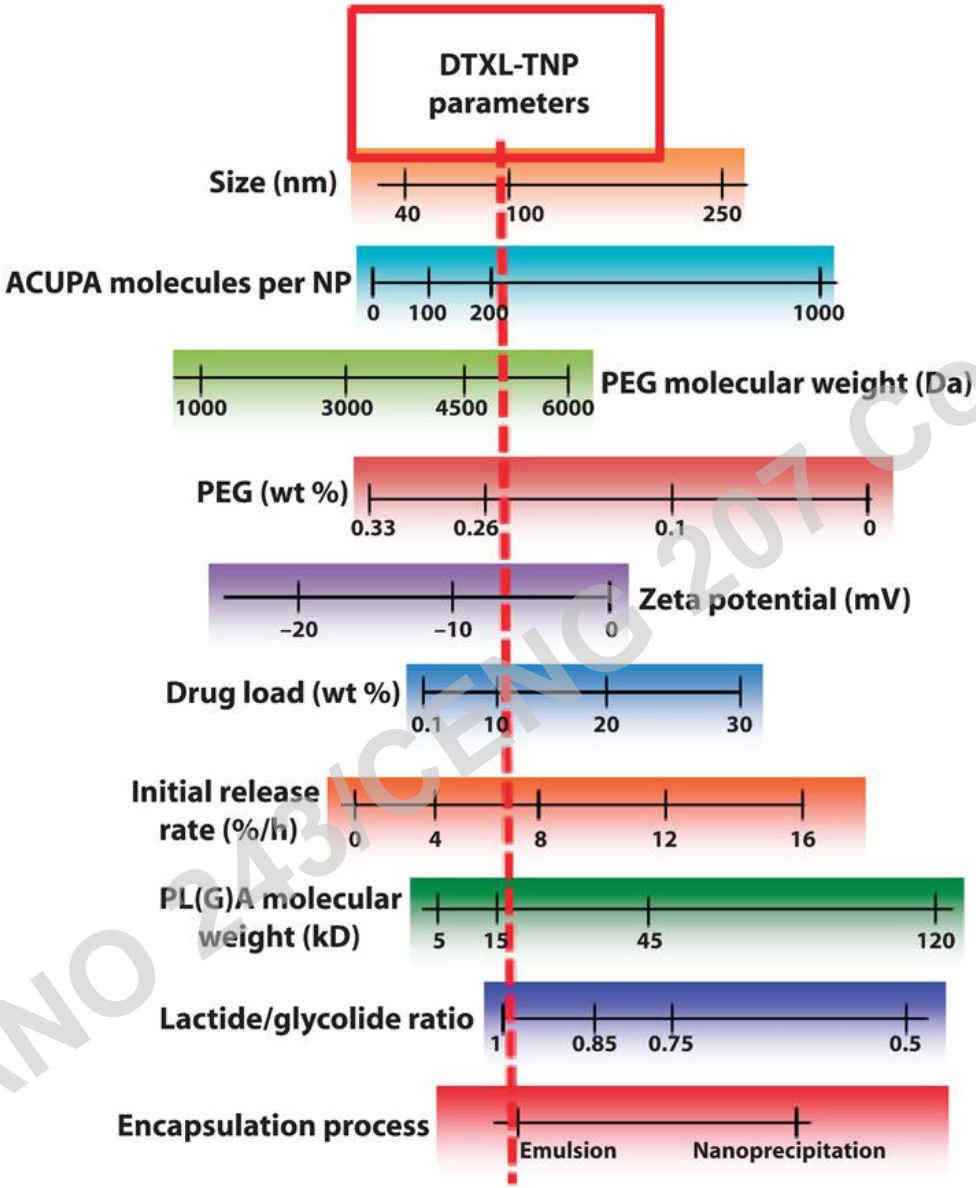
High Drug Loading Yield



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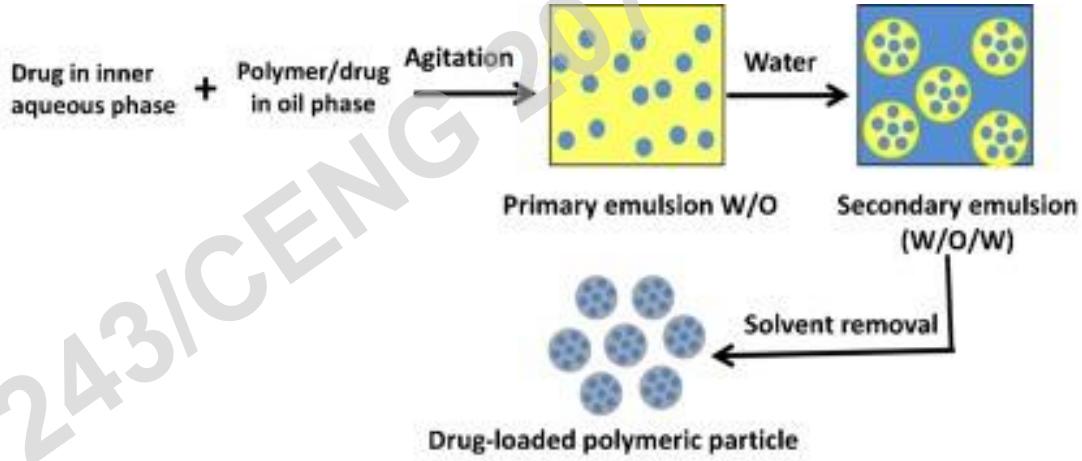
Sci Transl Med. 2012 Apr 4;4(128):128ra39

Physical Approach – Double Emulsion

Double emulsion (water-Oil-water) is one method by which polymers can be used to encapsulate hydrophilic drugs in micro- or nano-scale form. Briefly, hydrophilic drugs are first dissolved in water, which is then added to into an organic phase (oil) containing the polymers. The mixture is emulsified to form a primary emulsion. The resulting emulsion is added to a larger aqueous phase and stirred for several hours, which allows the solvent to evaporate. Hardened nanoparticles are collected and washed by centrifugation.



Encapsulation of hydrophilic and lipophilic molecules via double emulsion techniques

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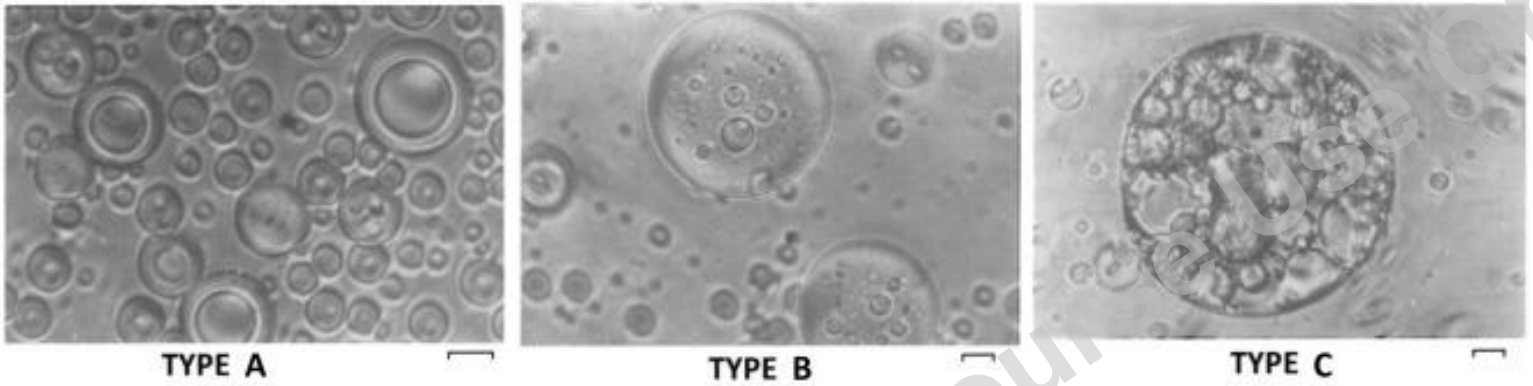
Int. J. Pharmaceutics, 2015, 496 (2), 173

Three Types of Double Emulsions



The type A was found to be the simplest system consists of relatively small droplets with almost single droplet of the internal aqueous phase.

