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TOXICOLOGICAL STUDIES OF POLY (ANHYDRIDE) NANOPARTICLES FOR ORAL DRUG DELIVERY

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NANOTOXICOLOGY

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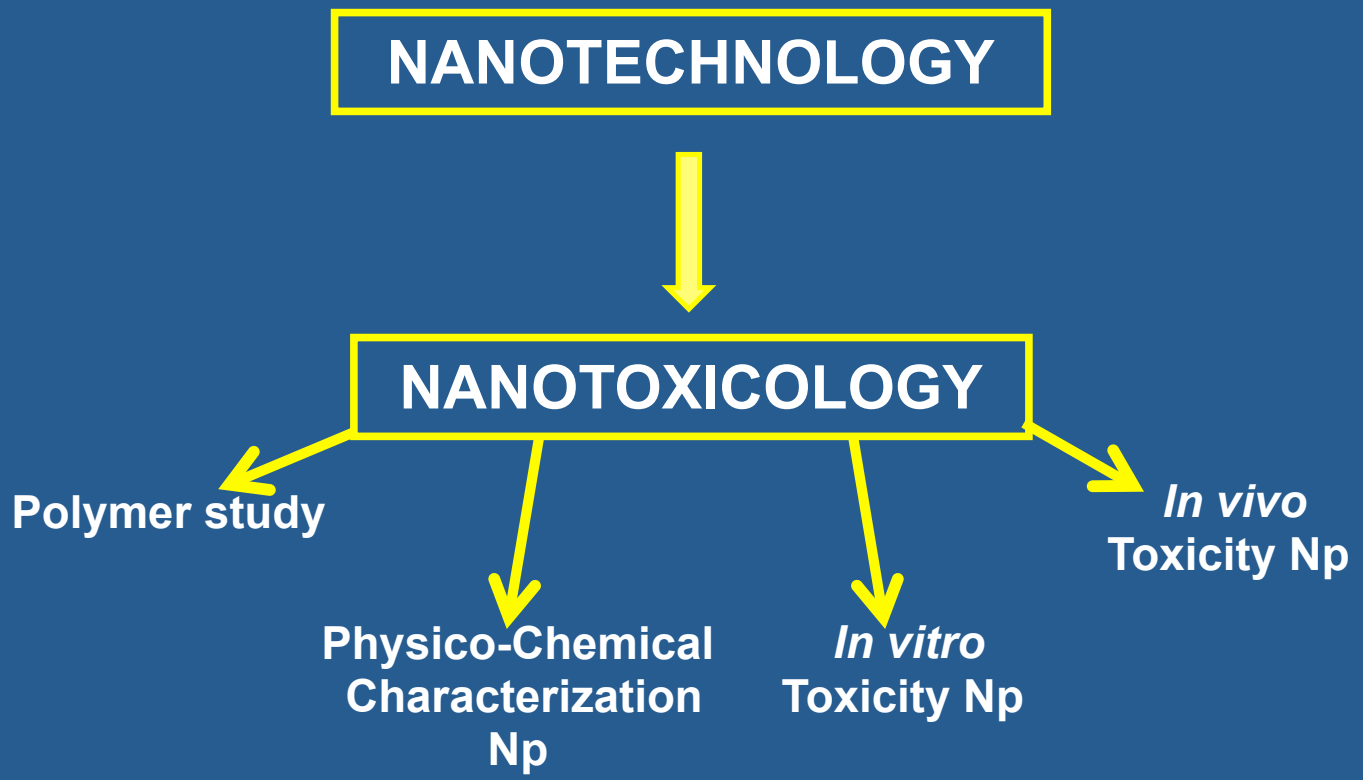

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NANOPARTICLES

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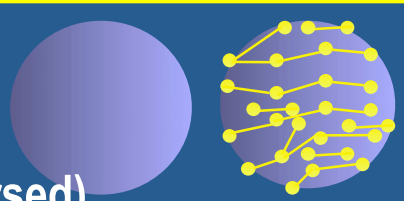
Solid particles colloidally dispersed sized between 1 and 1000 nm

