UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

POLYSACCHARIDE BASED NANOPARTICLES

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Chemistry for Biomedical Nanotechnologies
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Polysaccharides are natural polymers made up of monosaccharide units linked by glycosidic bonds.

They can be extracted from various sources:

- Plant origin
- Microbial origin
- Animal origin

Advantages:

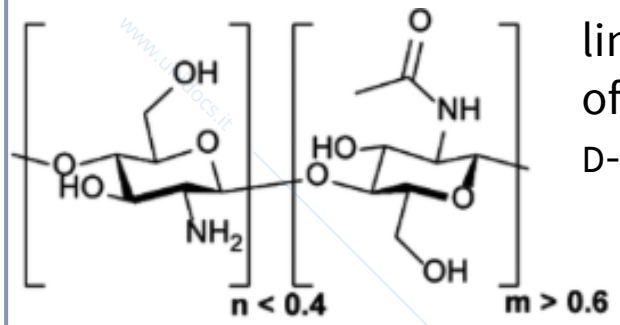
- Biodegradability
- Biocompatibility and low toxicity
- Abundance
- Ease of processing
- Natural origin

The main polysac

- Chitin
- Chitosan
- Cellulose
- Hyaluronic acid
- Dextran

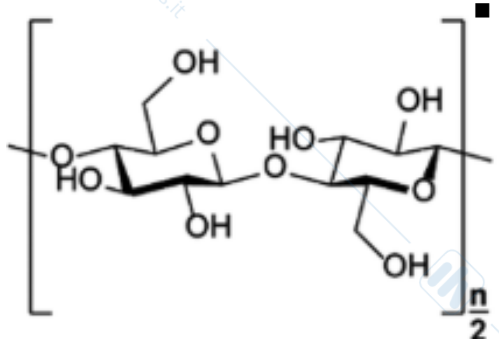
Polysaccharide	Water solubility	Molar mass range/kDa	Source
Chitin (CH)	Insoluble	1000-2500	Exoskeleton mammals
Chitosan (CS)	Soluble under acidic conditions	100-500	N-deacetylation p chitin
Cellulose (CL)	Insoluble	50-2000	Higher plants
Hyaluronic acid (HA)	Soluble	20-10000	Mammals
Dextran (DEX)	Soluble	3-2000	Bacteria

Chitin (CH)



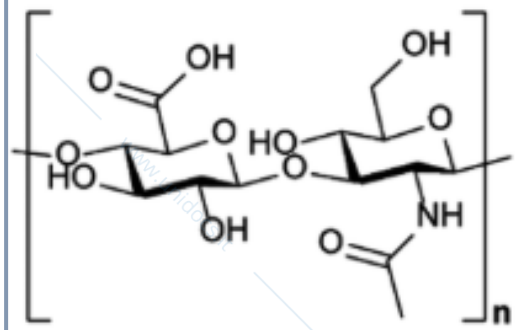
- CH has a hydrophobic linear structure composed of β-(1,4)-linked *N*-acetyl-D-glucosamine units.

Cellulose (CL)



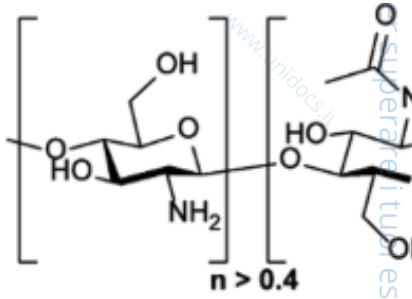
- CL is a linear poly composed of β-(1, glucose units.

Hyaluronic acid (HA)



- HA is a hydrophilic linear polysaccharide composed of alternately linked D-glucuronic and *N*-acetyl-D-glucosamine units *via* β-1,3 and β-1,4 glycosidic bonds .
- At physiological pH, it is a negatively charged polysaccharide→Hyaluronic acid-based NPs (HAMP) can be formed using cationic molecules (CS) as ionic crosslinkers.

Chitosan (CS)

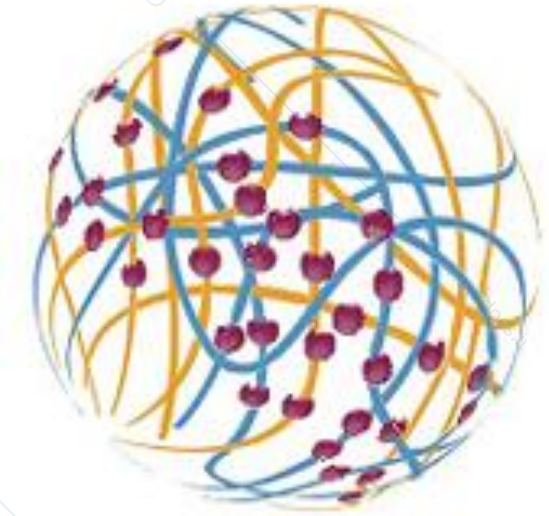


- CS is mainly based linked glucosamine acetyl glucosamine u

Definition of polysaccharide nanoparticle: It is a nanometric-sized particle made of polysaccharides.

Properties

- Biocompatibility and low toxicity
- Biodegradability
- Easy modificability
- Renewable origin
- Versatility in preparation
- Stability (combining NPs with synthetic polymers can improve stability).
- Mucoadhesion and cellular uptake (they can adhere to mucous membranes, facilitating cellular absorption and improving drug and gene delivery).



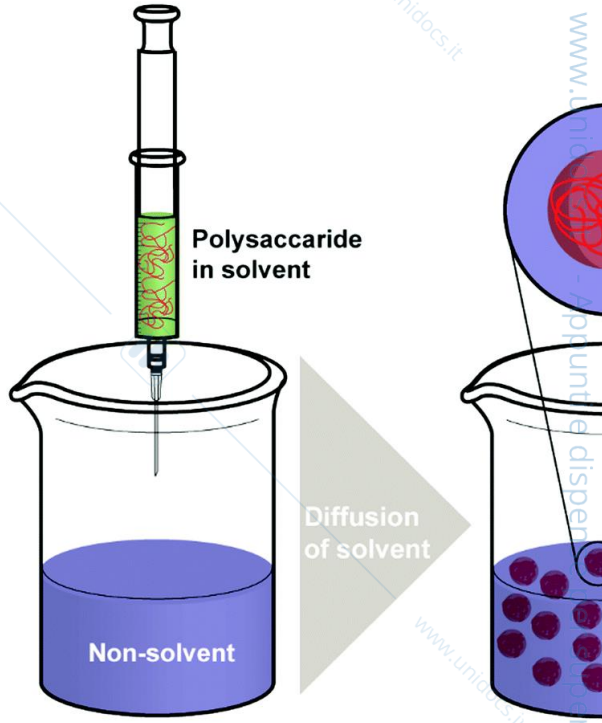
The main methods of nanoparticle synthesis are:

1. Nanoprecipitation
2. Complex coacervation
3. Emulsion-based methods
4. Self-assembly

1. Nanoprecipitation

Mechanism

I. Solute dissolution	A hydrophobic solute is dissolved in a solvent miscible with the solute and water (THF. EtOH...)
II. Addition of anti-solvent	An anti-solvent, such as H2O, is added to the solute solution.
III. Precipitation	The solubility of the solute decreases, and NP precipitation is observed.
IV. Removal of the solvent results in NPs	The solvent and anti-solvent are removed throught evaporation,dialysis and lyophilization.



