Nano-in-macro system for the oral administration of exenatide

Prof. Stefano Salmaso

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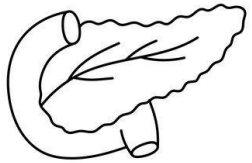
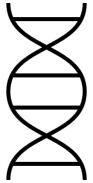
Diabetes: causes

High blood pressure

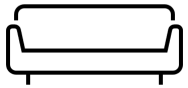


Pancreatic diseases

Unhealthy food



Genetic predisposition



Sedentary lifestyle

Age

Impaired or **insufficient insulin production** to meet metabolic needs.

Insulin resistance due to adipose cell deposition in the patient's liver or muscles.

Nakrani, Mihir N., Robert H. Wineland, and Fatima Anjum. "Physiology, glucose metabolism." (2020).

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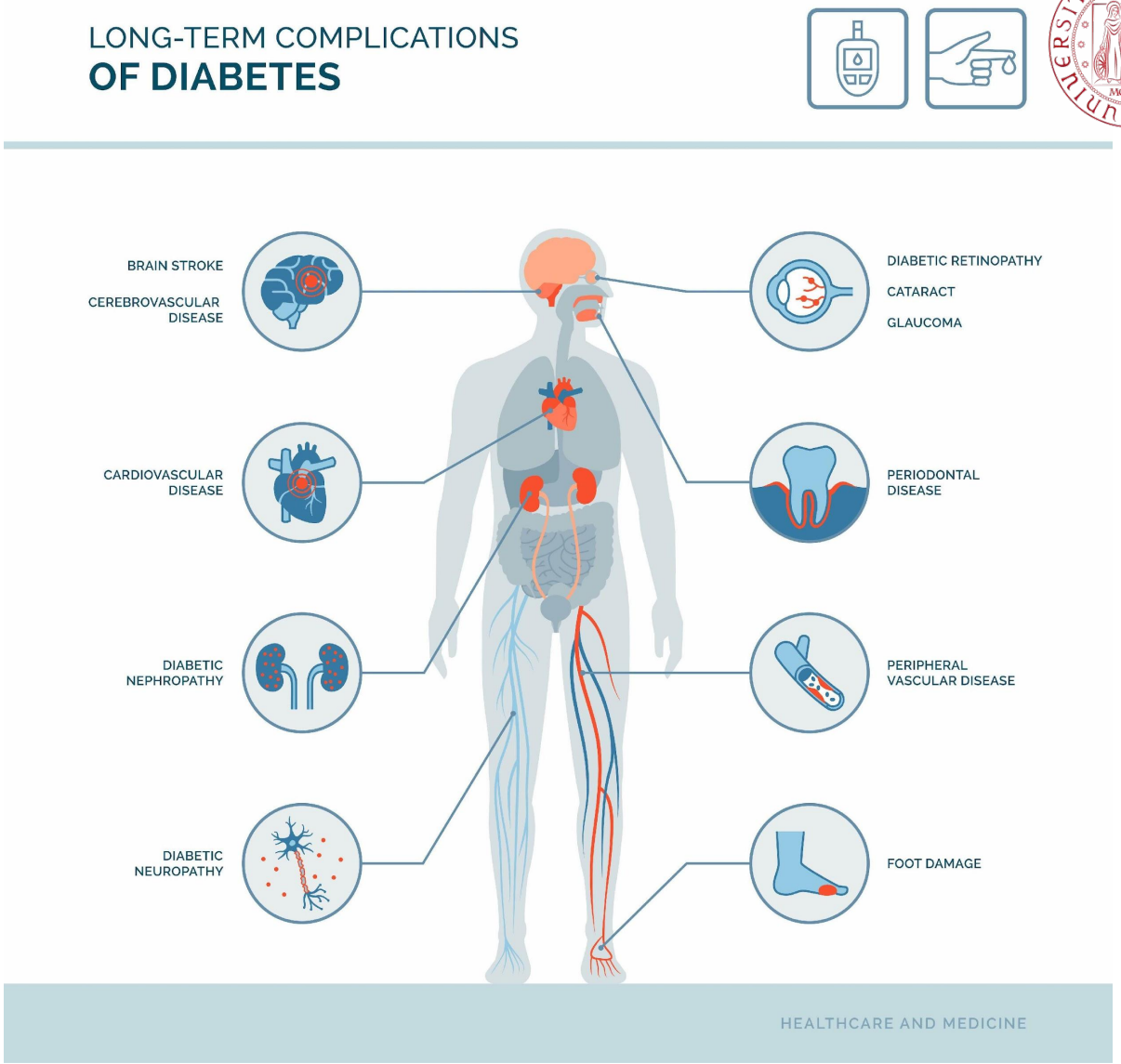
Diabetes: key facts

- About **500 million** people suffer from diabetes worldwide.
- In 2019, diabetes and kidney disease due to diabetes caused an estimated 2 million deaths.
- The major causes of blindness, kidney failure, heart attacks, stroke, and lower limb amputation.