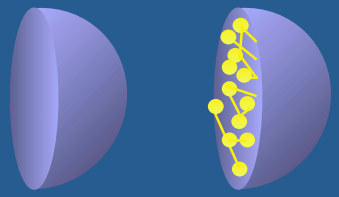
NANOSPHERES

(Drug uniformly dispersed)



NANOCAPSULES

(Encapsulated drug)



APPLICATIONS

Drug delivery:

↓ dose administration

Minimize drug degradation

↑ efficacy

ORAL DRUG DELIVERY

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Poly (anhydride) GANTREZ® AN 119

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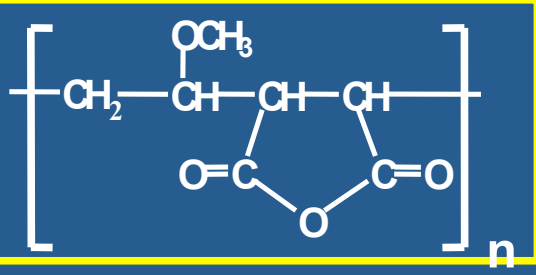
GANTREZ® AN 119

- Poly (methyl vinyl ether - co - maleic anhydride)
- Synthetic and biocompatible
- Low cost



PHYSICO-CHEMICAL PROPERTIES OF GANTREZ® AN 119

- Specific viscosity (1% MEK): 0.1 - 0.5
- Tg: 152 ºC
- Viscosity of 5% w/w solution at 25ºC: 15 mPas

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Aplications of GANTREZ® AN 119

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PHARMACEUTICAL APPLICATIONS

- **Dental adhesives**

  high quality bioadhesive performance

- **Controlled-release coatings, enteric coatings and ostomy adhesives**

  Excellent film-forming properties

- **Transdermal patches, toothpastes, mouthwashes and transdermal gels**

  Thickeners, complexing agents and hydrophilic colloids

- **Specific bioadhesive ligand-nanoparticle conjugates for oral drug delivery**

  Fabrication of nanoparticles for oral drug delivery: Spanish patent, Arbos, P. et al, 2002

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Poly (anhydride) nanoparticles

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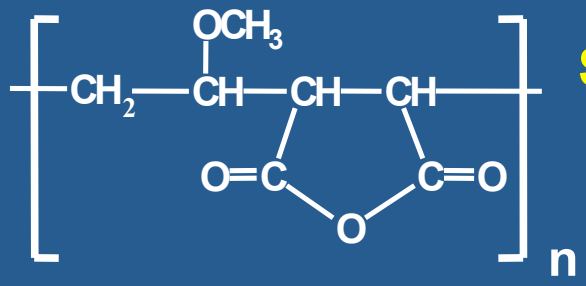
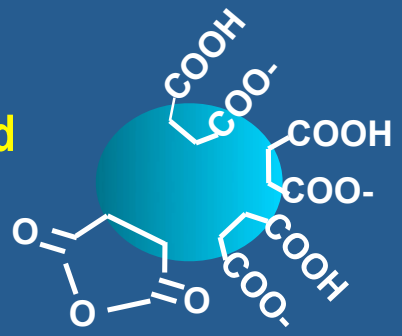
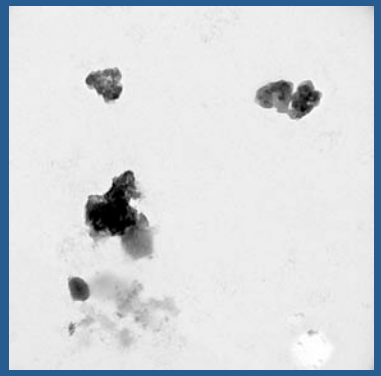