The droplet size in the type B emulsion system is larger composed of several small droplets (less than 50) of internal aqueous phase.

The system became more complex (type C) when majority of droplets achieve relatively largest size, encapsulating numerous droplets of internal aqueous phase. The system C showed slow release of entrapped moiety than A or B.

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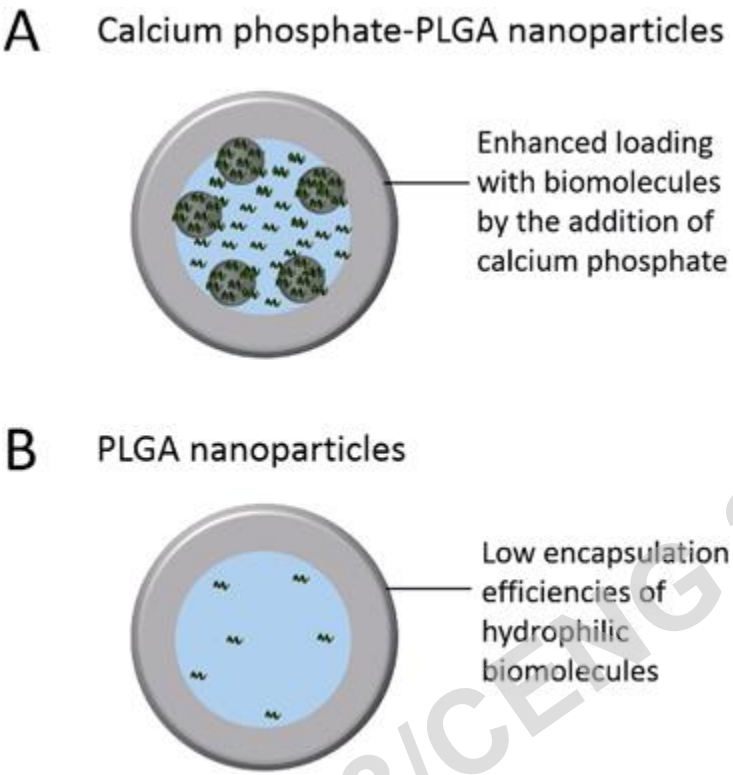
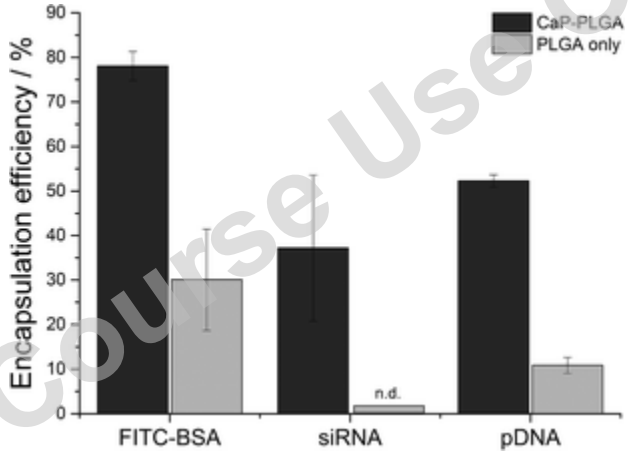
Int. J. Pharmaceutics, 2015, 496 (2), 173

High Loading of Hydrophilic Drugs



Schematic illustration of the improved loading efficiency of nucleic acids by the addition of calcium phosphate nanoparticles



Comparison of the encapsulation efficiency of FITC-BSA, siRNA, and pDNA into PLGA nanoparticles with (black) and without (grey) the addition of calcium phosphate nanoparticles. Values are given as the mean ± SD of triplicates.

J. Mater. Chem. B, 2014, 2, 7250

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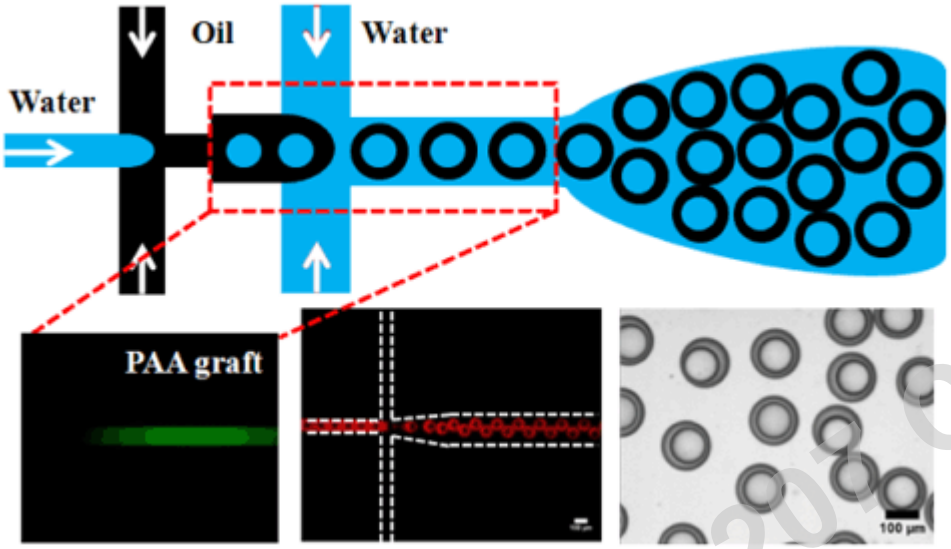
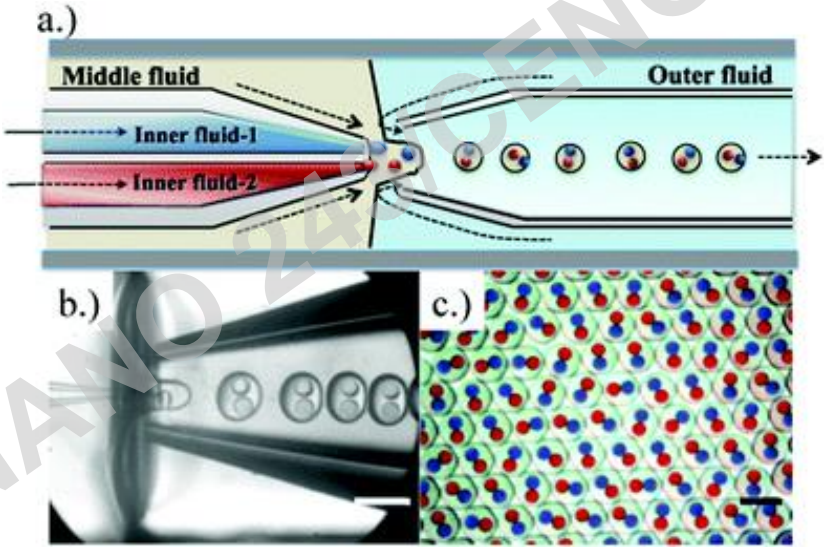
Double Emulsion Approach by Microfluidics



Microfluidic device for the generation of monodisperse double emulsions.

Macromolecular Res., 2012, 20, 422



Schematic of a microfluidic capillary device for preparation of multiple component double emulsions using a single-step emulsification.