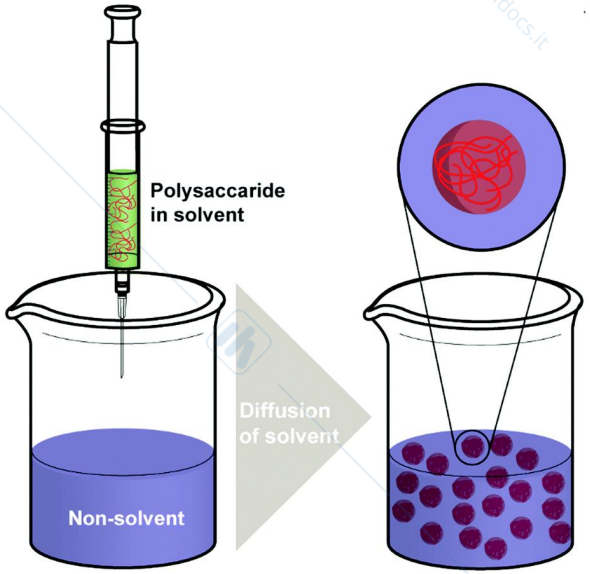
Factors influencing the size and forma

- Solvent mixing
- Surface tension
- Temperature, solvent/anti-solvent polymer properties

1. Nanoprecipitation

Nanoprecipitation methods

- Traditional nanoprecipitation
- Flash nanoprecipitation
- Micro-fluidic nanoprecipitation



Traditional nanoprecipitation

- Mixing of the solute solution with the anti-solvent slowly through diffusion.
- Precipitation time: seconds to minutes.
- Produces particles with variable size.
- Difficult to control the size.

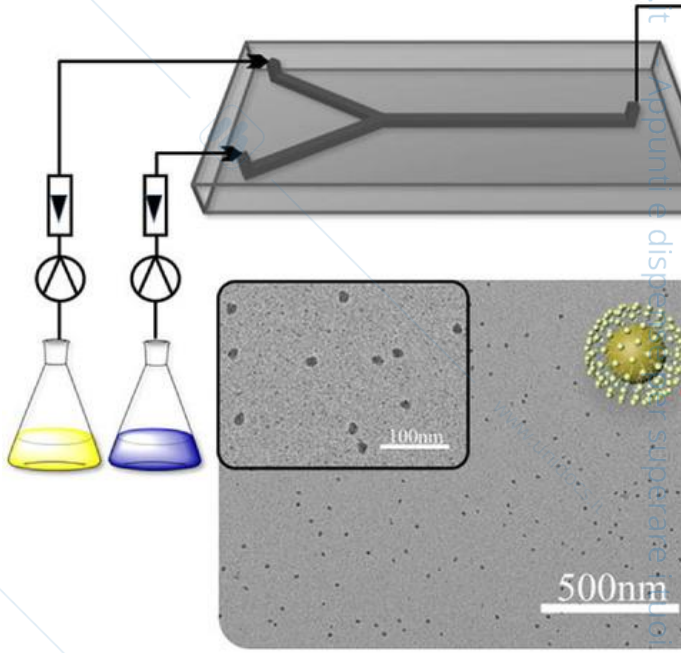
Flash nanoprecipitation

- Mixing of the solute solution wi
  solvent quickly through turbulen
- Precipitation time: milliseconds.
- Produces NPs with smaller si
  narrow size distribution.
- The particles produced may
  adequate stability for some appl

1. Nanoprecipitation

Micro-fluidic nanoprecipitation

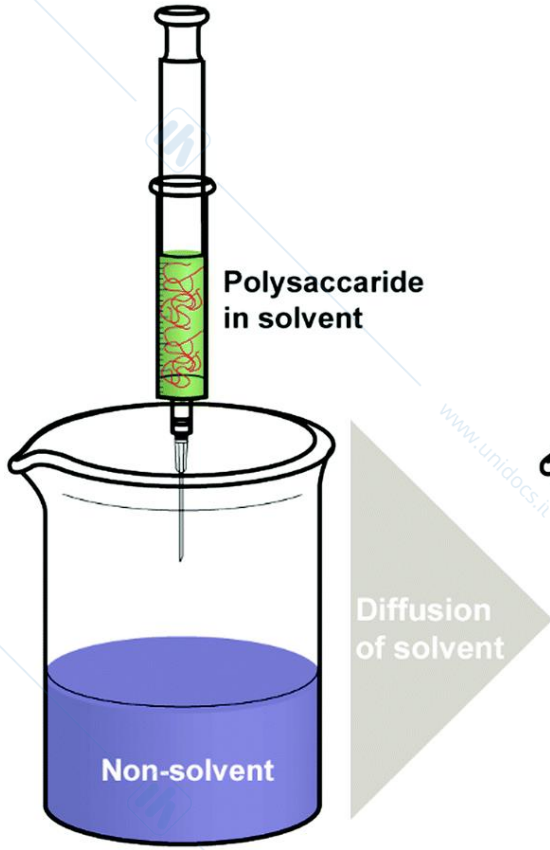
- Mixing of the solute solution with the anti-solvent in microfluidic channels.
- Inside the microfluidic channels, the flow is laminar, and diffusion between two phases is fast and uniform.
- The small channels allow for rapid phases contact, leading to efficient mixing in a short time.
- Precipitation time: milliseconds.
- Physicochemical properties of NPs can be controlled by modifying the micromixer configuration or adjusting the flow rate ratio.
- The small volume and low flow rates result in low productivity.



1. Nanoprecipitation

Characteristics

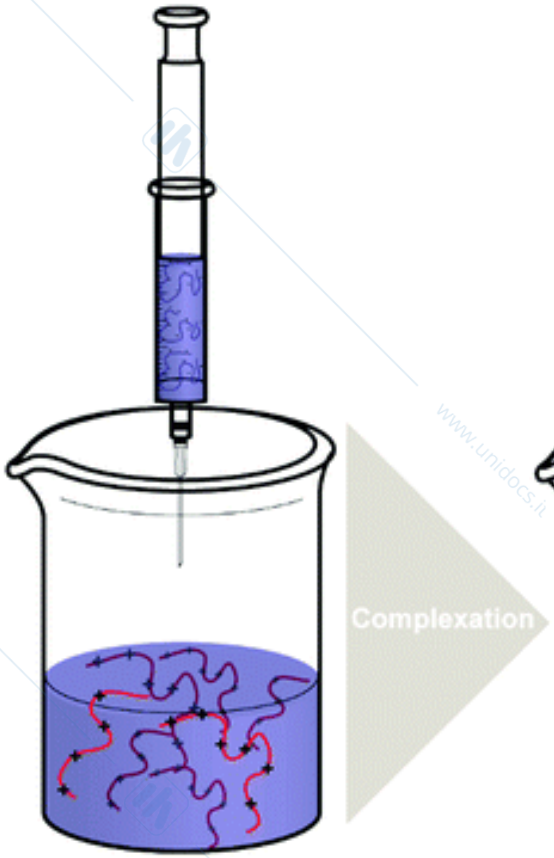
- It is one of the first techniques developed for the encapsulation of drug molecules.
- It is an easy technique to conduct, with a fast processing time.
- It requires simple equipment requirements.
- It is difficult to use for encapsulating water-soluble compounds.
- Low quantity of NPs produced.
- Microfluidic channels may get clogged.