Solvent Displacement Method

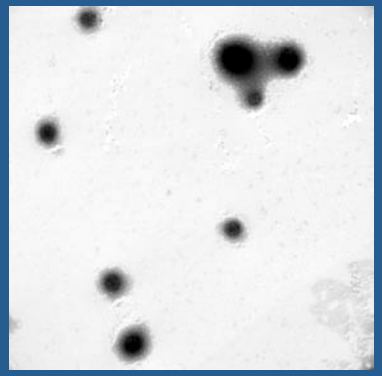


Gantrez® AN 119 polymer

Gantrez® AN 119 Nanoparticles



Gantrez® AN 119 x12500



Gantrez® AN 119 Nanoparticles x 5000

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OBJECTIVES

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- Preparation of different types of nanoparticles:
  - ✓ Conventional nanoparticles (NP)
  - ✓ Pegylated nanoparticles (PEG-NP)
  - ✓ Cyclodextrin nanoparticles (HPβCD-NP)
- Physico Chemical characterization of nanoparticles prepared:
  - ✓ Size
  - ✓ Surface charge
  - ✓ Shape
  - ✓ Stability
- Evaluate the cytotoxicity of Gantrez® AN poly (anhydride) nanoparticles by MTS assay using the Hep G2 cell line

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Types of poly (anhydride) nanoparticles

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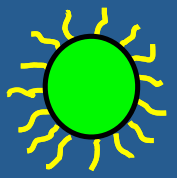
- **Conventional nanoparticles (NP)**

↑ Bioavailability of the presystemically metabolised drug

- **Pegylated nanoparticles (PEG-NP)**

↓interaction of nanoparticles with components of the lumen



- **Cyclodextrin nanoparticles (HPβCD-NP)**

↑ Loading capacity of lipophilic drugs in the nanoparticles

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Types of poly (anhydride) nanoparticles

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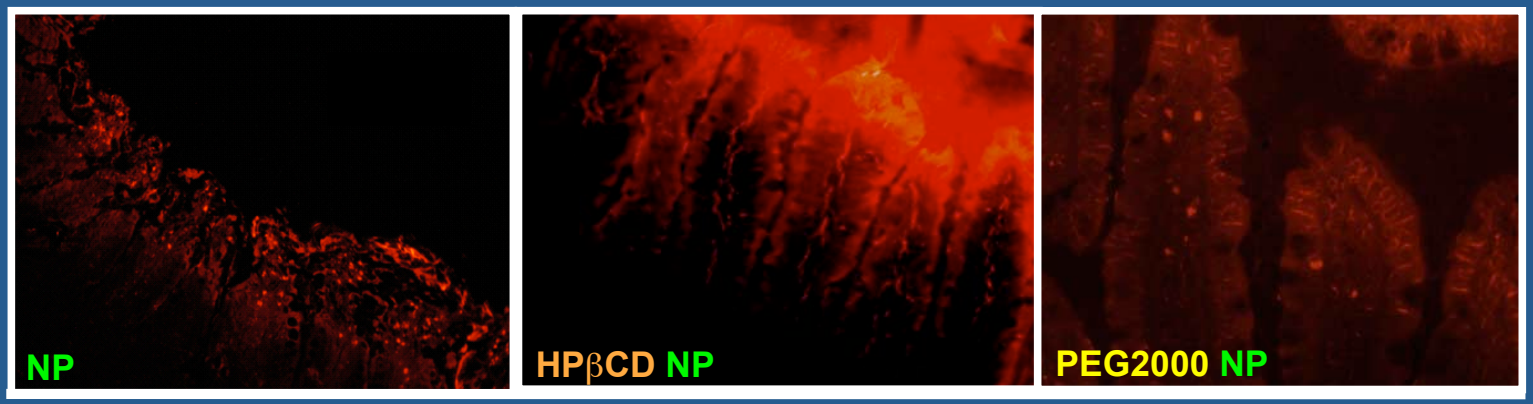
Fluorescence microscopic visualization



- Poly (anhydride) nanoparticles (**NP**) displayed a restricted location at the mucosa, mainly on the mucus layer of the ileum, and a low ability to cross this barrier
- Nanoparticles associated to hydroxypropyl-β-CD (**HPβCD NP**) and pegylated nanoparticles (**PEG2000 NP**) distributed homogeneously along the ileum mucosa and show high ability to establish bioadhesive interactions.