Soft Matter, 2012, 8, 10719

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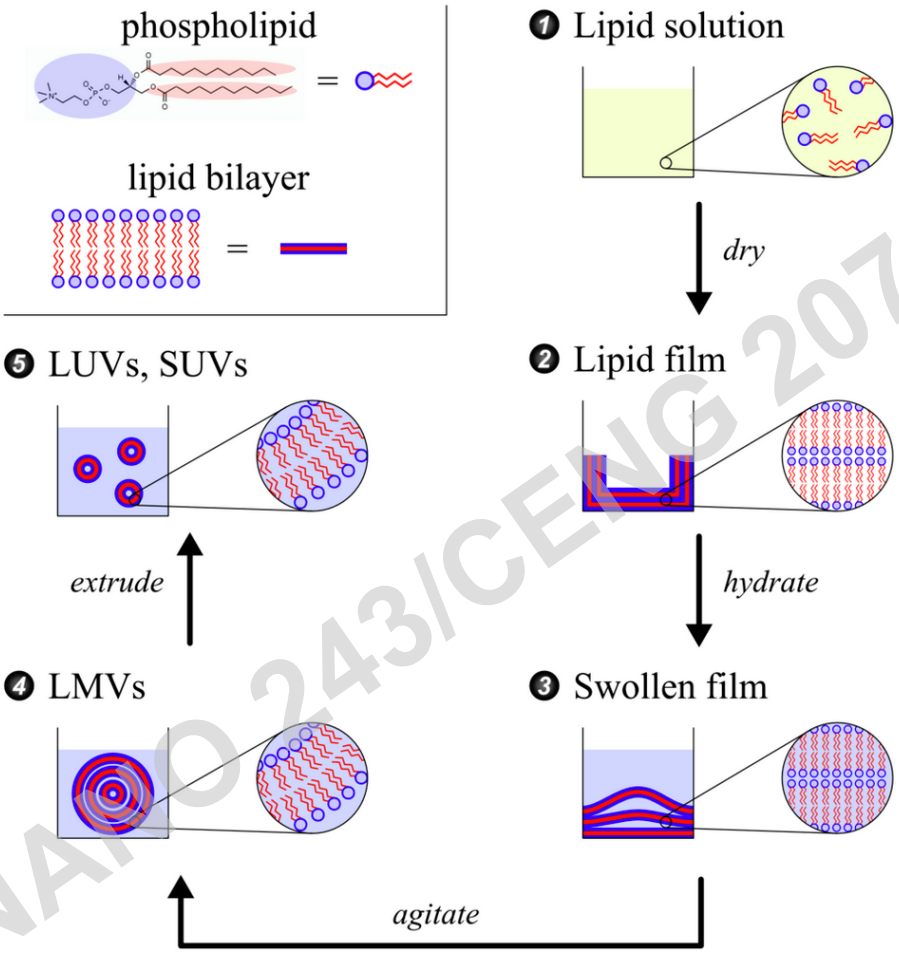
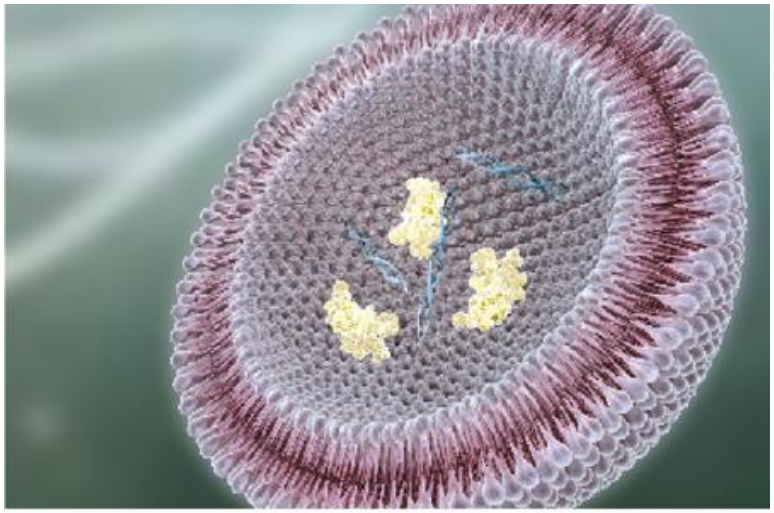
Physical Approach – Encapsulation

Encapsulation (passive loading) involves dissolution of dried lipid films in aqueous solutions containing the drug of interest. This approach can only be used for water-soluble drugs, and the efficiency of loading is often low.





Example of a liposome (Photo courtesy and copyright of The Gates Foundation)

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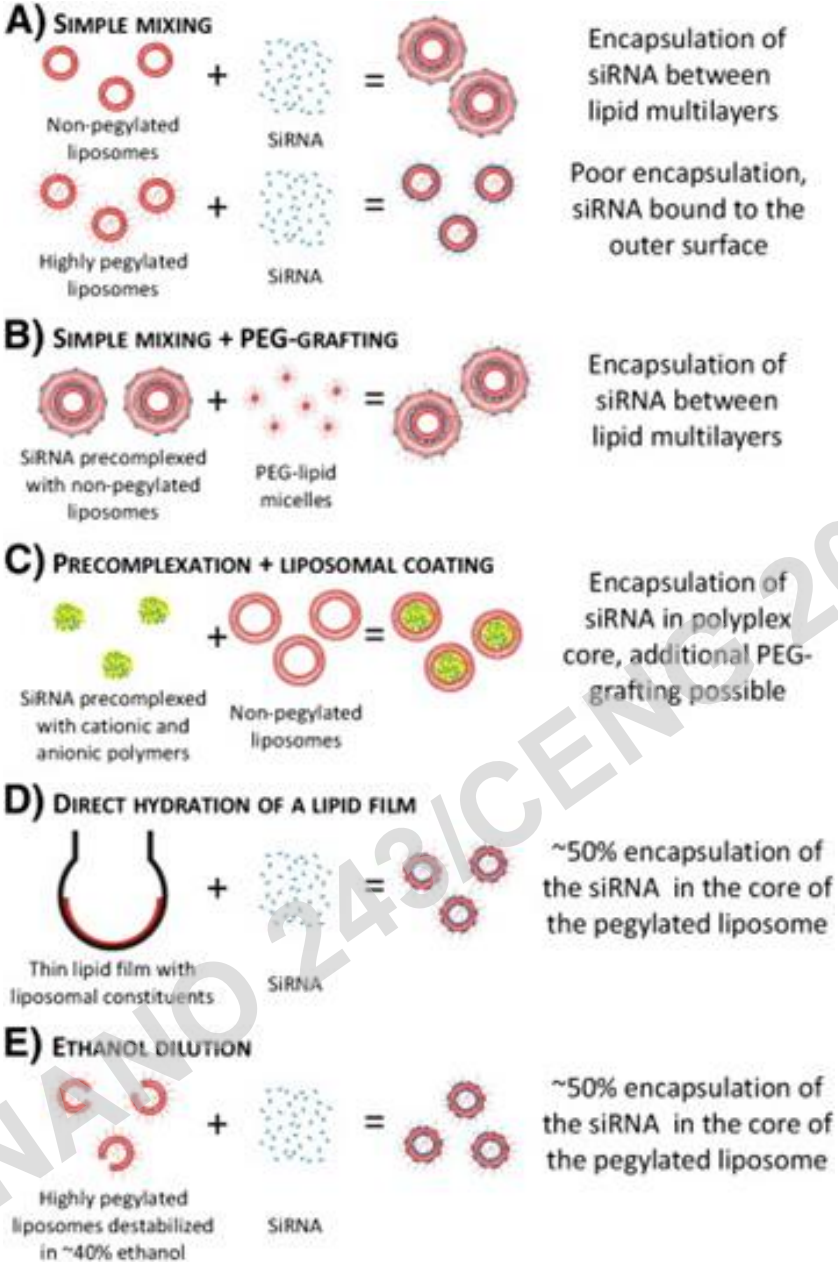
Liposomal siRNA Delivery



Schematic representation of complexation strategies (left) of siRNA with cationic liposomes and resulting complexes (right).

J. Control. Release 2012, 158 (3), 362

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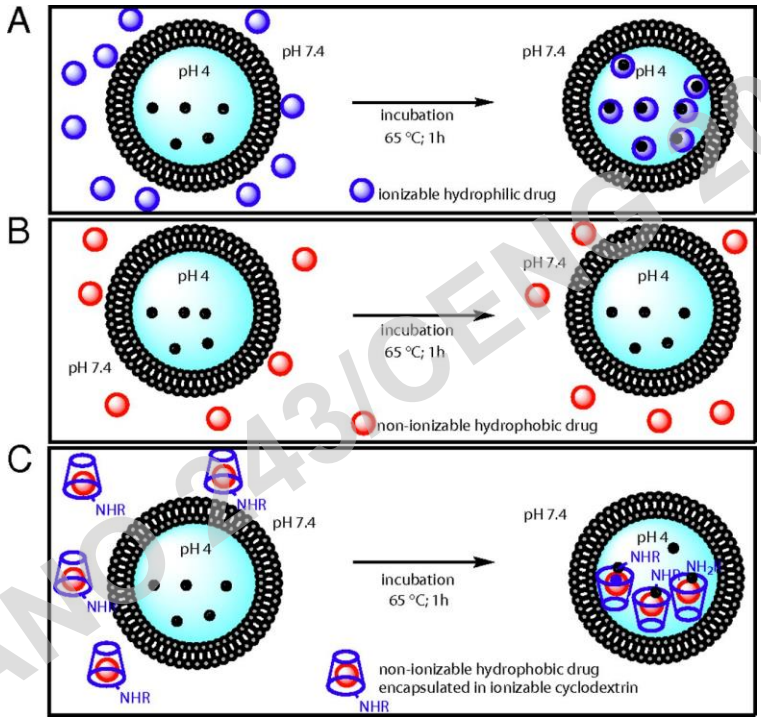
Physical Approach – Remote Loading

Remote loading (active loading) can be extremely efficient, resulting in high intraliposomal concentrations and minimal wastage of chemotherapeutic agents. In active loading, drug internalization into preformed liposomes is typically driven by a transmembrane pH gradient. The pH outside the liposome allows some of the drug to exist in an unionized form, able to migrate across the lipid bilayer. Once inside the liposome, the drug becomes ionized due to the differing pH and becomes trapped there.