2. Complex coacervation

Mechanism

I. Preparation of solutions of oppositely charged polymers	Preparation of aqueous solutions of two polysaccharides, one cationic and the other anionic.
II. Mixing solutions and formation of the coacervate	Mixing of the two solutions and subsequent electrostatic interaction between the charged polymers. A dense liquid phase rich in polyelectrolytes, called a coacervate, is formed.
III. Self-assembly of polyelectrolyte complexes into NPs	The polyelectrolyte complexes self-assemble and nanoparticles are formed.
IV. Stabilization of nanoparticles	If the electrostatic interaction alone is not sufficient for stabilization, crosslinking can be used. Additionally, by varying the ratio of the polyelectrolytes, the net charge of the nanoparticles can be modulated.



2. Complex coacervation

Factors that influence the process

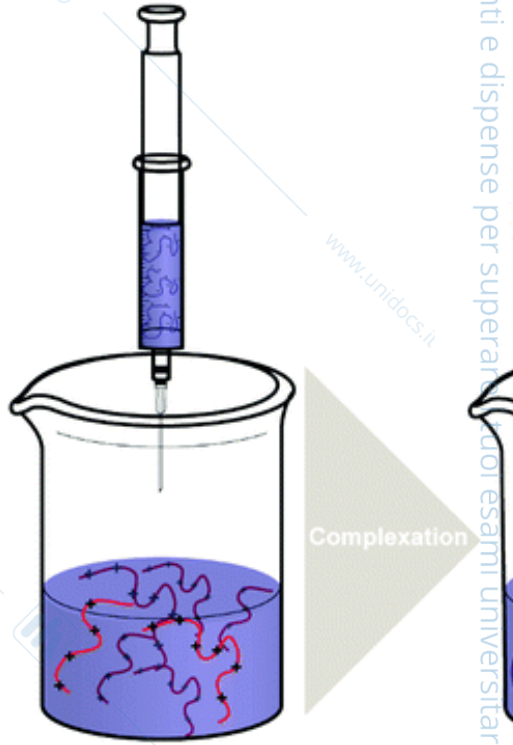
- The pH of the solution
- The temperature
- The ionic strength
- The charge density of the polymers
- Their molecular weight

Process characteristics

- This method preserves the structure and properties of biochemical drugs, including genes and proteins, thanks to the mild conditions required for coacervate formation and their native environment.
- It is difficult to control the size and shape of the NPs due to the numerous factors that influence the process.
- Polysaccharides can be mixed with other polymers to modulate the properties of the NPs.

Commonly used polysac

- CS as a cationic poly combined with HA or anionic polysacchari



3. Emulsion-based methods

An emulsion is defined as a metastable dispersion of two immiscible liquids, such as oil and water. This c
thermodynamically unstable and tends to separate into its original phases over time, unless emulsifiers ar
improve its stability. Depending on the dispersed phase and the dispersion medium, emulsions are classif
water (O/W) direct emulsions or water-in-oil (W/O) inverse emulsions.

Based on the droplet size, emulsions can be classified as:

- **Microemulsions** (10 -100 nm), thermodynamically stable
- **Miniemulsions** (100 nm - 1 μm), thermodynamically unstable
- **Macroemulsions** (>1 μm), thermodynamically unstable.

3. Emulsion-based methods

Mechanism

I. Preparation of a polysaccharide solution

Preparation of a solution of polysaccharides, which are dissolved in water or other solvents

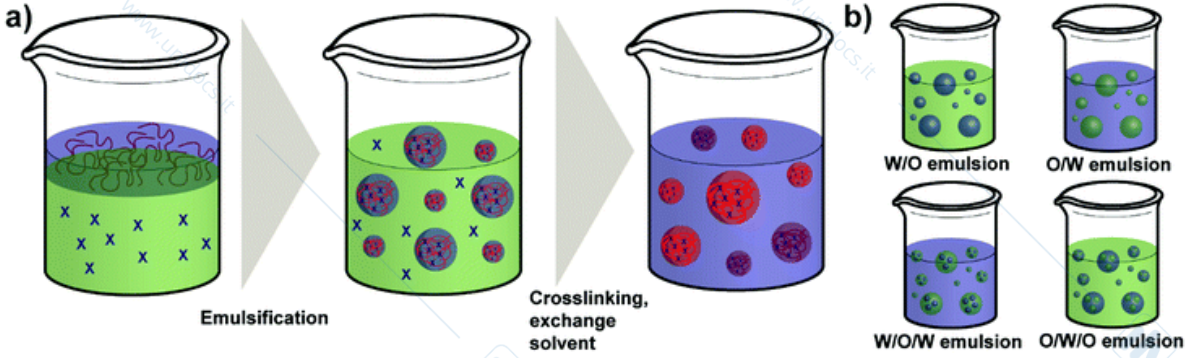
II. Formation of emulsion

The solution is dispersed in an oily phase to make O/W or W/O emulsions by shaking or using ultrasound.

III. Gelation

Through internal or external gelation, polysaccharide NPs are generated.

- **External gelation:** crosslinking agents diffuse from an external phase (the continuous phase of the emulsion) into the polysaccharide droplets.
- **Internal gelation:** crosslinking agents are already present within the polysaccharide droplets before gelation occurs.

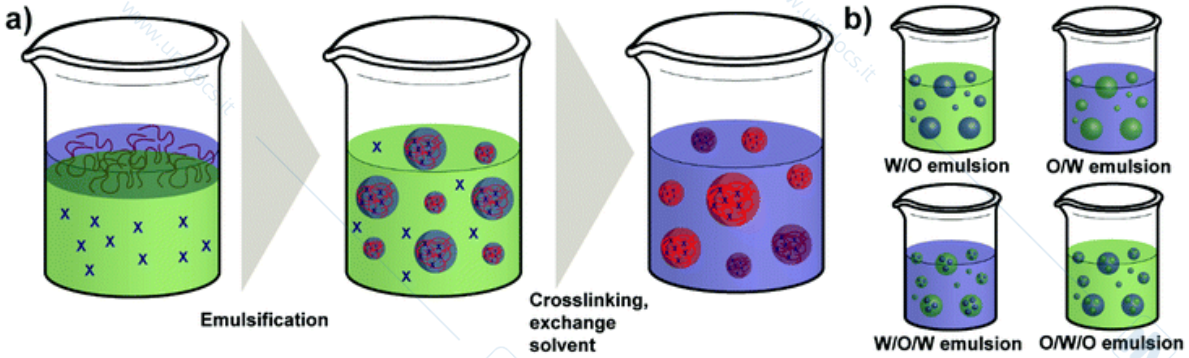


3. Emulsion-based methods

Characteristics

- Advantage in controlling the size and loading of synthesized polysaccharide NPs.
- Emulsification methods are more complicated than nanoprecipitation and complex coacervation due to the additional emulsification step.
- Large amounts of organic solvents are required, which limits the use of this method.