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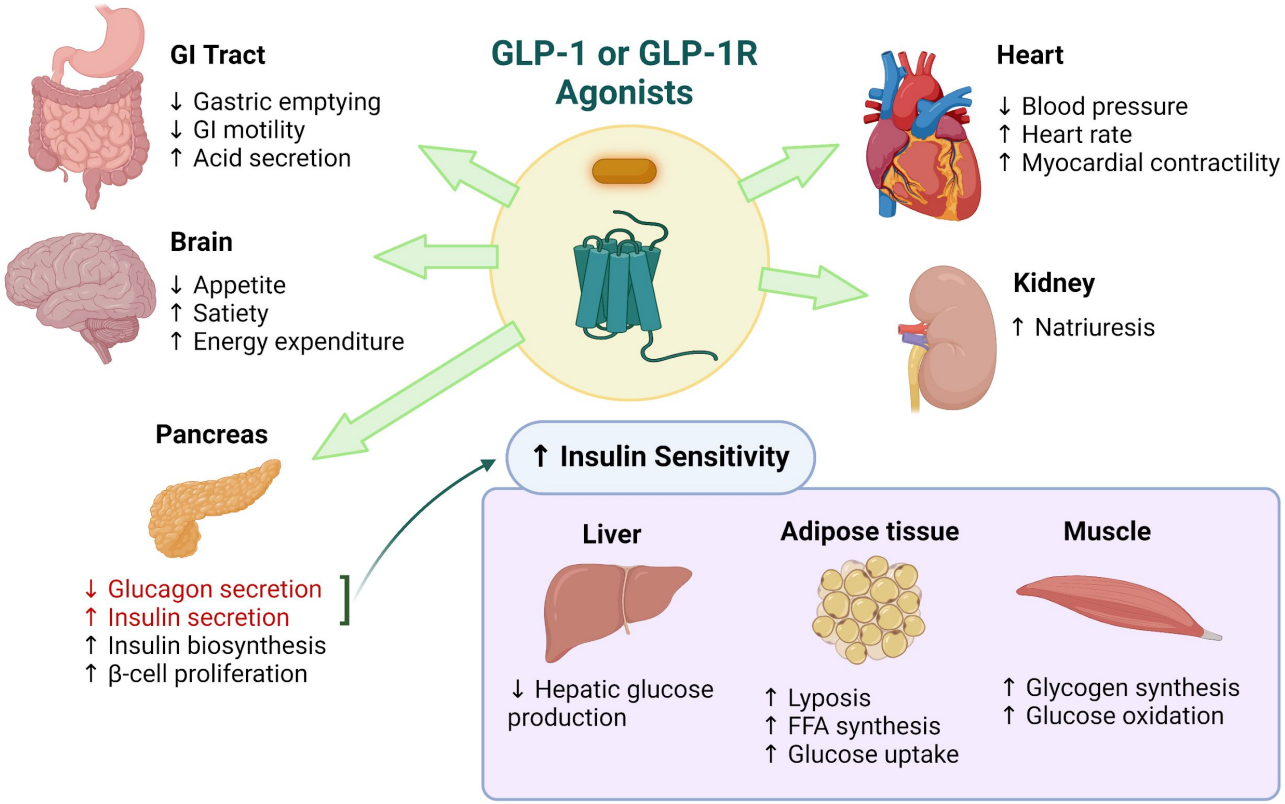
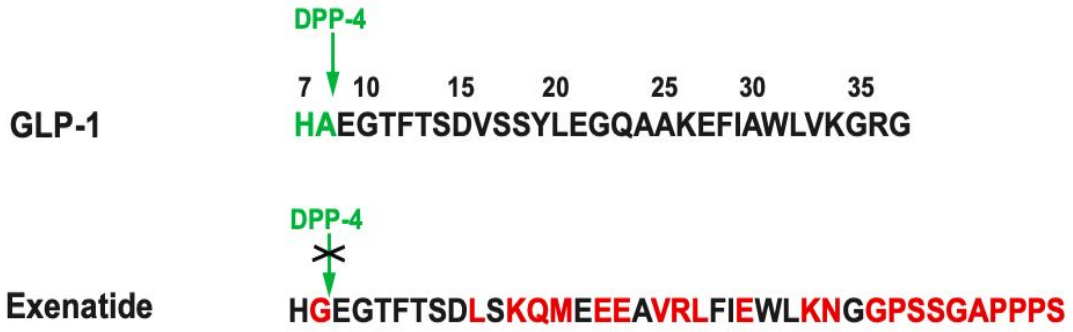
Type 2 diabetes: GLP-1 agonist treatment



Saraiva, F. K., & Sposito, A. C. (2014). *Cardiovascular Diabetology*, *13*, 1-11.

Structures of native GLP-1 and Exenatide



Chia, C. W., & Egan, J. M. (2009). *Diabetes, metabolic syndrome and obesity: targets and therapy*, 37-49.

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Treatment: s.c. vs oral administration

Semaglutide is a GLP-1 agonist that is 94% identical to the natural human GLP-1.