Schematic representation of active loading of a liposome. (*A*) Remote loading of an ionizable hydrophilic drug using a transmembrane pH results in efficient incorporation. (*B*) A poorly soluble hydrophobic drug results in meager incorporation into preformed liposomes under similar conditions. (*C*) Encapsulation of a poorly soluble drug into an ionizable cyclodextrin (R = H, ionizable alkyl or aryl groups) enhances its water solubility and permits efficient liposomal loading via a pH gradient.

PNAS 2014, 111 (6), 2283

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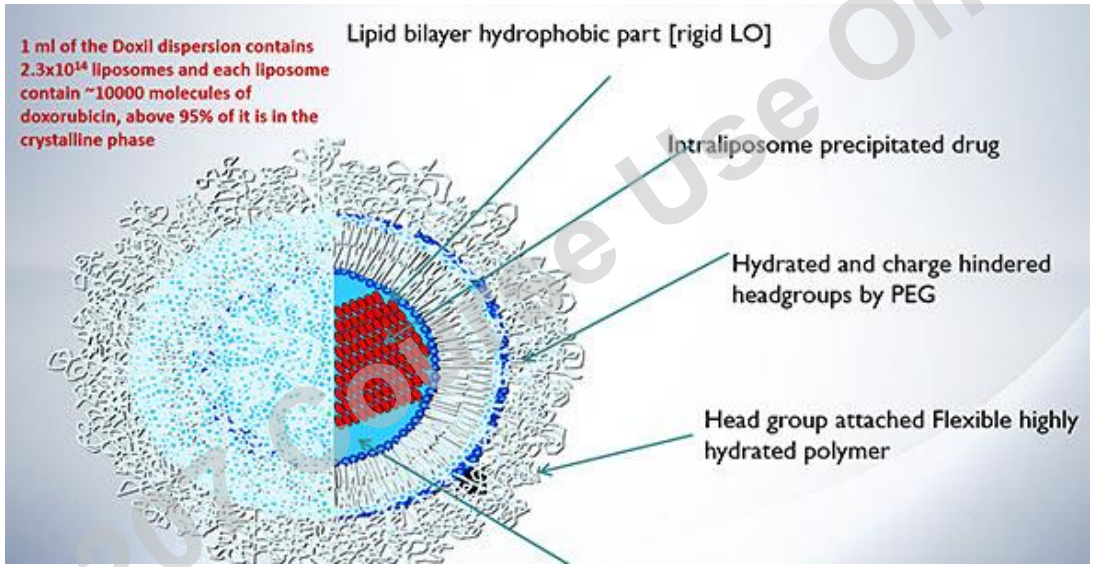
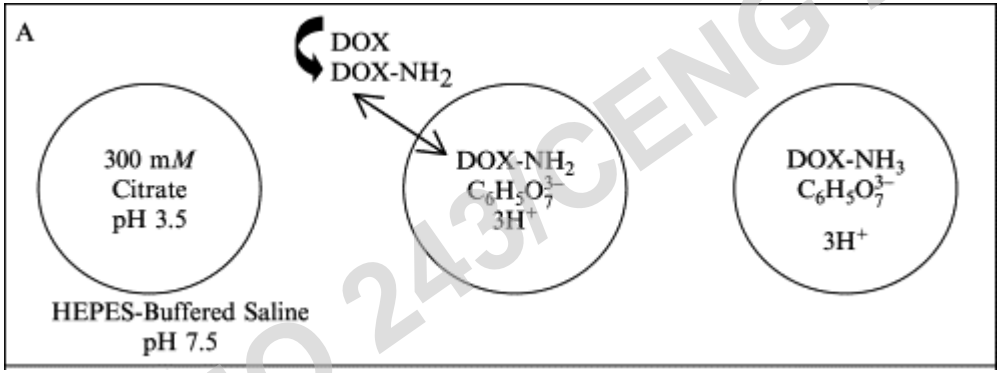
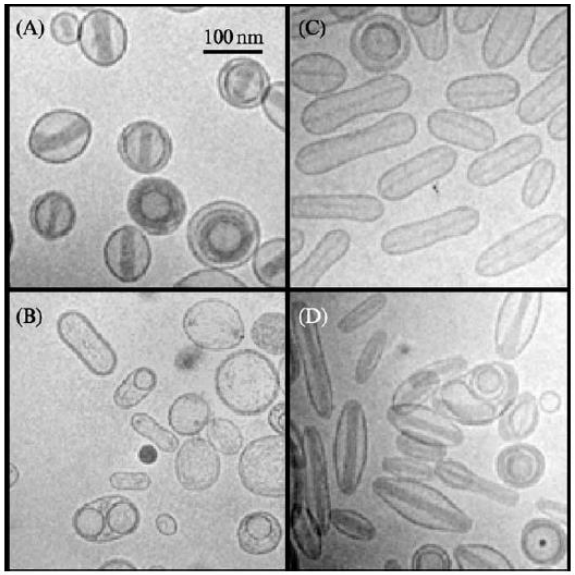
Remote Loading of Doxorubicin to Liposome







The citrate loading procedure. Liposomes are prepared in 300 m*M* citrate buffer, pH 3.5, and outside buffer exchanged to HEPES-buffered saline at pH 7.5.

Methods Enzym. 2005, 391, 71.