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Nanoparticle preparation

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Solvent Displacement Method

1

Gantrez® AN
+
PEG 2000
ACETONE
(incubation 1 hour)

Evaporation
EtOH / Water

Purification
Centrifugation

5 % sucrose
Liophilization

PEG2000-NP

2

Gantrez® AN
ACETONE
+
HP-β-CD
water

Water

Excipient
Spray-Drying

Mini Spray Dryer

NP

HPβCD-NP

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Physico-Chemical Characterization

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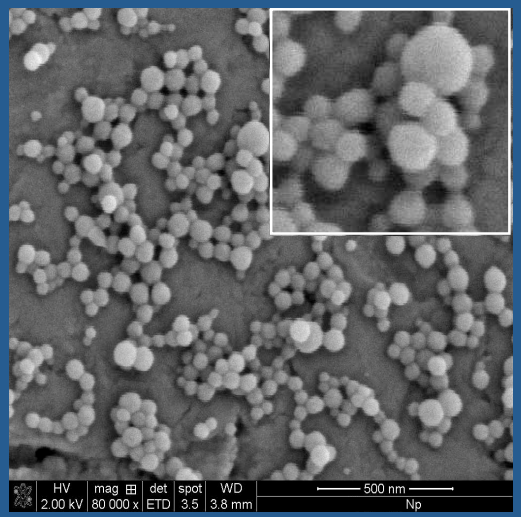
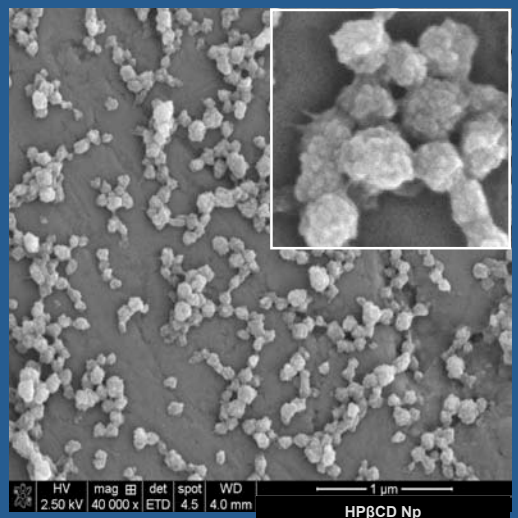
	Size (nm)	Zeta Potential (mV)	µg ligand/mg Np
NP	192 ± 2	-56.8 ± 0.5	
HPβCD-NP	168 ± 1	-52.8 ± 0.5	219.3 ± 3.2
PEG 2000-NP	205 ± 1	-57.1 ± 0.7	25.7 ± 0.5

Mean values ± SD (n=6)

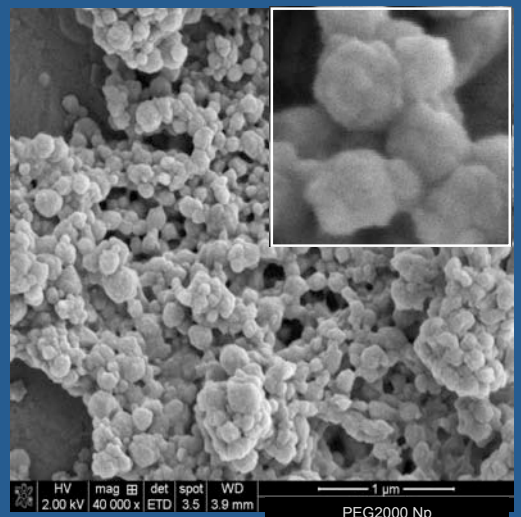
NP SEM IMAGES



NP



HPβCD-NP



PEG 2000-NP

In vitro toxicity

In cytotoxicity of nanoparticles it is important to :

- **Develop the complete physico-chemical characterizacion of the nanoparticles**
- **Choose cell cultures sensitive to changes in their environment**
- **Controlling the experimental conditions**