Semaglutide

Rybelsus and Ozempic

Subcutaneous administration

Once a week

Cost per month **$1235.9**

Price for injector (2mg/1.5 mL): **$308.97**

Oral administration

Once a day

Cost per month **$927**

Price for 14 mg tablet: **$30.90**

https://emedz.net/rybelsus-ozempic-semaglutide

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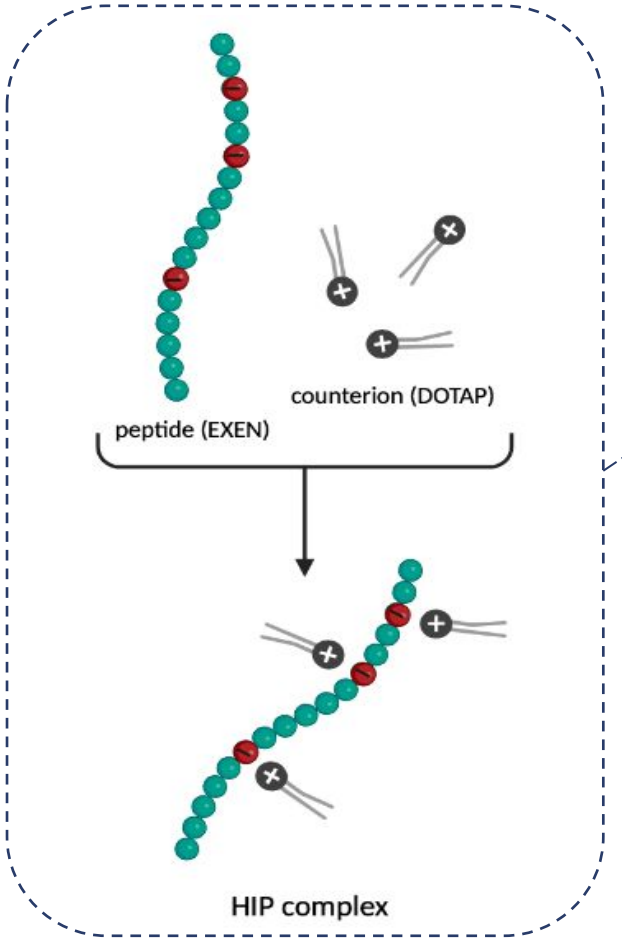
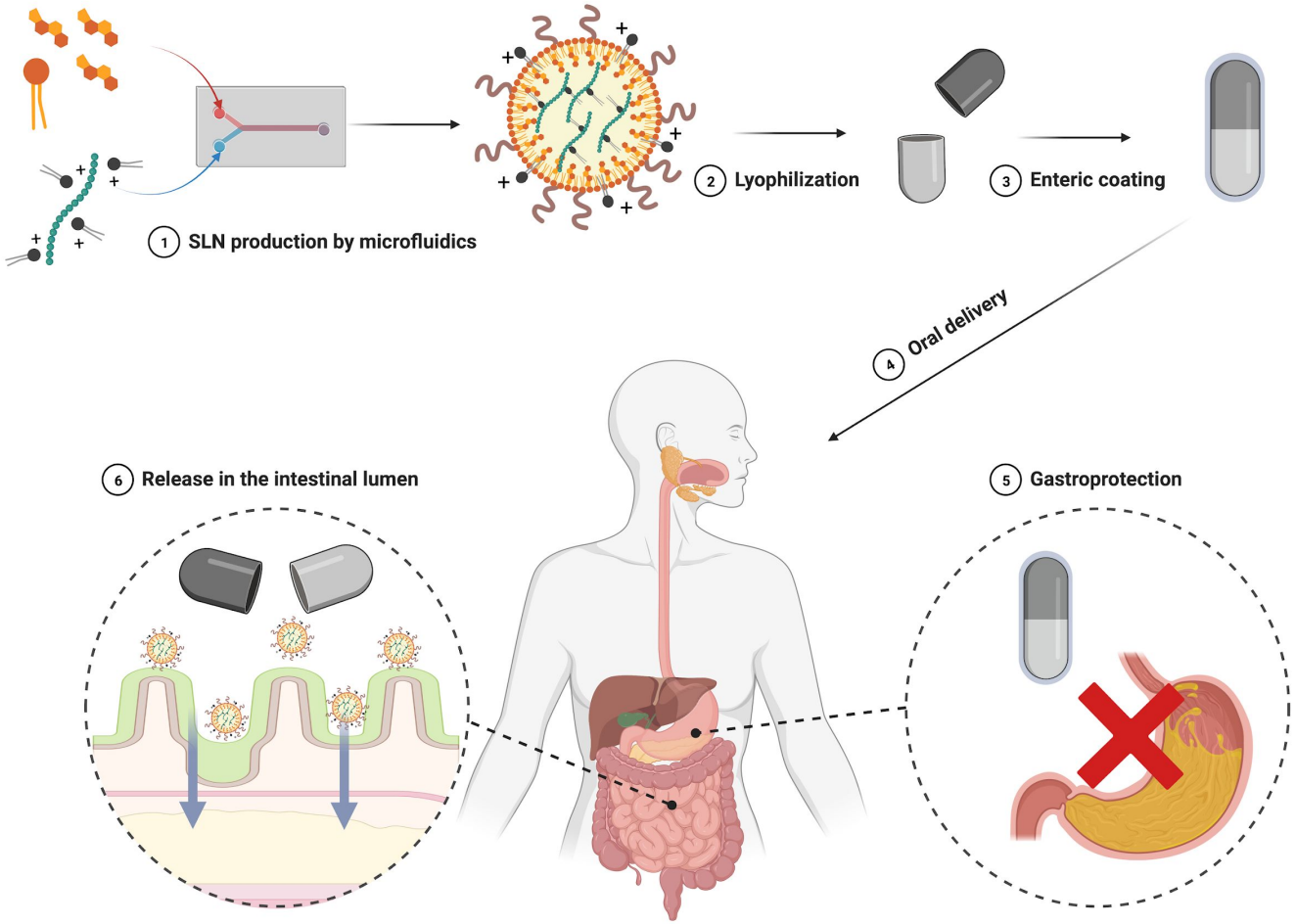
Oral delivery platform: nano in macro





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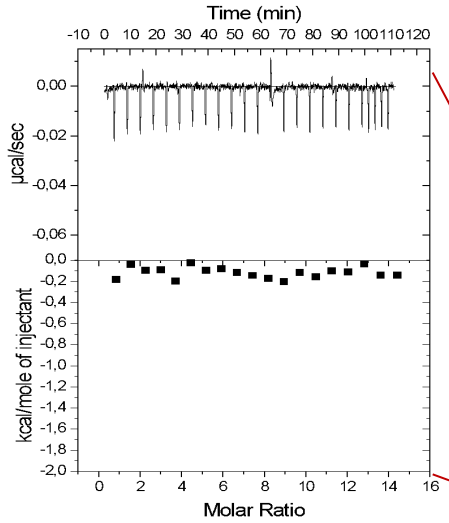
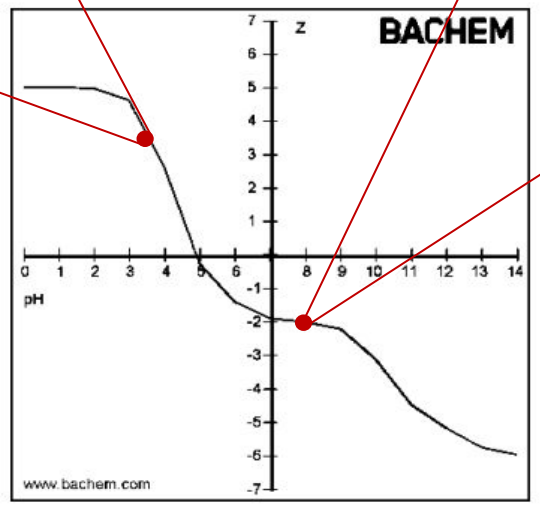
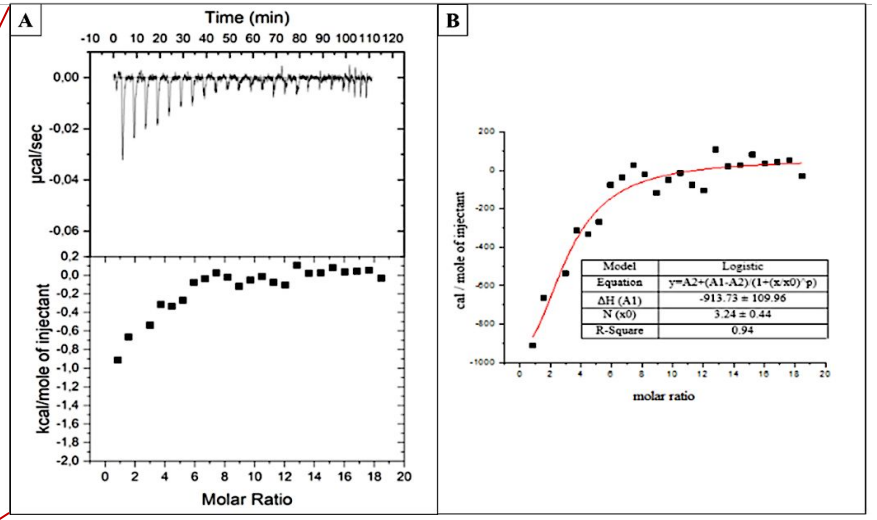
DOTAP/EXEN interaction by ITC