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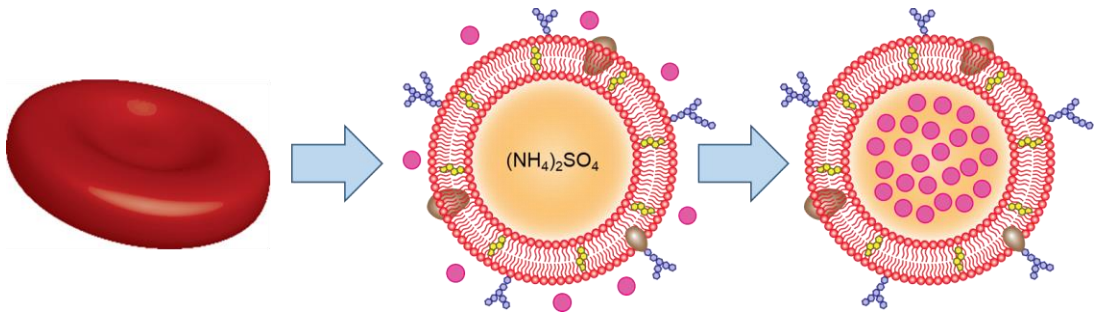
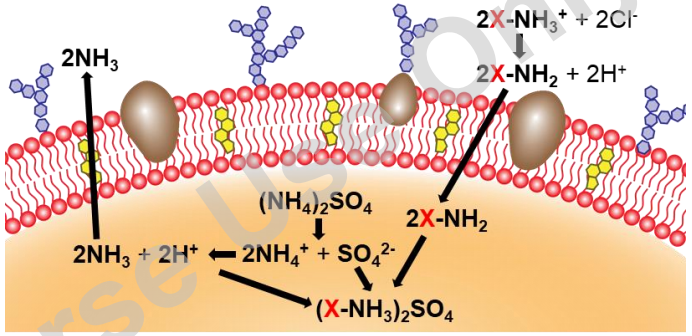
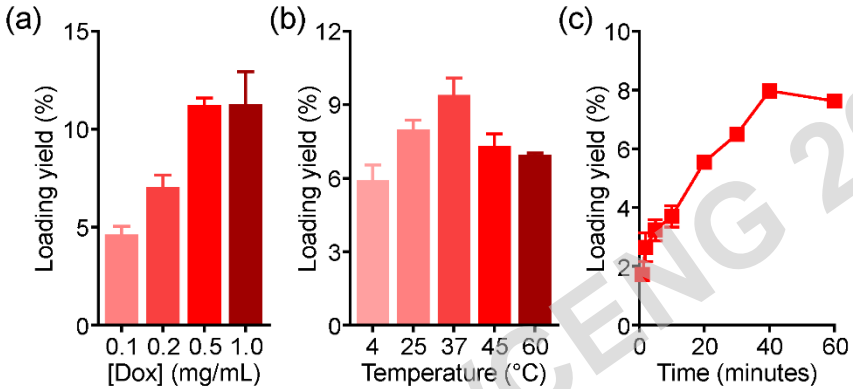
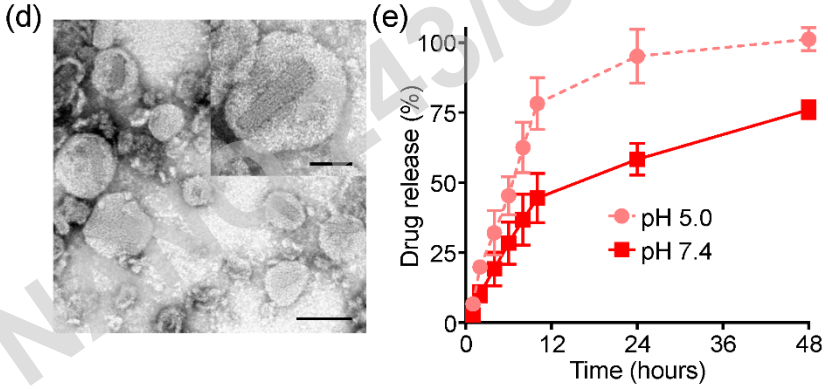
Remote Loading of Cell Membrane Vesicles





Remote loading into RBC vesicles. Natural cell membrane vesicles are fashioned from RBC ghosts enriched with cholesterol (yellow). Ammonium sulfate (orange) is used to generate a pH gradient, which facilitates accumulation of the drug (**X**) inside the cholesterol-enriched RBC vesicle.





Dox loading into RBC vesicles. a) Loading yield at different drug inputs. b) Loading yield at different temperatures. c) Loading yield over time. d) TEM images of Dox-RBC after negative staining with uranyl acetate (scale bar = 100 nm). Inset depicts a single Dox-RBC particle (scale bar = 50 nm). e) Dox release at pH 5.0 or pH 7.4.

Angew Chem Int Ed. 2017, 56, 14075.

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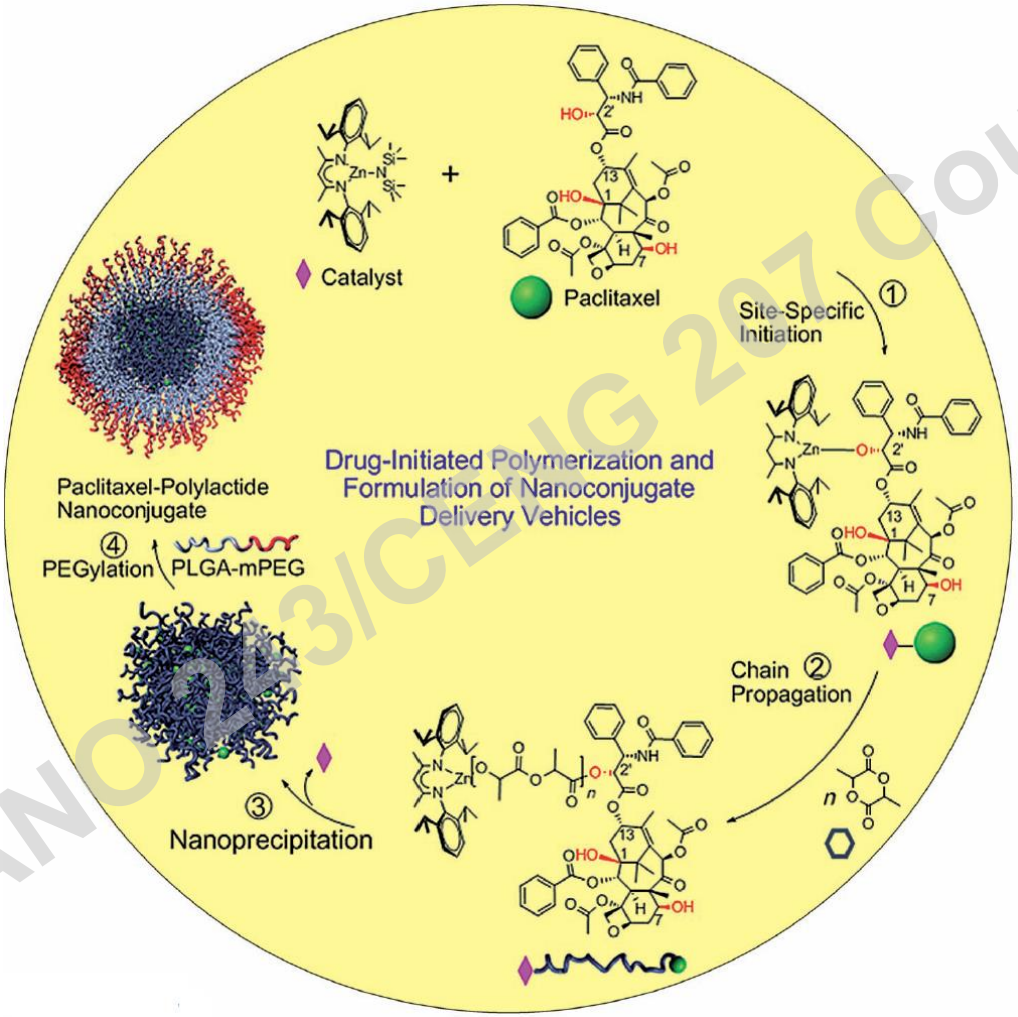
Chemical Approach – Pre-conjugation

Pre-conjugation involves directly conjugating drug molecules to building blocks (e.g. polymer chain) prior to the formation of nanoparticles. For example, polymer-drug conjugate has been one of the major platforms for the design of drug delivery systems and the development of new therapeutics.



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Angew Chem Int Ed, 2008, 47, 4830