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Stability studies

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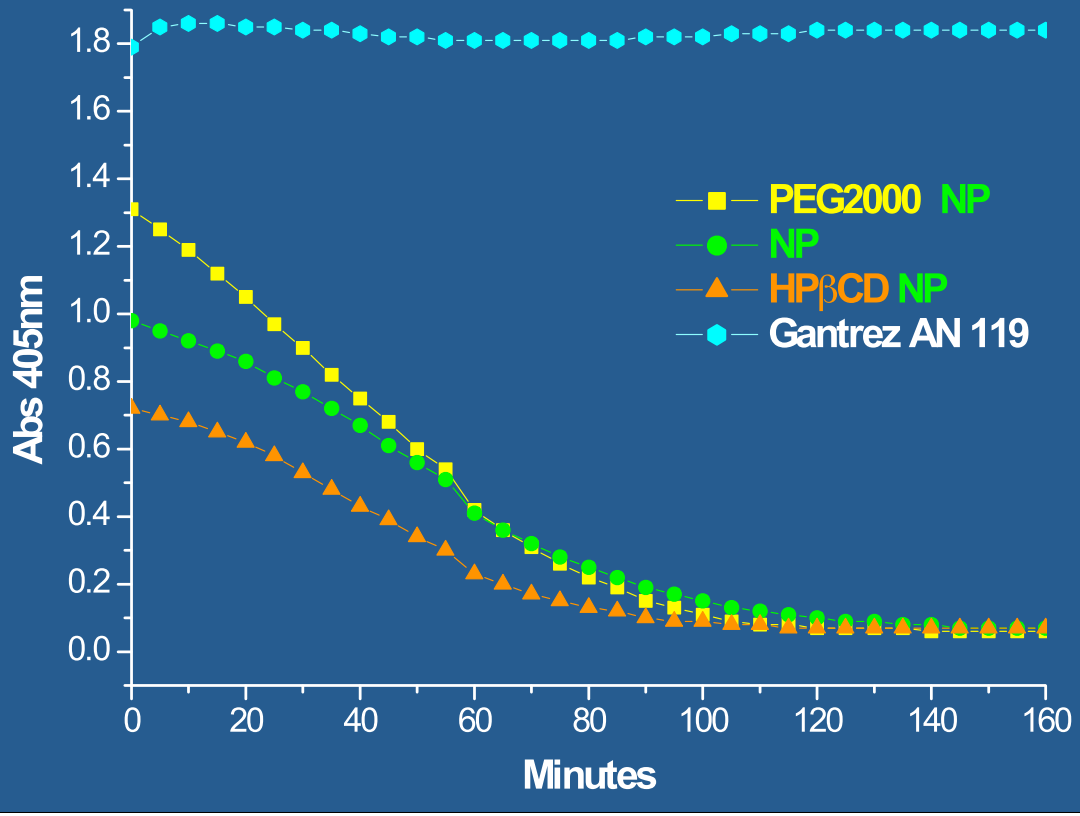
NANOPARTICLES STABILITY

- Measure Turbidity change as a function of time
- 15 mg NP/mL DPBS w/o Ca^{++}/Mg^{++}
- $t_{1/2}$ = 50 minutes



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In vitro toxicity: MTS ASSAY

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- Cell line: **Hep G2** (Human liver adenocarcinoma)

Oral route

Sensitive to damage

- Cell viability: **MTS ASSAY**

("CellTiter 96® Aq_{ueous} Non-Radioactive Cell Proliferation Assay")

- Concentrations tested: **0.0625 – 0.125 – 0.25 – 0.5 - 1 and 2 mg/mL**

- Incubation time: **30 minutes, 3, 24, 48 and 72 hours**

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In vitro toxicity: MTS ASSAY

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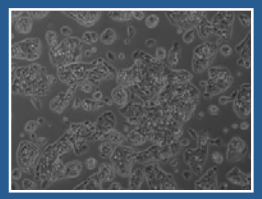
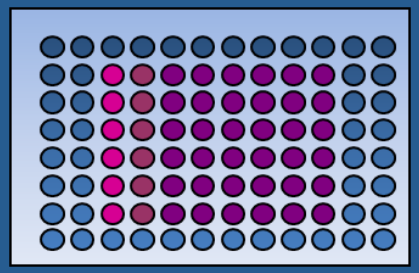


Hep G2 cells

Cultured in 96 well-plates



2 x 10^4 cs/well

Nanoparticles addition

- (NP)
- (HPβCD-NP)
- (PEG 2000-NP)
- (Gantrez ® AN polymer)