The size of the NPs is closely rela
size of the droplets in the emu
energy emulsification techniques
and microfluidic methods) apply h
leading to the formation o
nanoparticles (NPs).

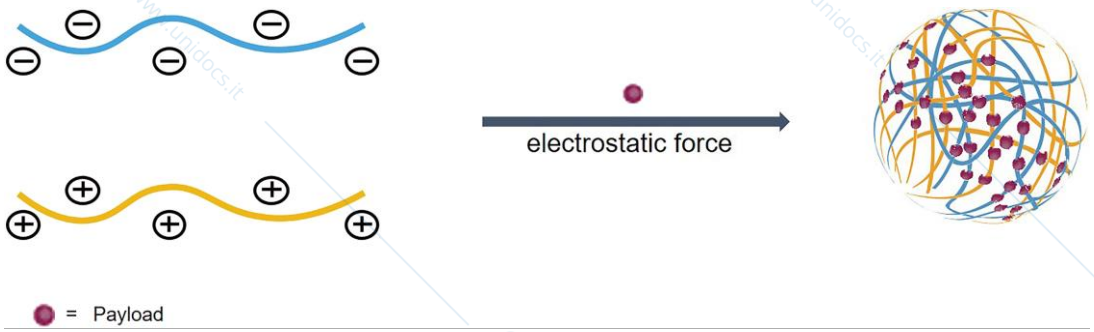


4. Self-assembly

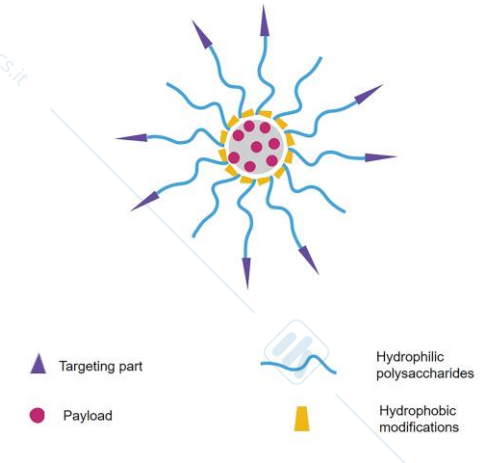
It is a process in which molecules spontaneously organize or aggregate into a stable structure. The interactic that govern this process are:

- Hydrophobic interactions
- Van der Waals forces
- Hydrogen bonds
- Electrostatic forces
- π–π aromatic interactions

- **Elettrostatic forces**



- **Hydrophobic interactions**



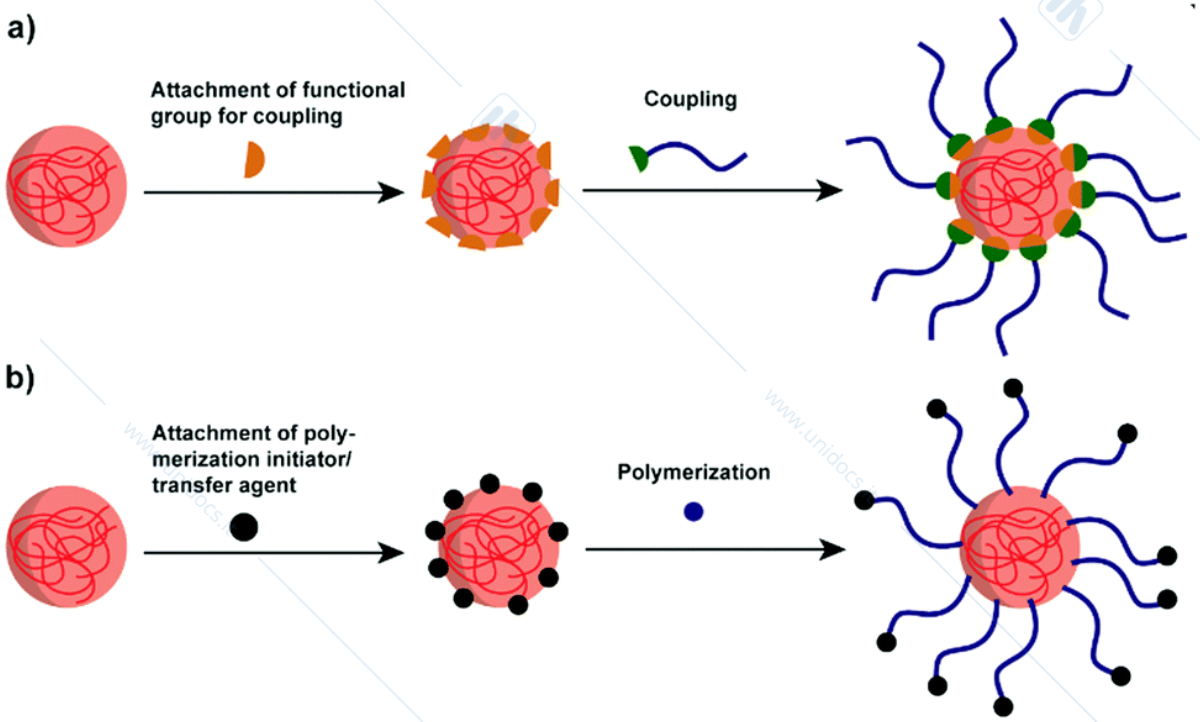
4. Self-assembly

Characteristics

- **Spontaneous process**
- **Versatility:** it can be applied to natural polysaccharides, polymers, and other biomolecules.
- **"Green" method:** It is considered an eco-friendly method, reducing the possibility of toxic cross-linkers that would be harmful in biomedical applications.
- **Versatility in drug delivery:** The self-assembled NPs can improve the solubility of insoluble drugs, enhance tissue targeting, and enable controlled and sustained drug release.
- **Possibility of encapsulating different types of molecules:** Both hydrophilic and hydrophobic molecules can be encapsulated, expanding therapeutic possibilities.
- NPs can be designed to carry multiple therapeutic agents simultaneously.
- **Reduced toxicity**
- **Sensitivity to environmental conditions**
- **Lack of in vivo studies**

Polymers bound to polysaccharide NPs

- The combination of polysaccharides and polymers allows for the creation of systems with combined prope overcoming the limitations of individual materials.
- There are two main grafting methods used to modify polysaccharide nanoparticles:



a) "Grafting to" → Reaction between the prepared polymer and th groups of the polysacchar

b) "Grafting from" → The polymerization of the occurs directly on the su polysaccharide. The triggered by an initiator

Applications of polysaccharide NPs

In the biomedical field

1. Polysaccharide NPs for the delivery of cosmetic i
2. Polisaccharide NPs for tissue engineering
3. Polisaccharide NPs for drug/gene delivery

1. Polysaccharide NPs for the delivery of cosmetic ingredients

- Used as nanocarriers in cosmetic products for hair or body care.
- Bioactive substances in cosmetic formulations, such as vitamins, are often unstable (sensitive to temperature, pH, light, and oxidation). Therefore, encapsulation and protection are necessary to prevent unwanted degradation and ensure specific release.