Drug-polymer Conjugation

Drug A-Polymer

Drug B-Polymer

Drug A/Drug B with a desirable molar ratio

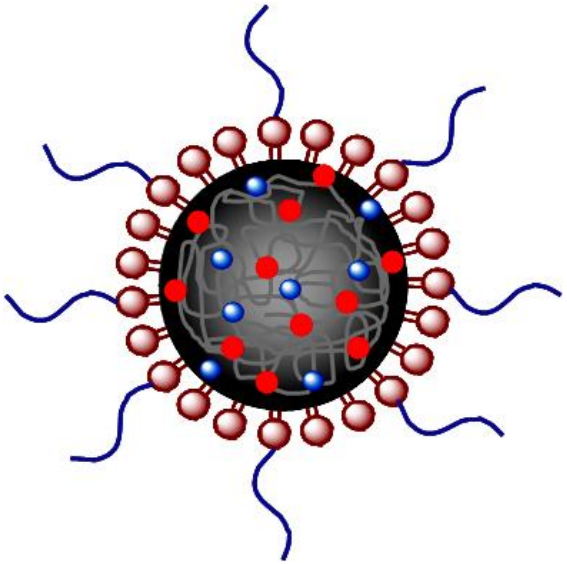
DOX-PLA

CPT-PLA

Lipid

PEG

Self assembly



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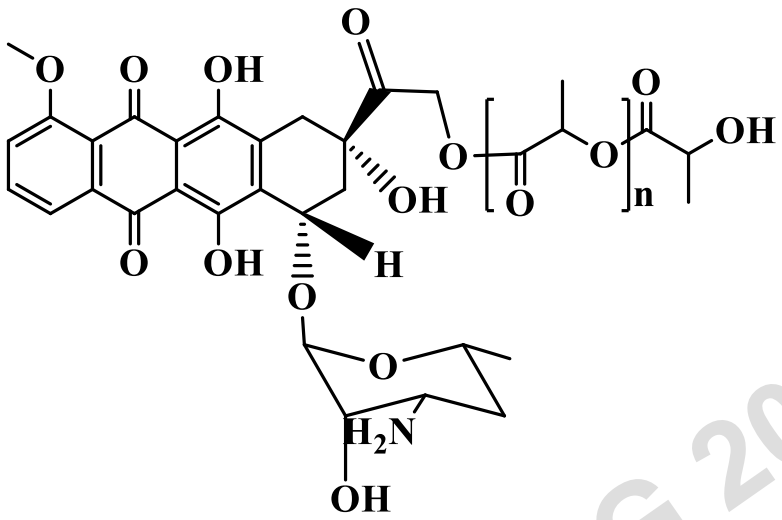
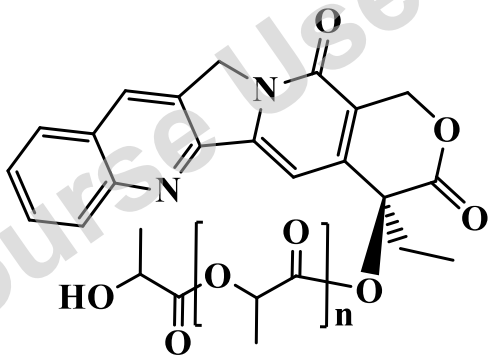
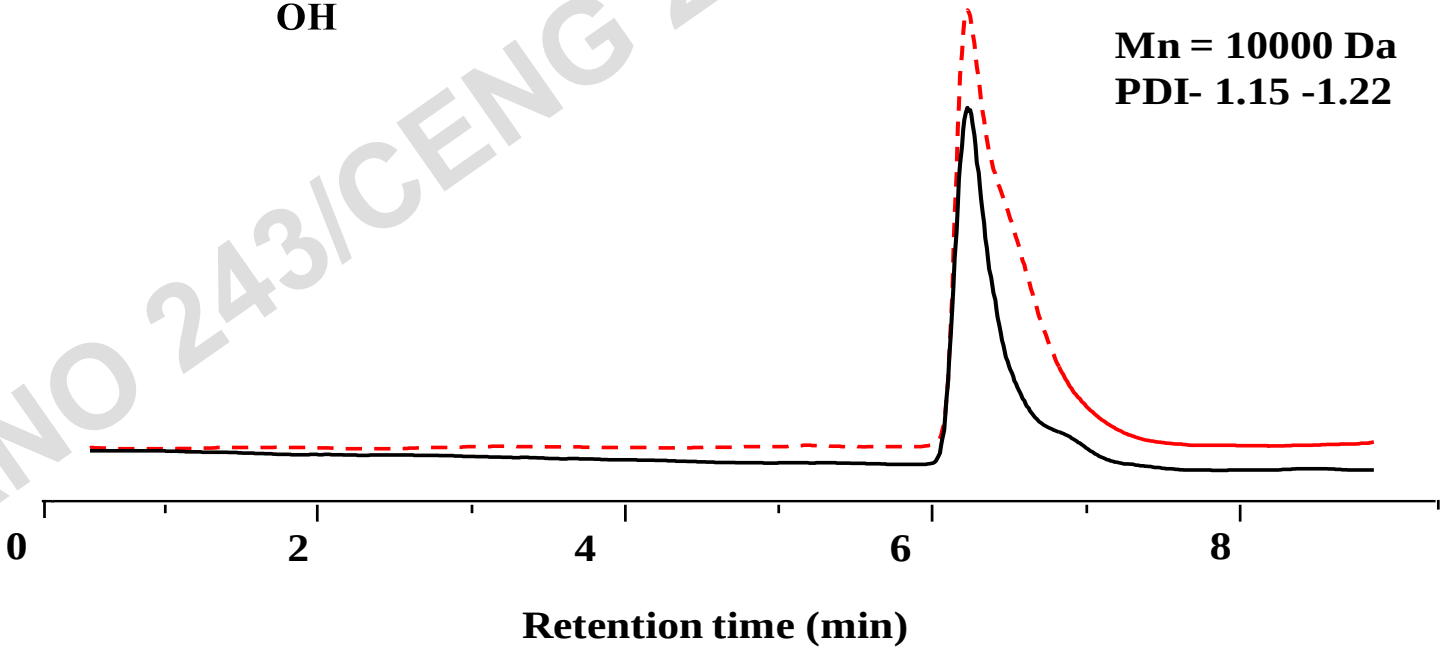
Synthesis of DOX-PLA and CPT-PLA Conjugates

DOX-PLA



CPT-PLA





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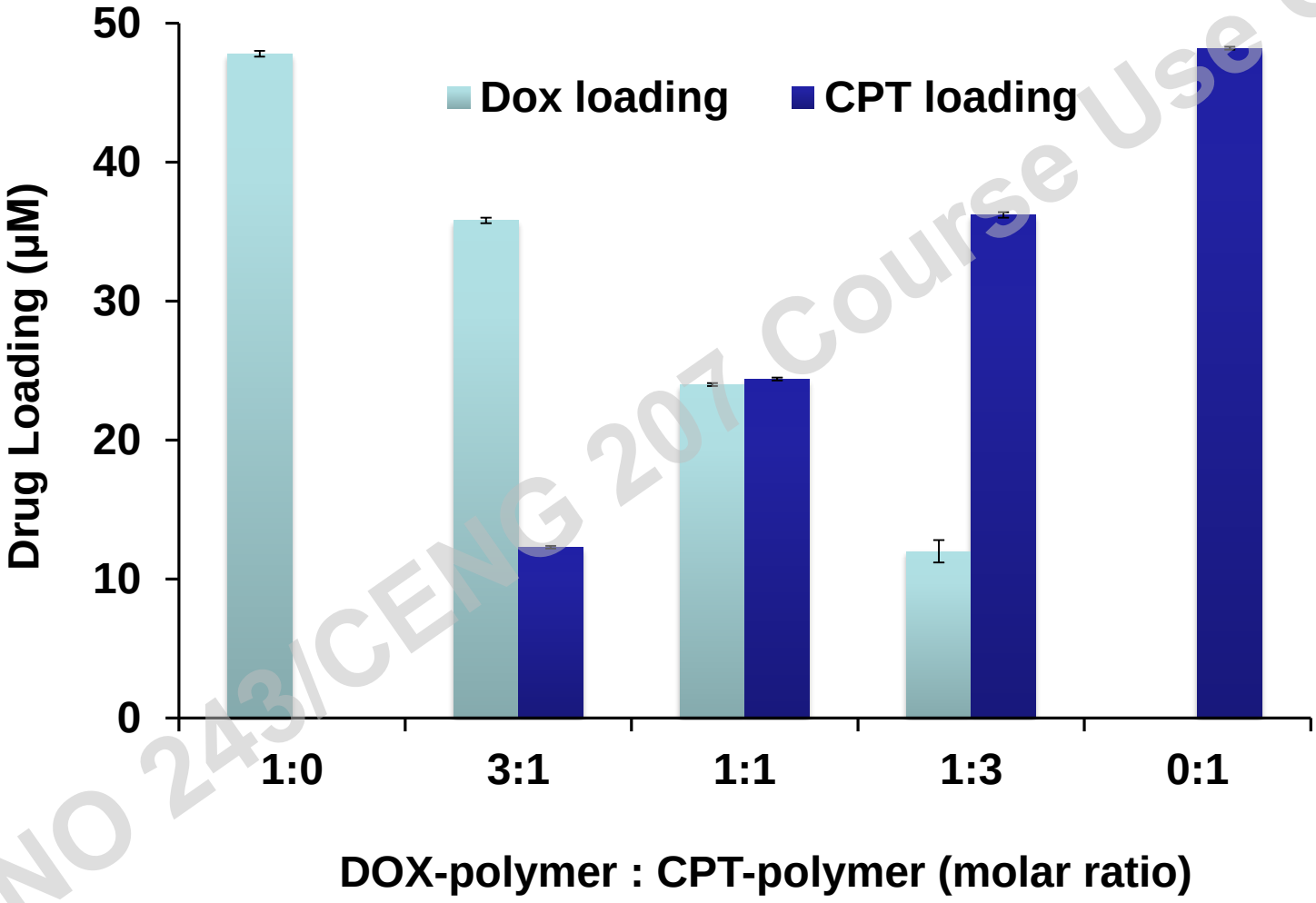
Ratiometric Loading of DOX and CPT