Cell-Nanoparticles Incubation

- 30 minutes
- 3 hours
- 24 hours
- 48 hours
- 72 hours

MTS ASSAY

Measure 96 well-plates at 490 nm

% Cell Viability

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MTS results: Cell Viability

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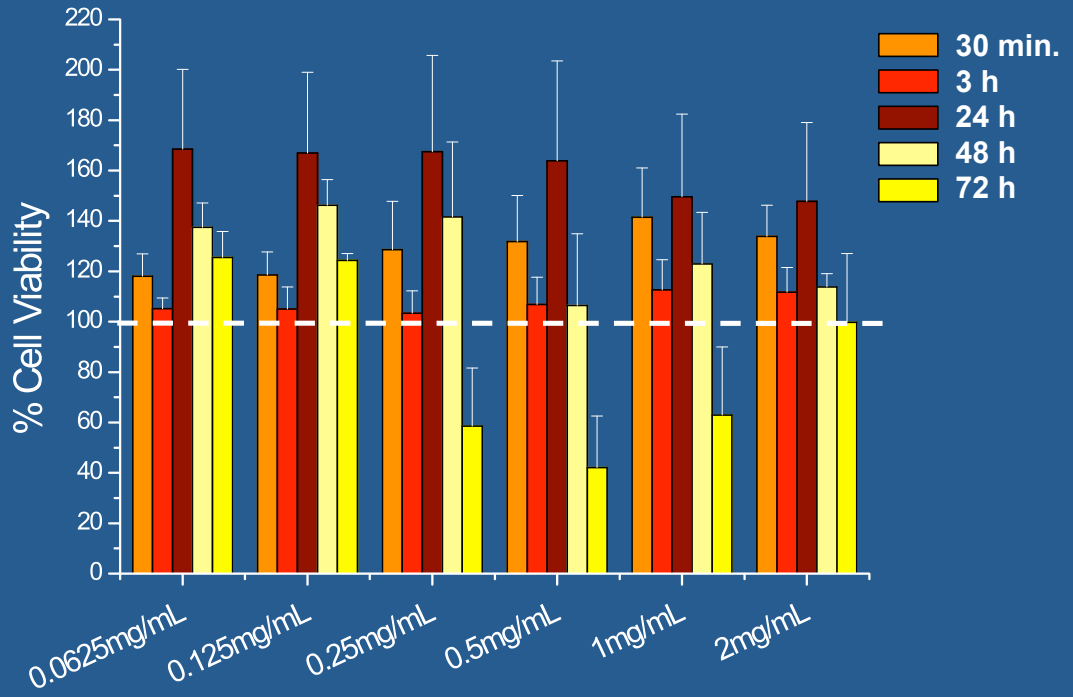
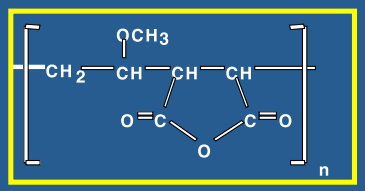


GANTREZ AN 119 Concentrations



- Cell viability increased more than 100%
- No dose-response relation was observed
- These results suggested polymer interference with the assay

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Hep G2 Images

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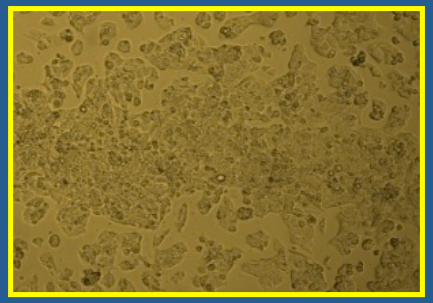
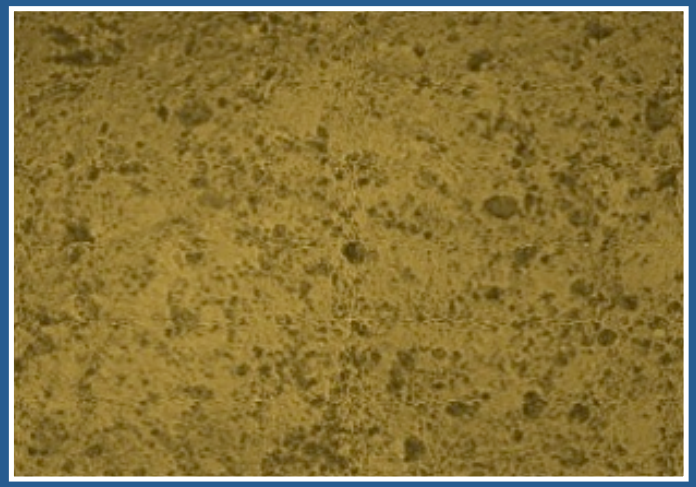
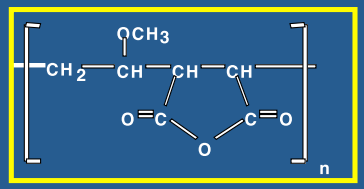
CONTROL