Microcalorimetric titration profile of DOTAP liposomes and EXEN solution at **pH 3.5**





Microcalorimetric titration profile of DOTAP liposomes and EXEN solution at **pH 8**

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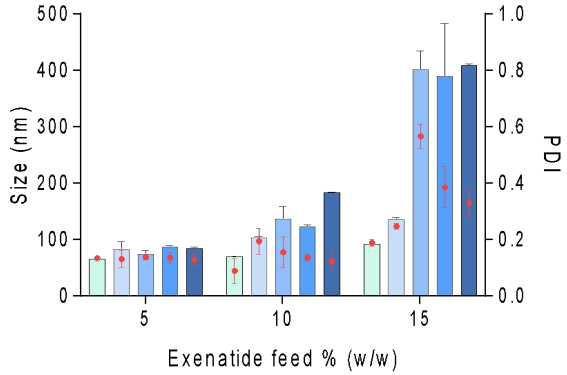
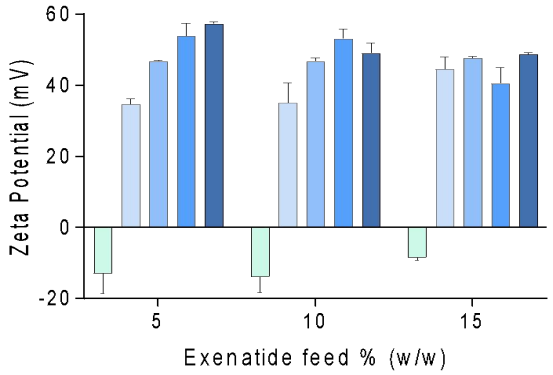
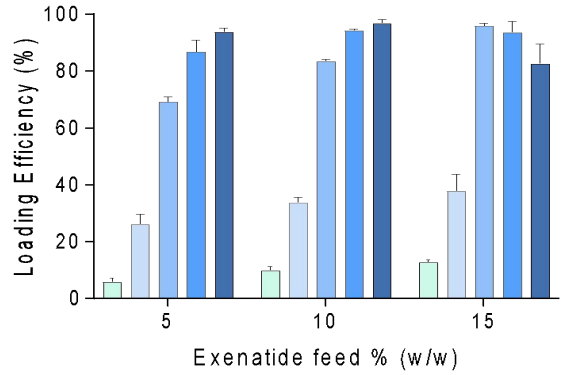
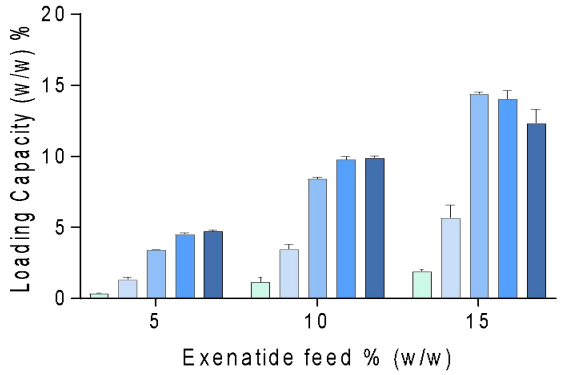
SLNs production and characterization









0:1 DOTAP/EXEN 2:1 DOTAP/EXEN 6:1 DOTAP/EXEN 12:1 DOTAP/EXEN 18:1 DOTAP/EXEN

<u>Selected parameters</u>

12:1 (mol/mol) DOTAP/EXEN
10% (w/w) EXEN/Total lipid feed

<u>Formulation Characteristics</u>

Particle size: ≈120 nm
PdI: <0.2
Zeta potential: ≈53 mV

Loading efficiency: >90%
Loading capacity: ≈10% (w/w)

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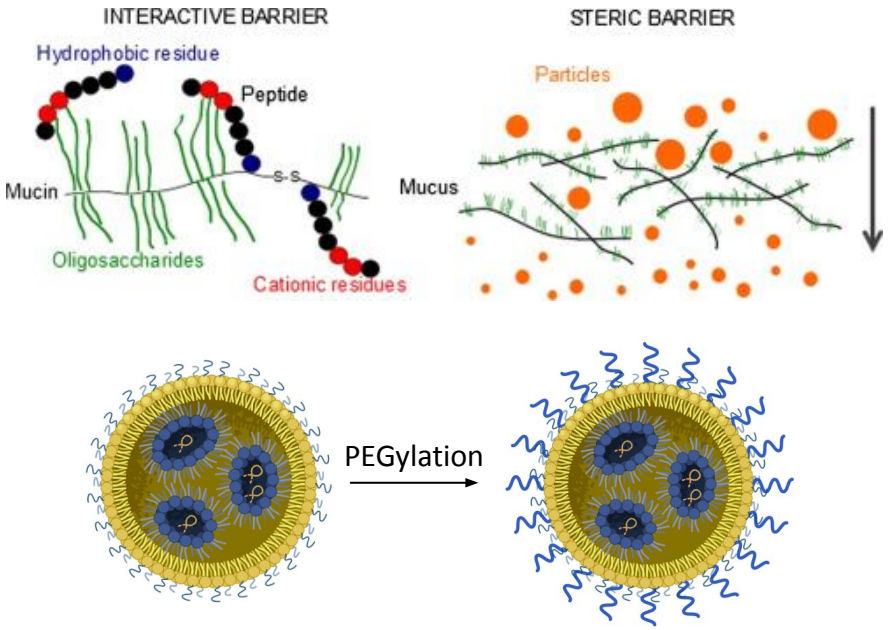
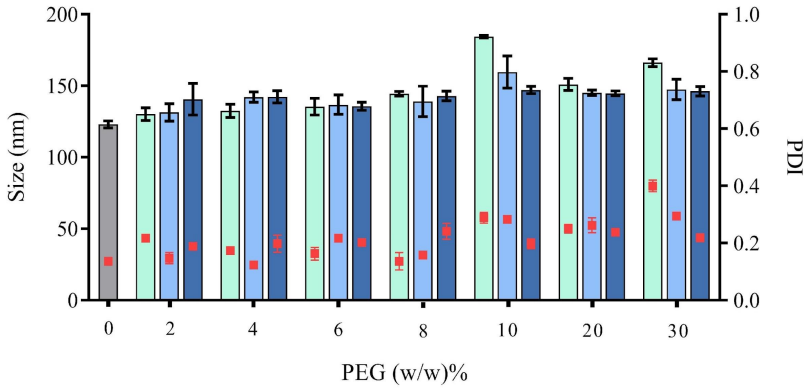
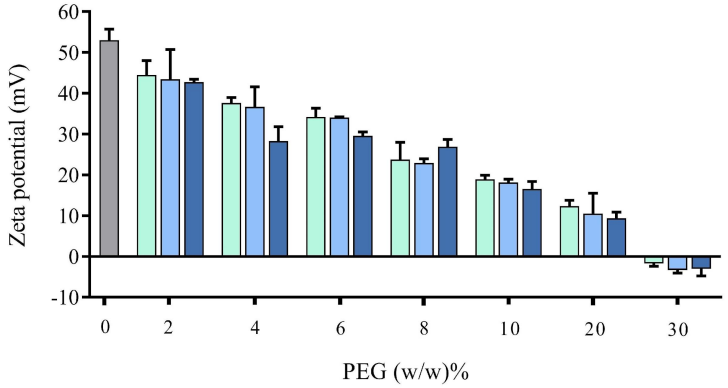