2. Polisaccharide NPs for tissue engineering

NPs can serve different functions:

I. Structural support
II. Cell adhesion and proliferation
III. Tissue integration
IV. Controlled release of growth factors
V. Cell delivery
VI. Protection of transplanted cells

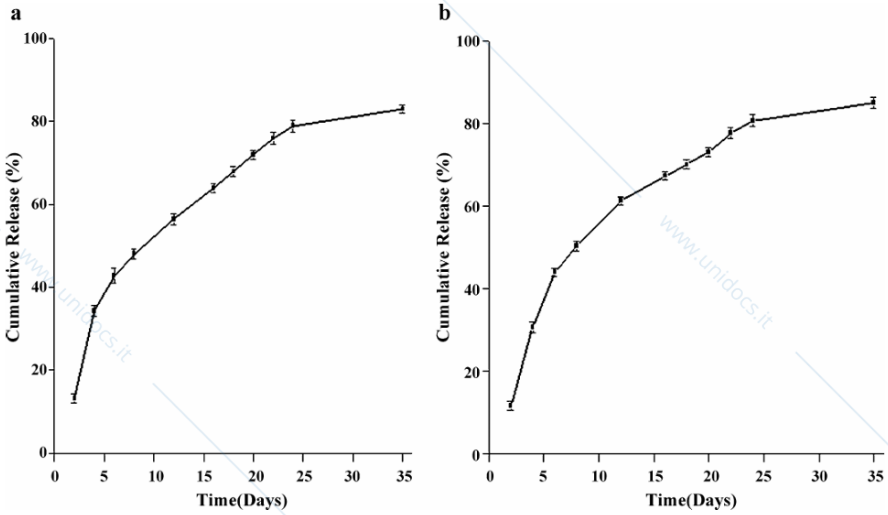


Fig. 4. Cumulative release profile of (a) EGF and (b) FGF from CNP in PBS, pH 7.4 at 37 °C. Each data point represents a mean ± standard deviation ($n = 3$).

Example

- Design of CS NPs to transport epidermal growth fact fibroblast growth factor (FGF).
- Objective: to create a controlled release system for gr to enhance tissue regeneration.
- Results: - CNPs are non-toxic up to 4 mg; at 6 m observed (Fig. 5a).
  - They promote fibroblast growth (Fig. 5b).
  - CNPs facilitate prolonged release (Fig. 4).

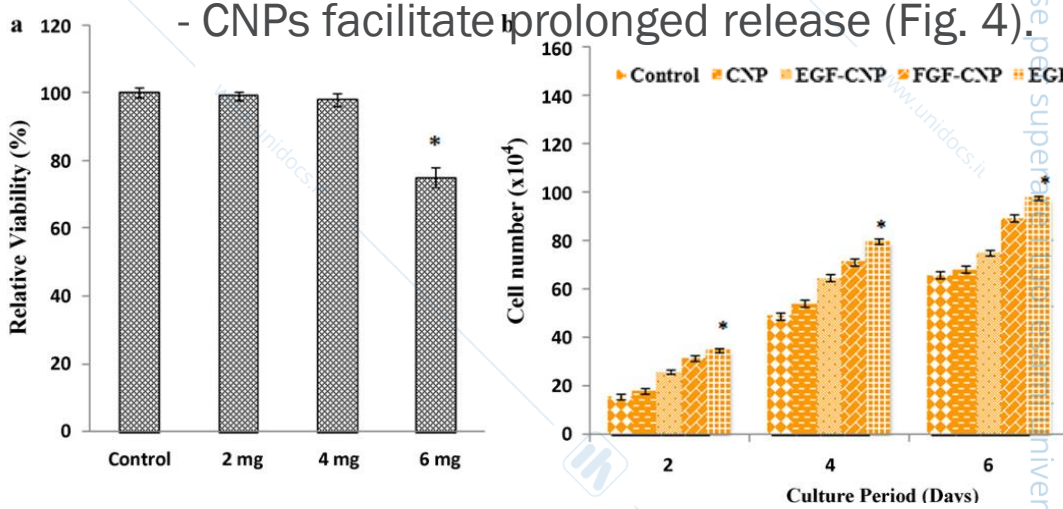


Fig. 5. Cytotoxicity of CNP in fibroblast cells: (a) the absence (control) or presence of different concentrations of CNP: 2 mg/ml, 4 mg/ represent the mean ± SD for $n = 3$. *$P < 0.01$ compared with control. (b) Proliferation of fibroblast cells on CNP with or without growth (control; CNP; EGF-CNP; FGF-CNP; and EGF + FGF-CNP). Each data point represents a mean ± standard deviation ($n = 3$). *$P < 0.01$ compa

3. Polisaccharide NPs for drug/gene delivery

Polysaccharide NPs are for:

- Improve the solubility of drugs in water.
- Transport the active drug molecules to a targeted pathological site.
- Release drugs in a controlled and sustained manner for drug/gene delivery.

Issues:

- Variations in NP size
- Heterogeneity of the EPR effect in tumo
- Uncertainty in delivery efficiency

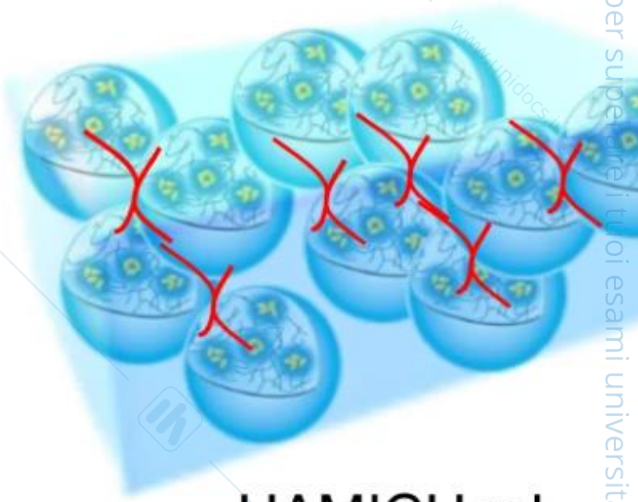
After injection into the body, drug/gene-loaded NPs cross epithelial barriers and circulate in the blood vesse reaching the target site.

EPR effect (Enhanced Permeability and Retention) → It is caused by → Permeable vasculature and dysfunctional lymphatic system that prevent nanoparticles from draining from the tumor site. → It allows → Drug accumulatio target sites.

Variable swelling behavior of and drug encapsulation in a maleimide-modified hyaluronic acid nanogel-bas

Hydrogels are polymeric materials with a three-dimensional structure capable of absorbing and retaining large amounts of water without dissolving.

Objective: To prepare HA nanogels using HA modified with cholesteryl and maleimide groups, aiming to build a new crosslinked hydrogel system through a Michael addition reaction using a poly(ethylene glycol) (PEG) crosslinker with a thiol functional group.