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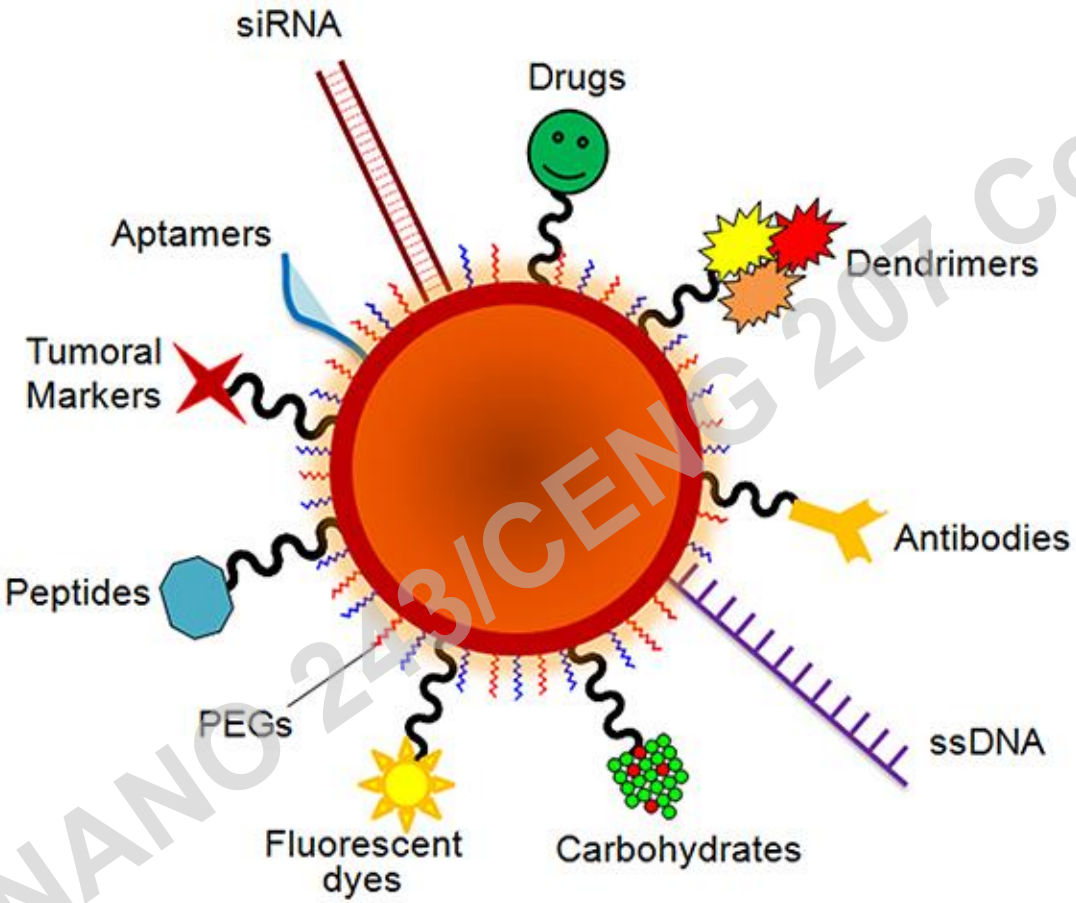
Mol. Pharma, 2011, 8, 1401

Chemical Approach – Post-conjugation

Post-conjugation involves conjugating drug molecules to the surface or interior of nanocarriers post the formation of the carriers.



Schematic representation of a nanocarrier with various agents covalently linked to its surface.

Front. Chem. 2014, 2, article 48

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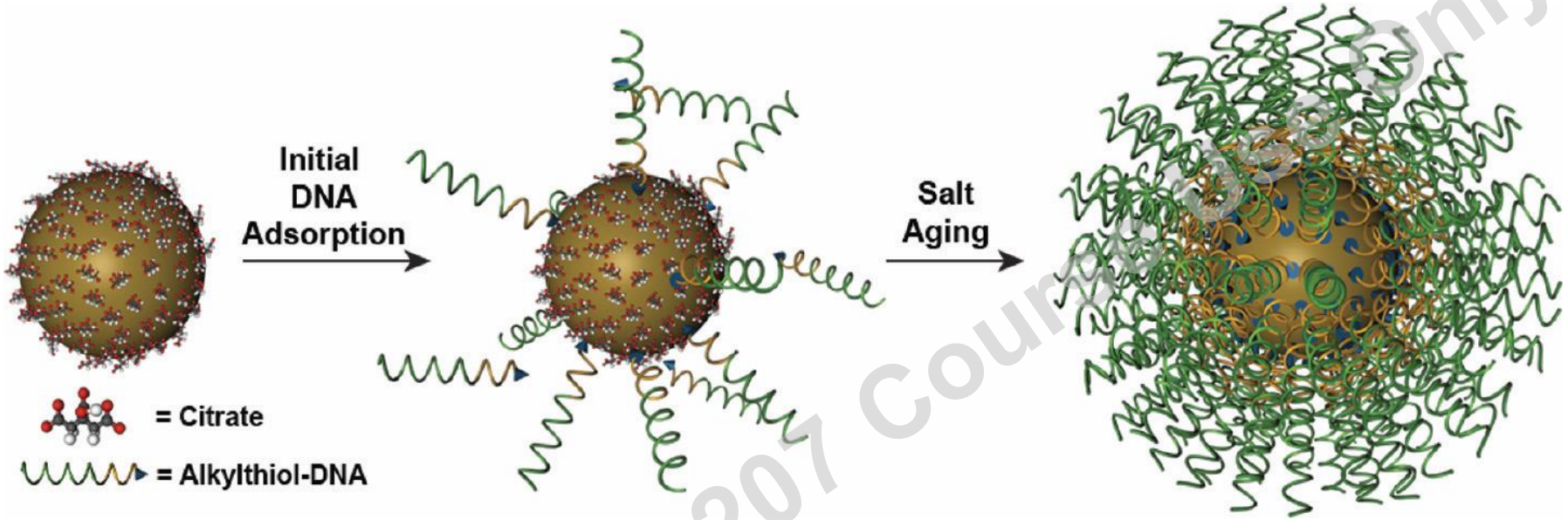
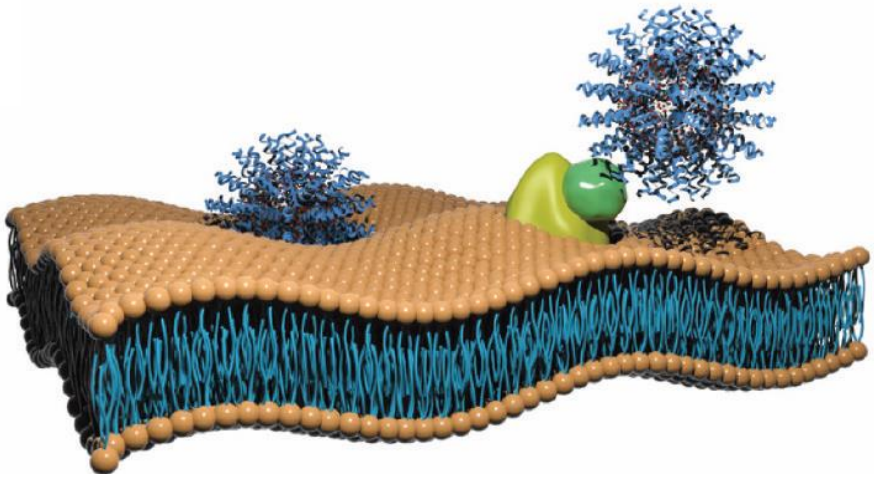
Spherical Nucleic Acids



Synthesis of SNA−Au NP conjugates. Citrate-stabilized particles are incubated with alkylthiol-functionalized oligonucleotides in water to form a low-density monolayer. By incubating the nanoparticles in aqueous solutions with successively higher concentrations of salt (typically 0.15−1.0 M) and surfactants over ~12 h, a high-density SNA shell is formed.