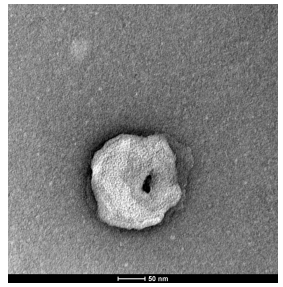




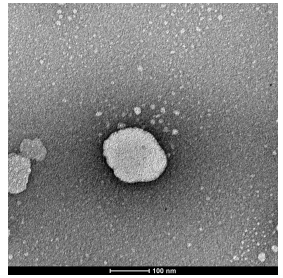
2 mg/mL SLNs with 2 - 30 % (w/w) DSPE – PEG_{2kDa} incubated at 37° C,

300 rpm for 5 min 10 min 15 min

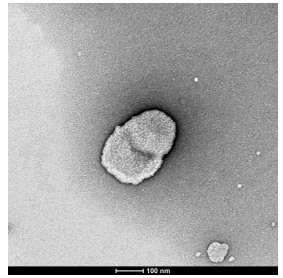
SLN-0% PEG



SLN-10% PEG



SLN-30% PEG



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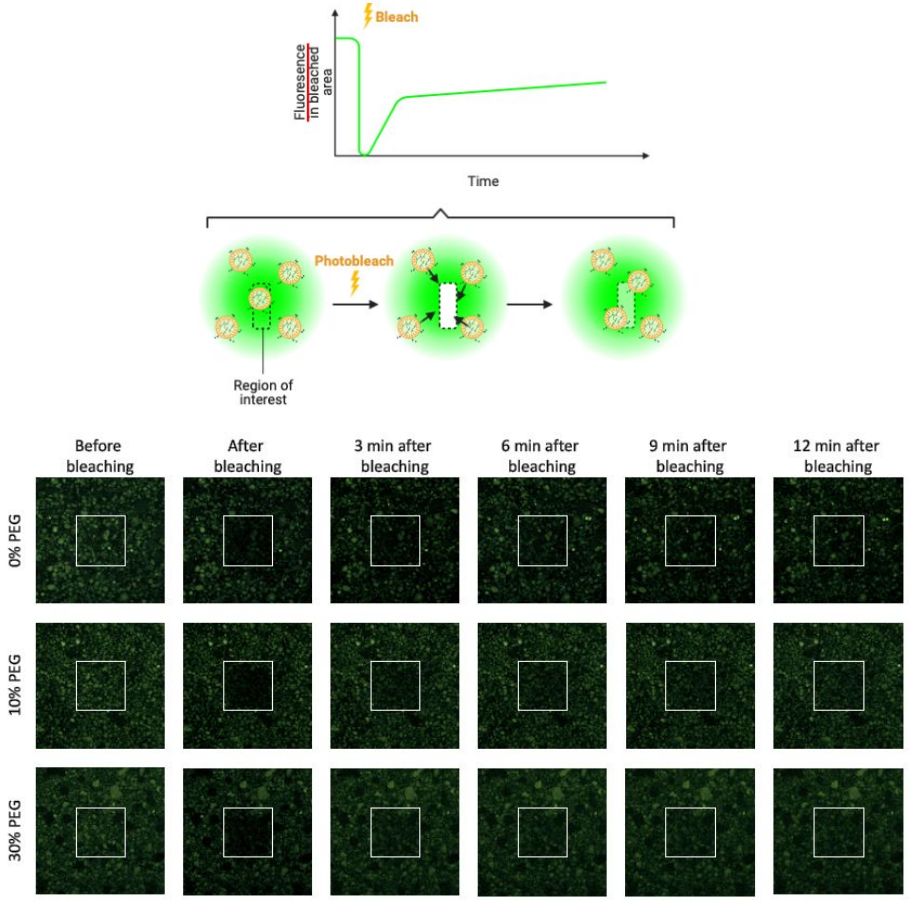
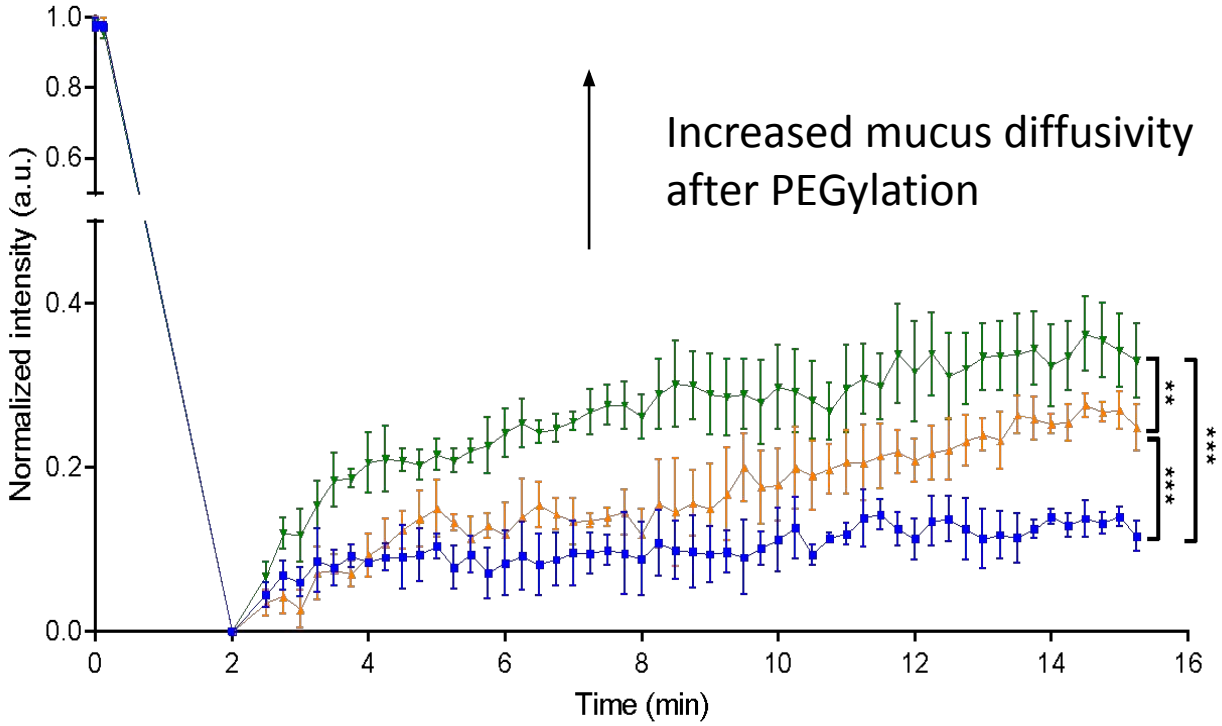
Effect of PEGylation on mucus diffusivity