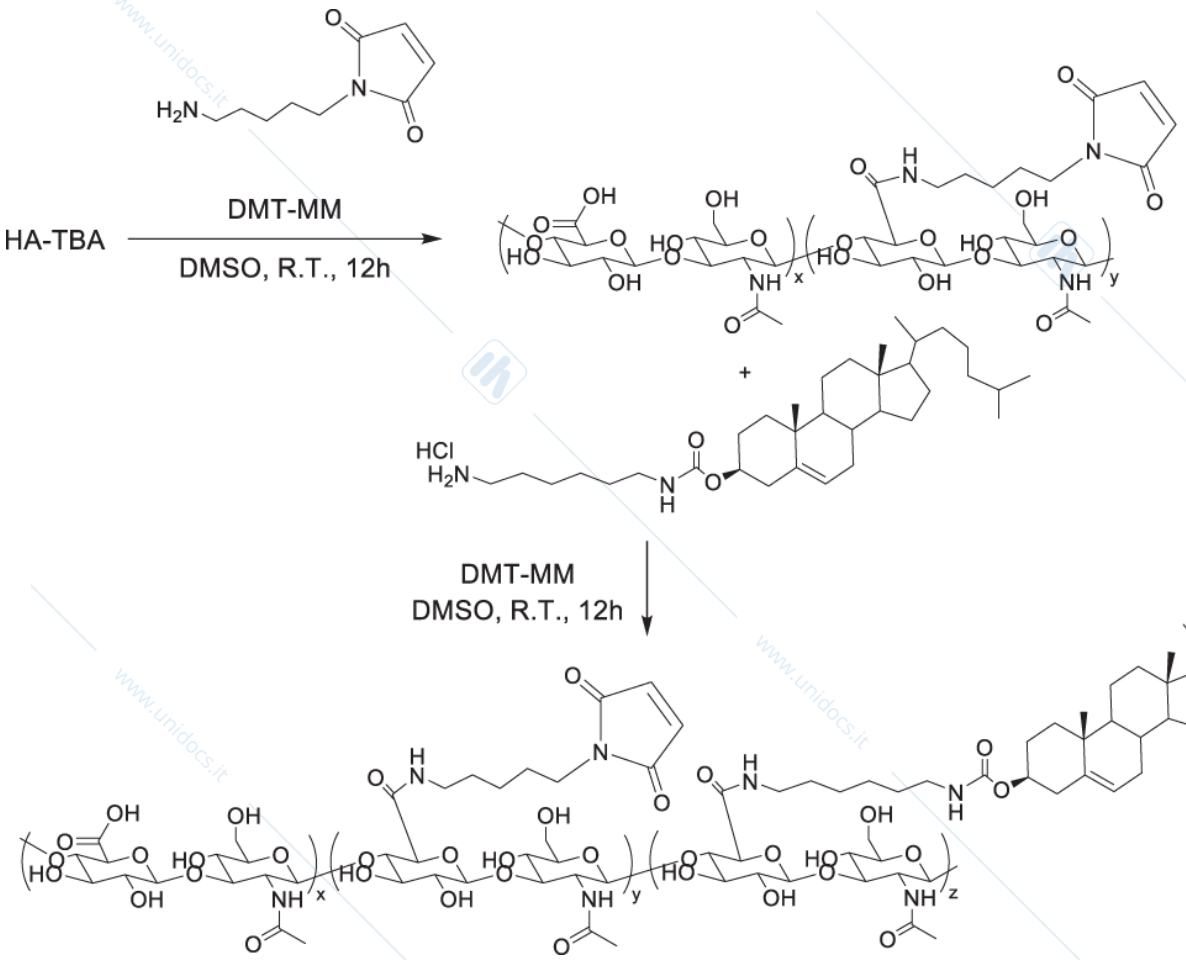


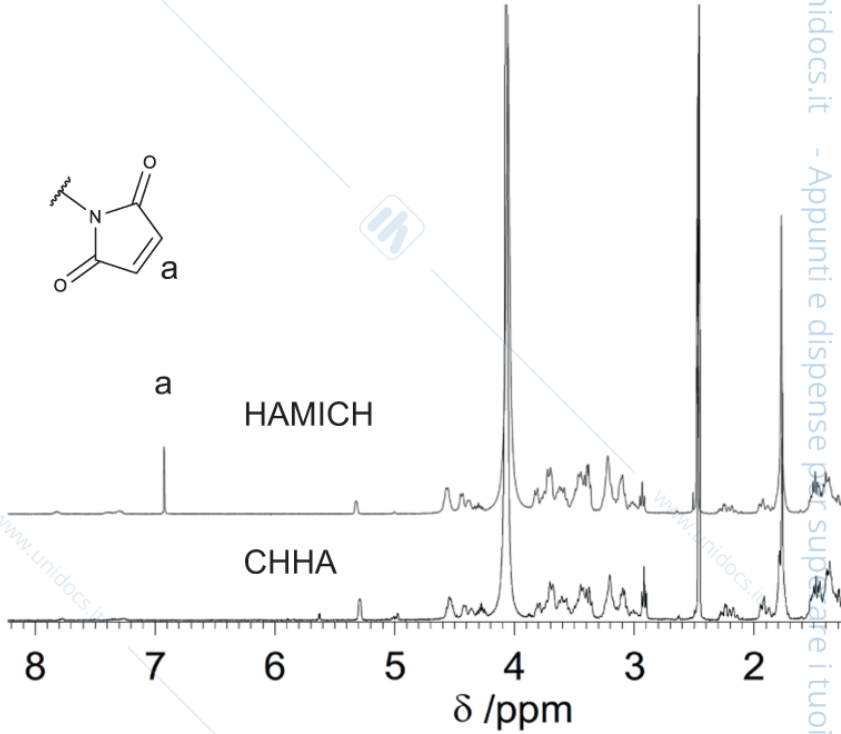
HAMICHgel

Synthesis of a novel crosslinked hydrogel composed of HA modified with cholesteryl, maleimide groups an

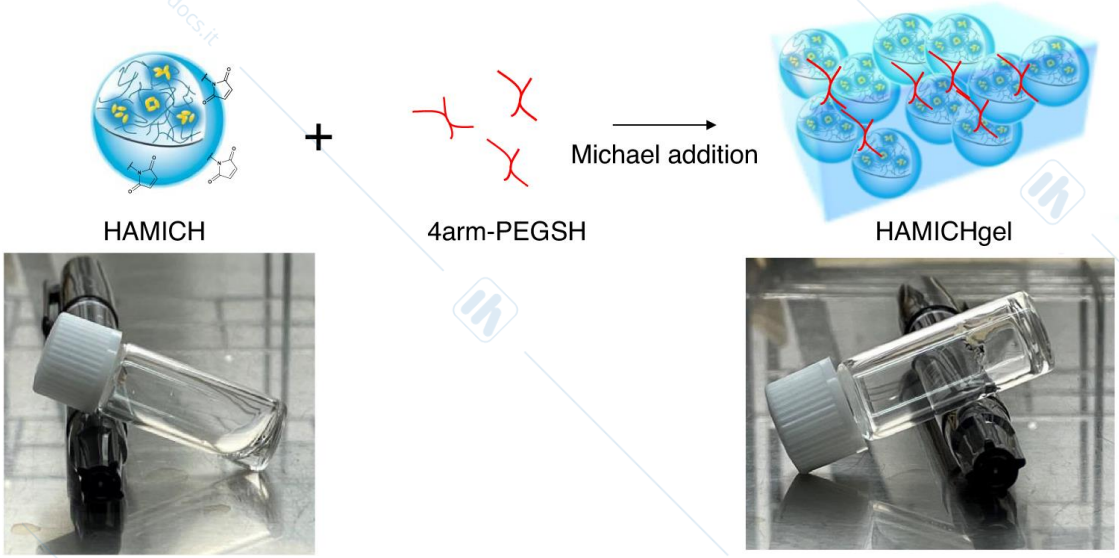
I. Synthesis HAMICH	HA is modified with cholesterol and maleimide derivatives.
II. Self-assembly into nanoparticles	HAMICH self-assembles into nanoparticles in water through hydrophobic interactions between cholesterol molecules.
III. Preparation of crosslinked gel and gelation	The hydrogel is prepared through a Michael addition reaction between HAMICH and PEGSH.