JACS 2012, 134, 1376

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Drug Quantification Techniques

- UV Spectroscopy
- High Performance Liquid Chromatography
- Fluorescence Spectroscopy
- Mass Spectrometry
- Scintillation Counter

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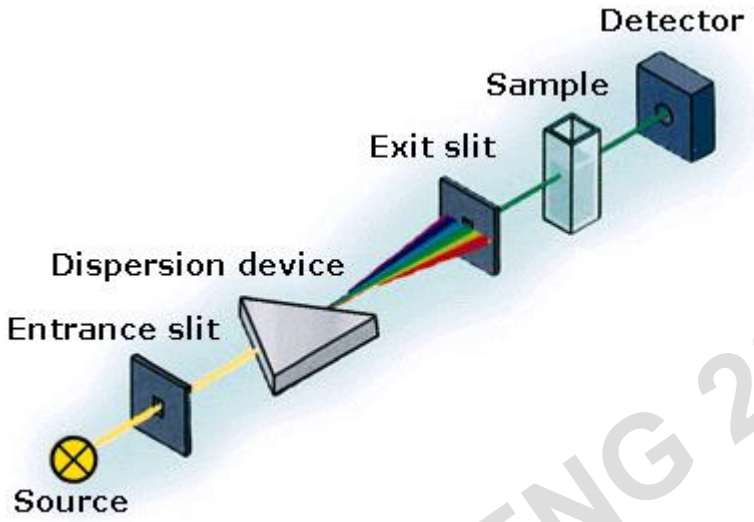
UV Spectroscopy



Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes.

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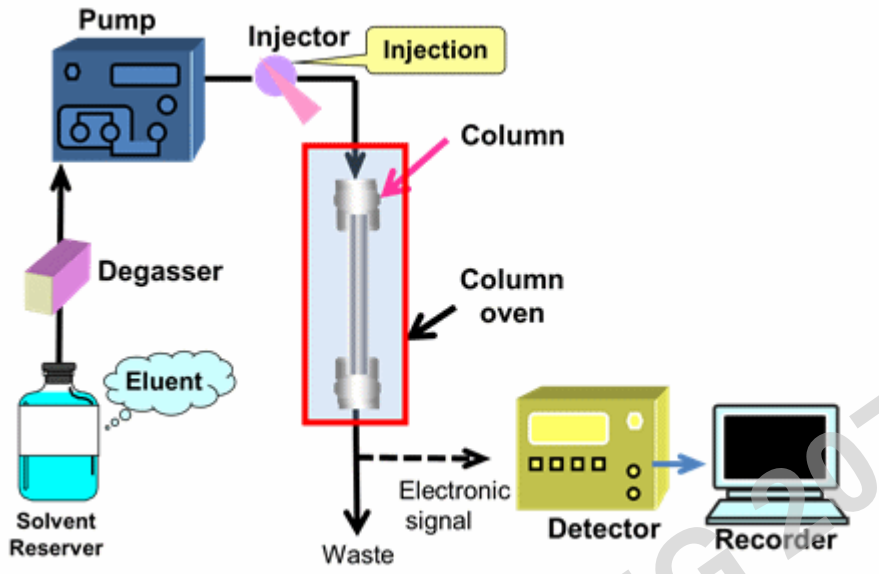
High Performance Liquid Chromatography (HPLC)



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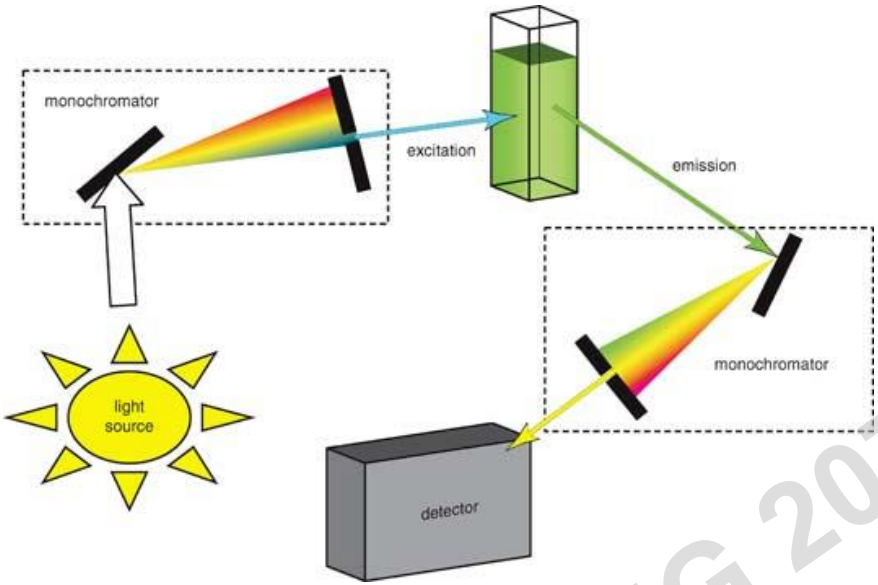
HPLC is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components.

Fluorescence Spectroscopy



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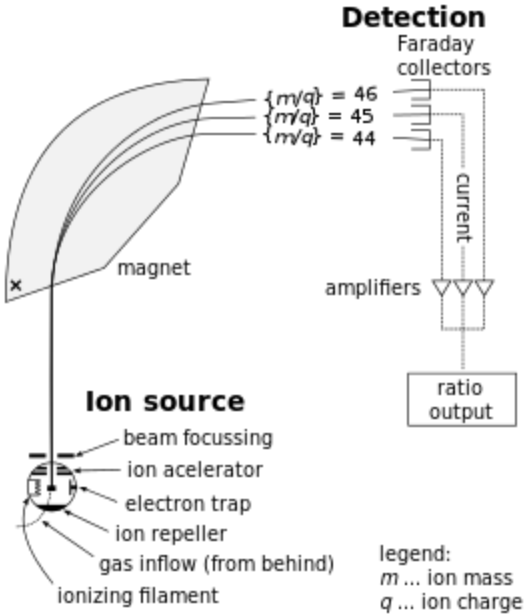
Fluorescence spectroscopy is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light, which is then detected. It complementary with UV absorption spectroscopy.

Mass Spectrometry



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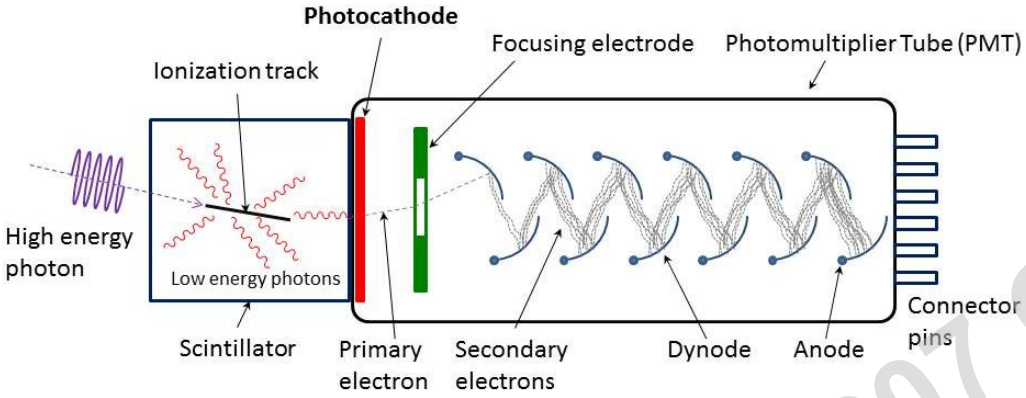
Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions based on their mass to charge ratio. In simpler terms, a mass spectrum measures the masses within a sample.

Scintillation Counter



Scintillation counter is an instrument for detecting and measuring ionizing radiation by using the excitation effect of incident radiation on a scintillator material, and detecting the resultant light pulses. It consists of a scintillator which generates photons in response to incident radiation, a sensitive photomultiplier tube (PMT) which converts the light to an electrical signal and electronics to process this signal.

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