SLN-0% PEG (–), SLN-10% PEG (–) and SLN-30% PEG (–)
p<0.01,*p<0.001

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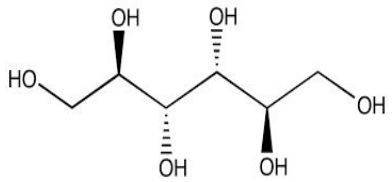
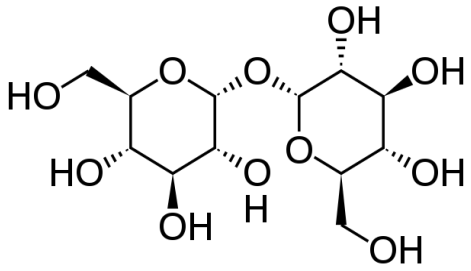
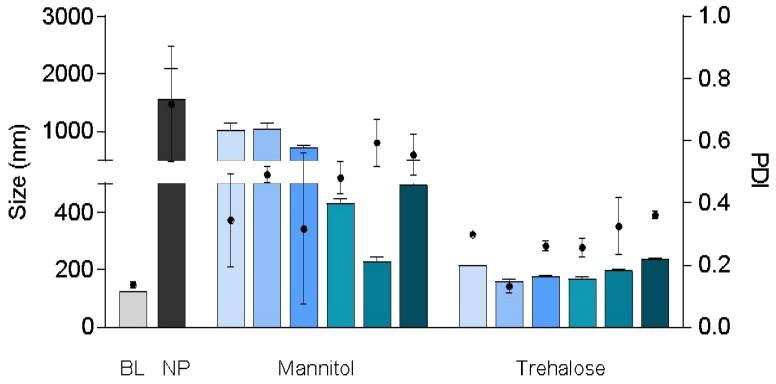
Freeze drying of the SLNs



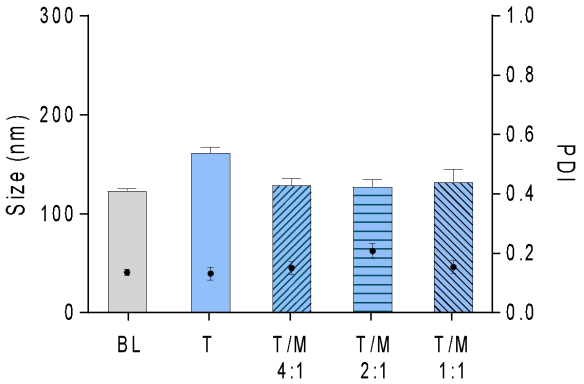
Mannitol



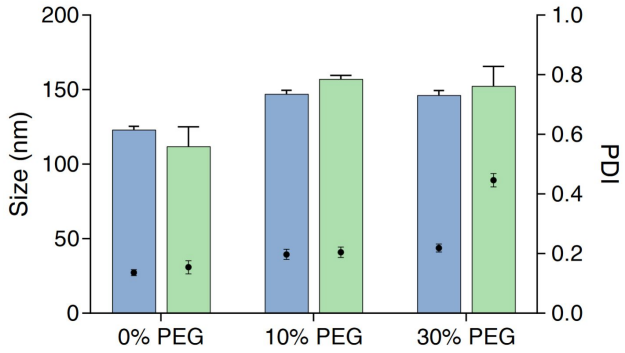
Trehalose



before lyophilization BL, ■
after lyophilization without cryoprotectant (no protectant, NP ■),
with 0.5 (■), 1 (■), 2.5 (■), 5 (■), 7.5 (■) and 10 (■) % w/v mannitol and trehalose concentrations.



The size and PDI profiles of the EXEN-loaded SLNs before lyophilization (■) and after lyophilization with 1 % w/v trehalose (■) and trehalose/mannitol mixtures (4:1, 2:1 and 1:1 w/w)



The size and PDI profiles of the EXEN-loaded SLNs with 0, 10 and 30 % (w/w) PEG before (■) and after (■) lyophilization with 2 % w/v lyoprotectant (1/1 T/M w/w).