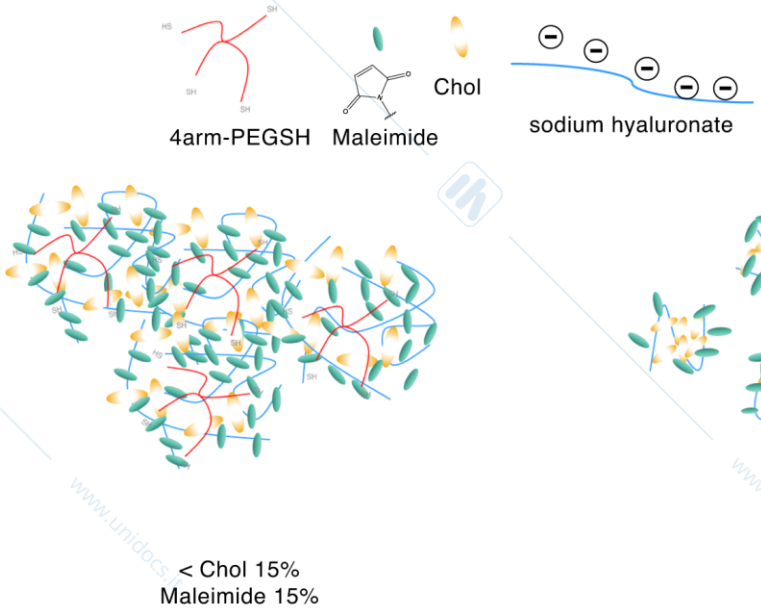
I. Synthesis of hyaluronic acid (HA) nanogels containing cholesterol and a maleimide derivative (HAMICH)





III. Preparation of crosslinked gel and gelation





- The Michael reaction leads to the formation of cross-links between HAMICH nanoparticles, creating a three-dimensional network that forms the hydrogel.
- The concentration of HAMICH required for gelation depends on the degree of cholesterol substitution and the concentration of 4-arm-PEG-SH.

Analysis and tests conducted

- **Size exclusion chromatography coupled with multiangle laser scattering (SEC-MALS)**

Results:

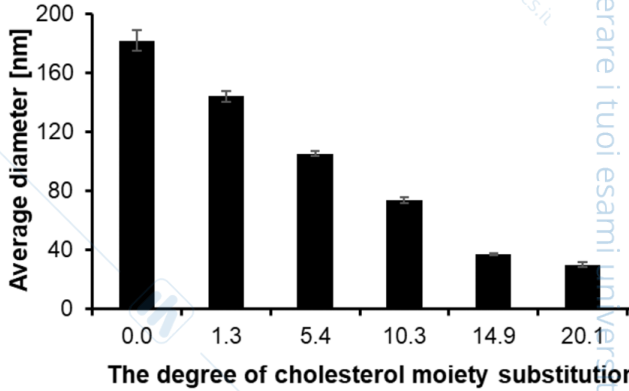
- The absolute molecular weight increases with the increase in the degree of substitution of choleste derivatives.
- With a substitution degree of 15-20%, a decrease in the number of aggregates is observed.
- When the degree of substitution is ≥28.6%, the absolute molecular weight increases, and the numbe associations also rises.

The cholesterol concentration affects the balance between hydrophobic and hydrophilic forces within system. An intermediate concentration (15-20%) favors the formation of well-defined nanoparticles, wh higher concentrations lead to increased interparticle aggregation.

- **Dynamic light scattering (DLS)**

Results

- It has been confirmed that HAMICH forms nanoparticles in aqueous solution with sizes ranging from 10 to 200 nm, depending on the degree of cholesterol substitution.



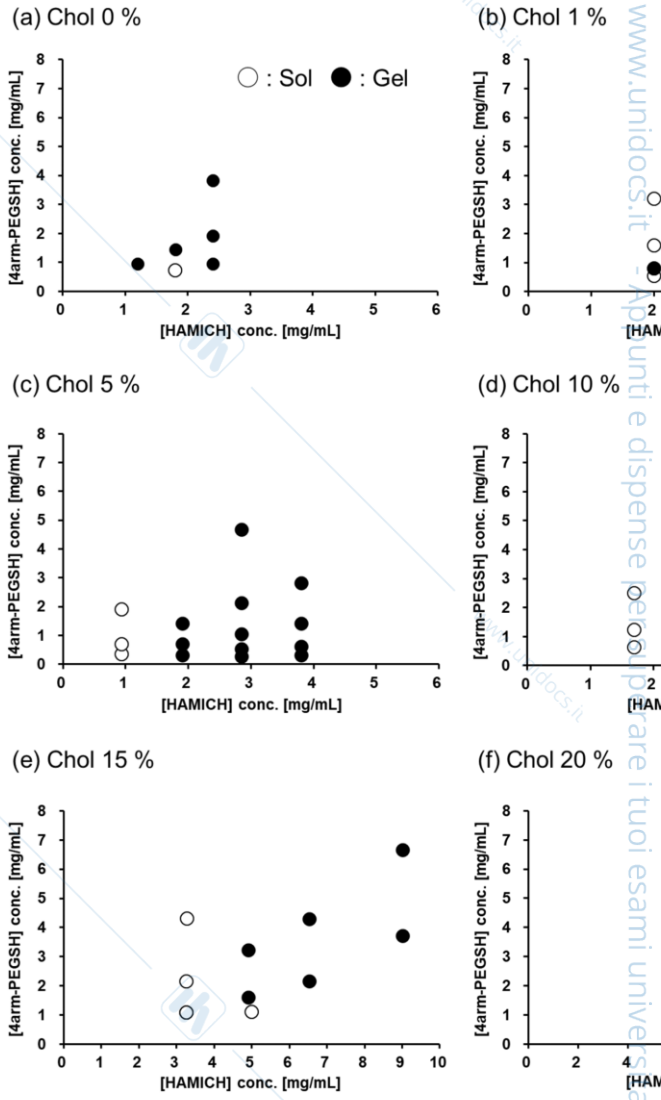
Characteristics of HAMICH. The results are expressed as the mean ± standard dev

- **Inverted vial tests (HAMICH gelation tests)**

It is used to assess the material's ability to form a gel under specific conditions.

Results

- The concentration of HAMICH required for gelation varies based on the degree of cholesterol incorporation.