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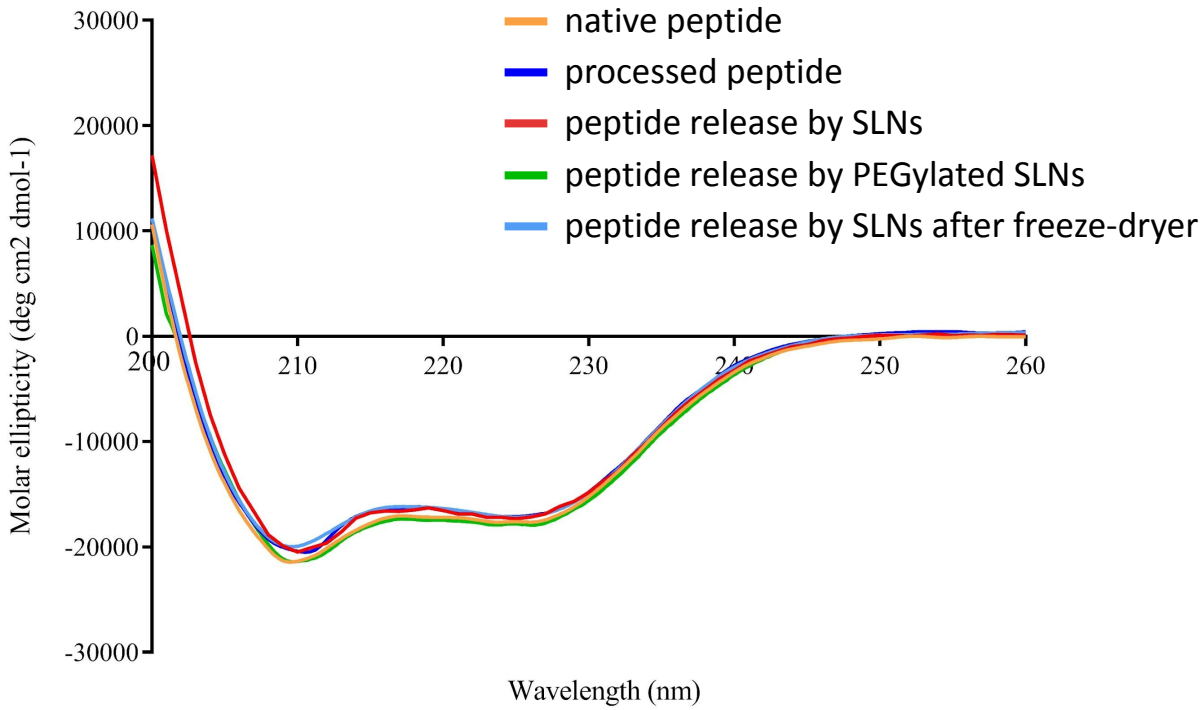
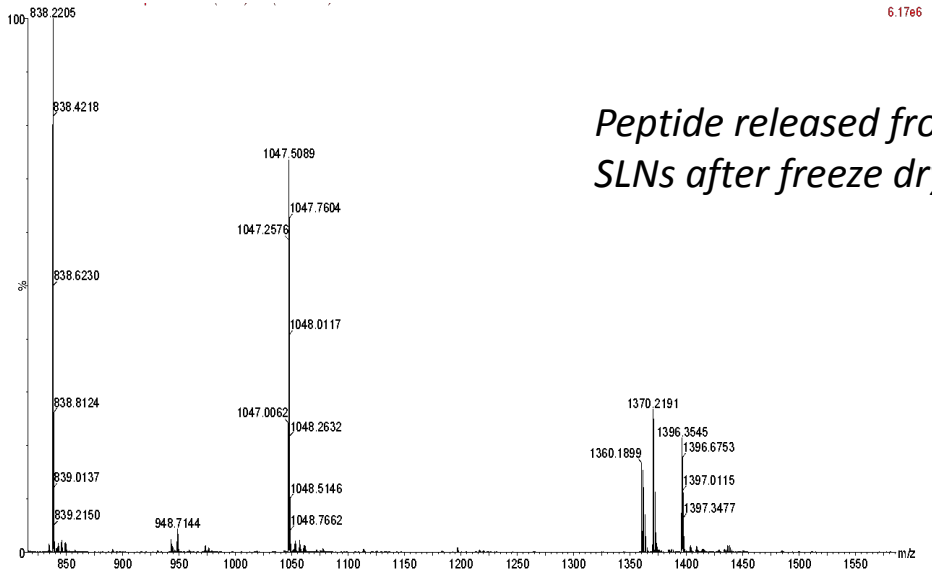
Peptide stability

Conformational stability by CD analysis



Chemical stability by ESI-TOF MS analysis



Peptide released from SLNs after freeze drying

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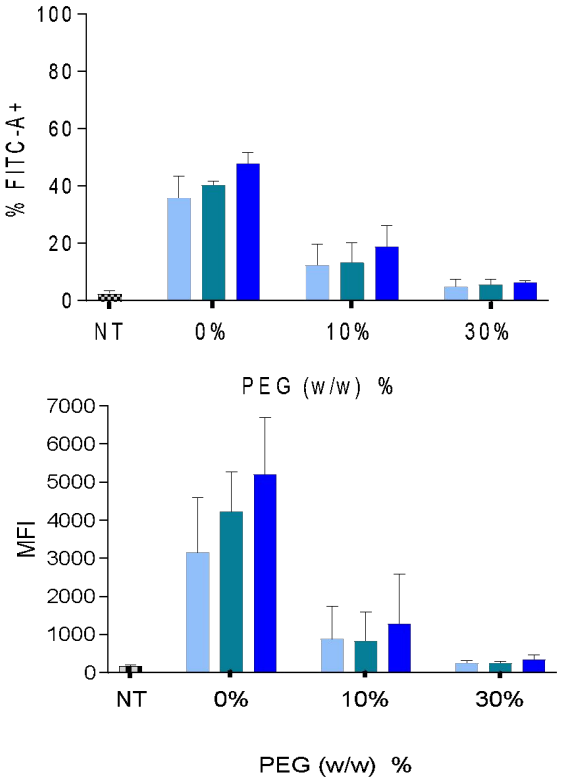
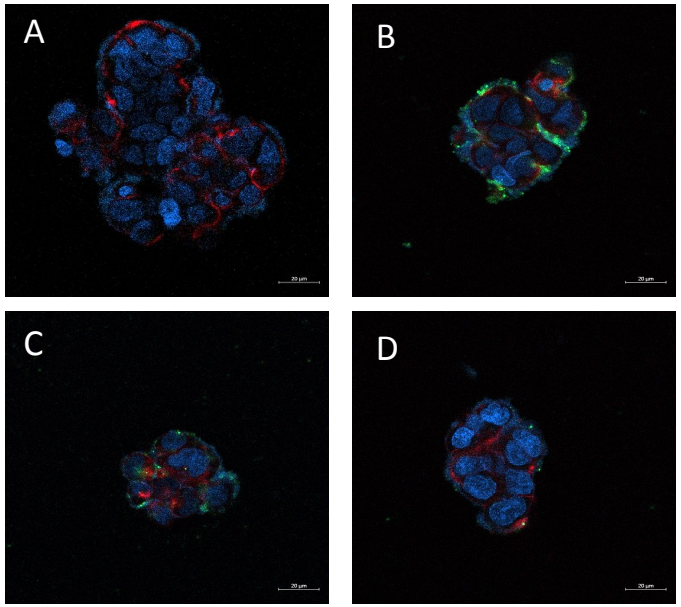
Cellular studies