Phase diagram of the gelation of HAMICH with different degrees of cho

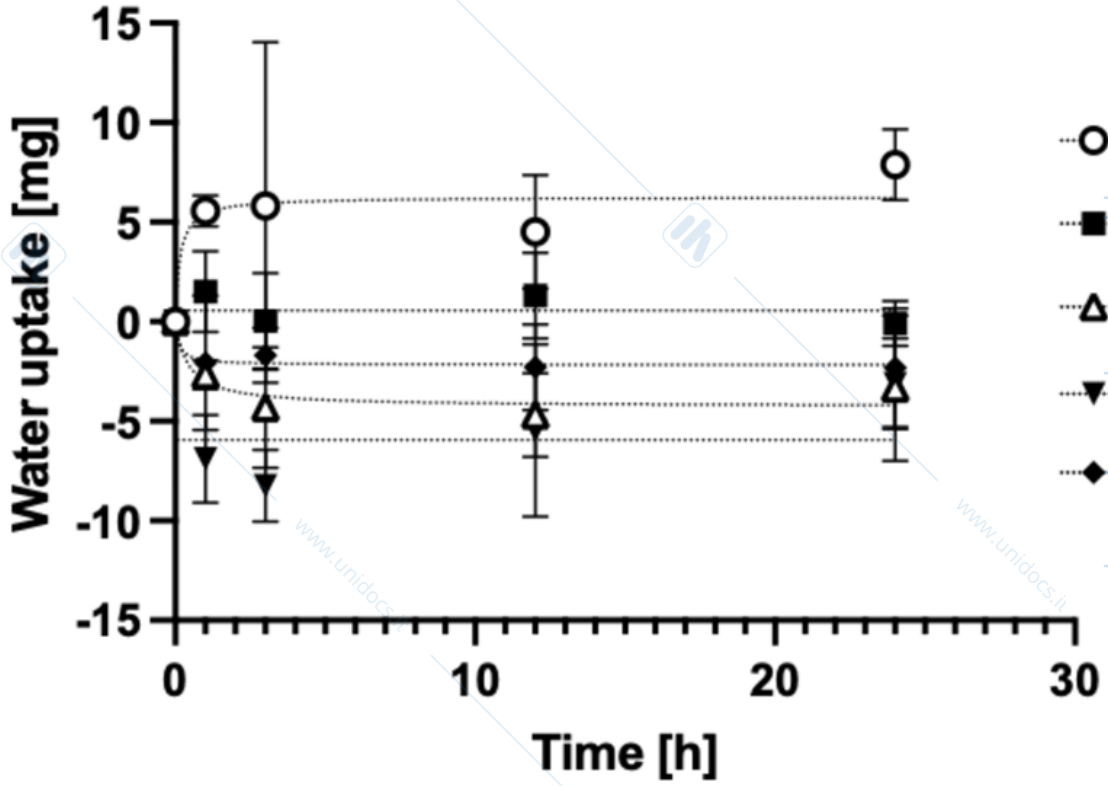
- **Water uptake by the hydrogel**

It is used to determine the gel's ability to absorb water and maintain its structure. HAMICH gels are immersed in phosphate-buffered saline (PBS) solution, and the weight change of the gel is measured over time.

Results

- The water absorption of the crosslinked gel decreased as the cholesterol content increased.

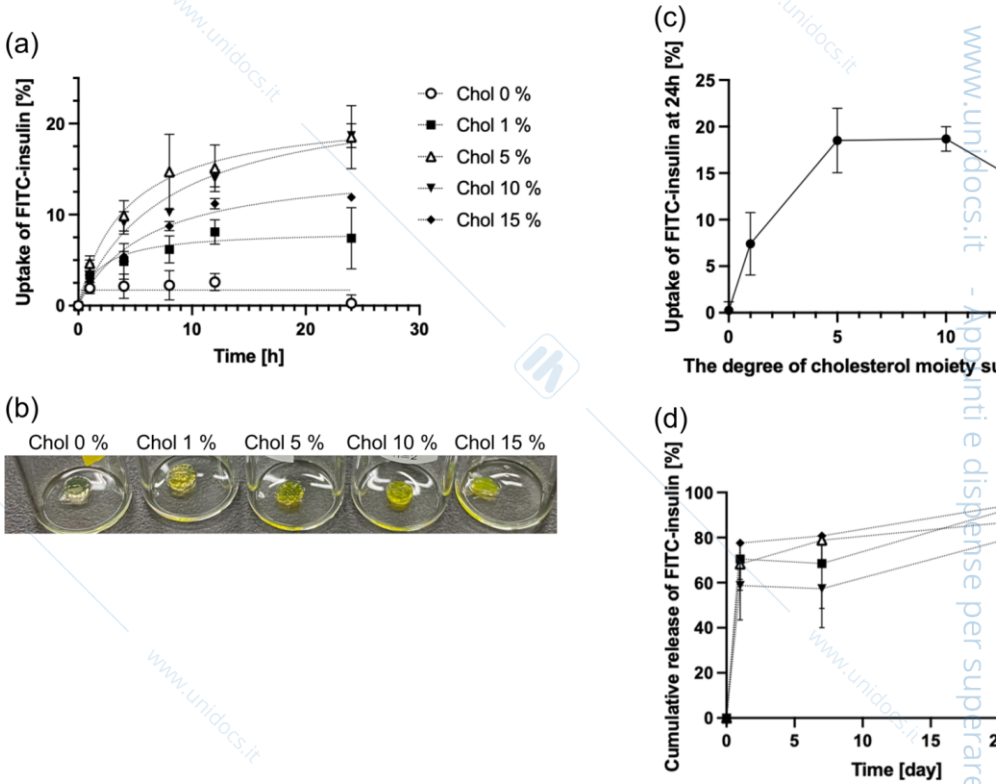
By varying the cholesterol content, it is possible to control the physical properties of the hydrogel. Controlling the swelling is crucial for controlled drug release and tissue engineering.



Water uptake by the HAMICH gel. The results are expressed as the mean ± standard

- **Protein loading and release**

- Protein loading
  - In cholesterol-crosslinked gels, the encapsulation of insulin increases due to hydrophobic interactions with the protein.
  - The highest inclusion amount was observed at a cholesterol incorporation rate of 5-10%, and the amount of encapsulated material was reduced with the HAMICH gel at 15%.
- Protein release
  - HAMICH gels with cholesterol release insulin more slowly (50% of insulin after 1 day, the remaining over 21 days) and in a prolonged manner compared to unmodified HA gels (1 day).



Insulin encapsulation profiles of the HAMICH gel. **a** Encapsulation of fluorescein iso
labeled insulin in the HAMICH gel, in which the concentration of HAMICH was 7.0 r
Images of hydrogels containing FITC-labeled insulin. **c** Correlation diagram betwee
cholesterol moiety substitution and the uptake of FITC-labeled insulin at 24 h (n = 4
of FITC-labeled insulin from HAMICH gels, in which the concentration of HAMICH w
3). The results are expressed as the mean ± standard deviation

Conclusions

- Polysaccharide NPs are biodegradable and biocompatible.
- They can find applications in the biomedical field (drug encapsulation and release, tissue engineering).
- HAMICH hydrogels have the functions of absorbing water and encapsulating and releasing insulin. These functions are influenced by the presence and concentration of cholesterol in the gel structure.
- Improvement of manufacturing methods.
- Customization of properties by combining NPs with polymeric materials.
- Expansion of applications.
- Advancing towards clinical studies.

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Thank you for your attention

Valenzini Giorgia Iole
Chemistry for Biomedical Nanotechnologies
Academic year 2024-2025