Cellular association



Not treated cells (■)
SLNs with 6 µg/mL EXEN (■)
SLNs with 8 µg/mL EXEN (■)
SLNs with 10 µg/mL EXEN (■)

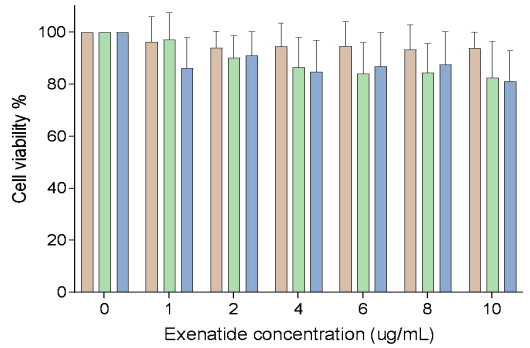


Untreated Caco-2 cells (A) and those after 4 hours of incubation with fluorescein-DHPE labeled SLNs decorated with 0 (B), 10 (C) and 30 (D) % (w/w) DSPE-PEG_{2kDa} at EXEN concentration of 10 µg/mL.

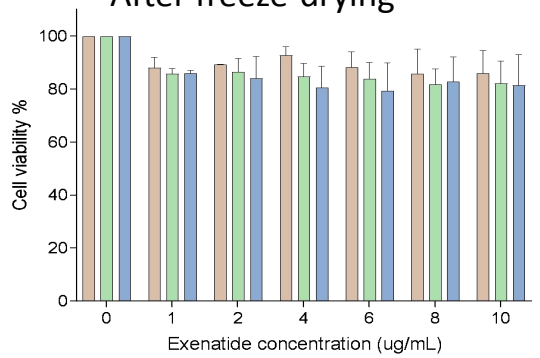
Nuclei are shown in ■, cell membranes are in ■, SLNs are in ■

Cytotoxicity

Before freeze-drying



After freeze-drying



The cell viability profile of the Caco-2 cells treated with EXEN-loaded SLNs (SLN-0% PEG (■), SLN-10% PEG (■) and SLN-30% PEG (■)) at increasing EXEN concentration

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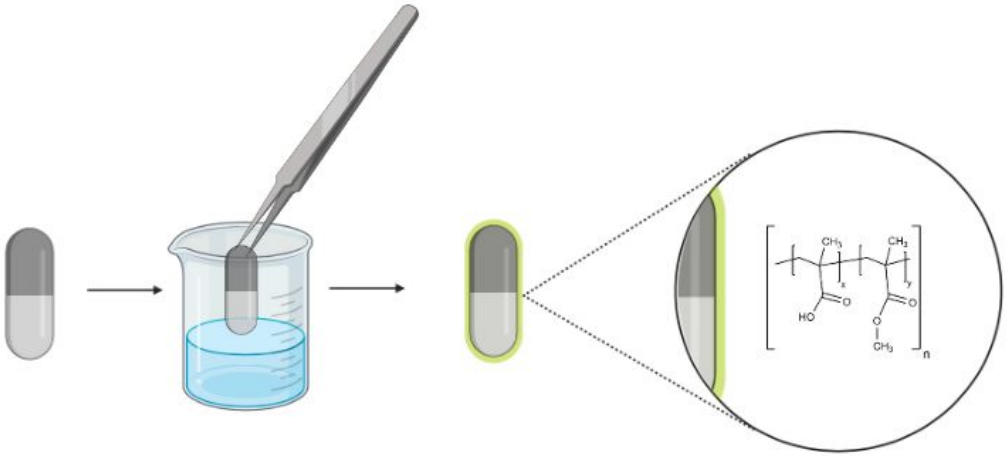
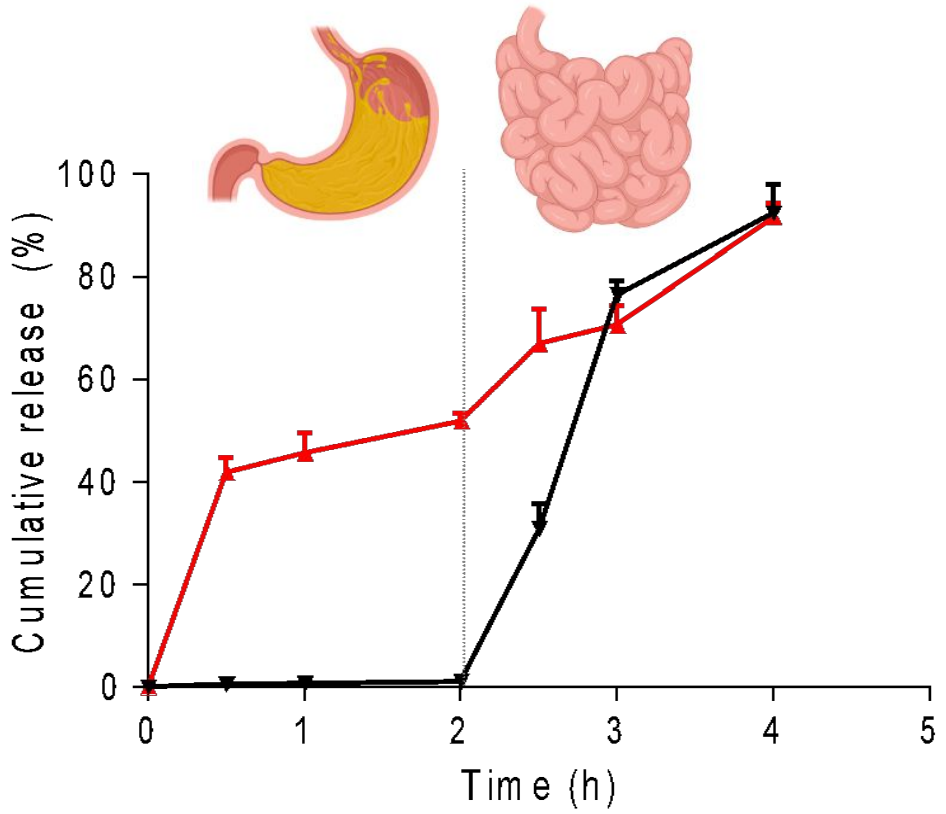
Enteric coating of macro formulation





Release profiles of the not coated (−) and coated (−) capsules filled with starch and Rhodamine B in pH 1.2 HCl (0-2h) and pH 6.8 SIF (2-4h)

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