POLY (ANHYDRIDE) GANTREZ® AN 119





For the poly (anhydride) Gantrez® AN, the morphology of the cells could not be observed probably due to the deposition of the polymer on the cells similar to a coating

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MTS results: Cell Viability

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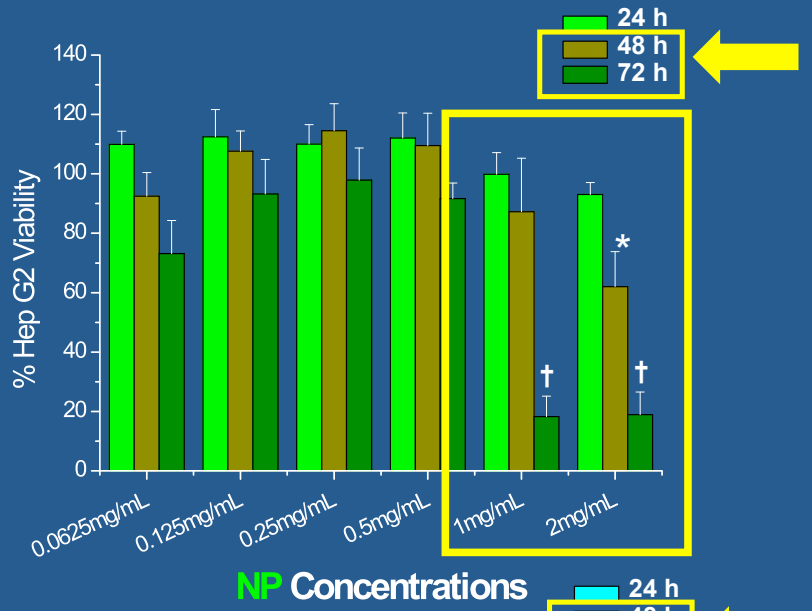

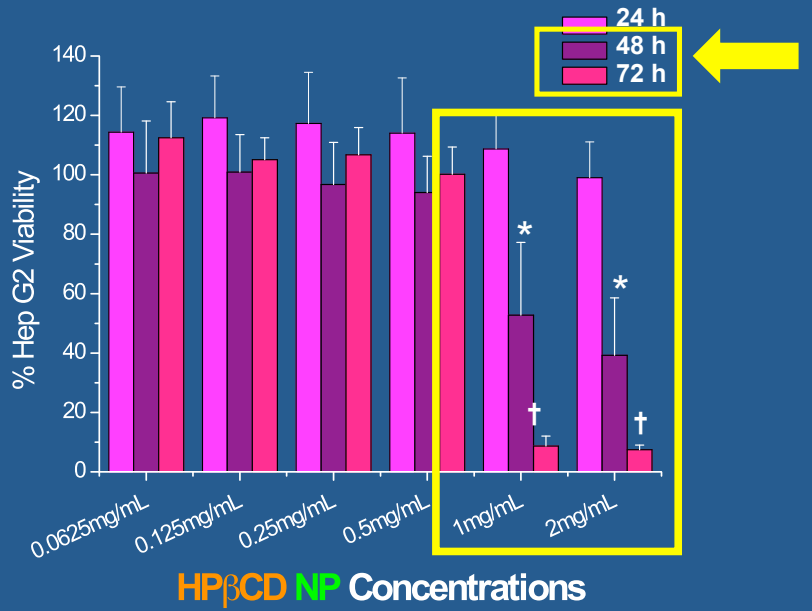

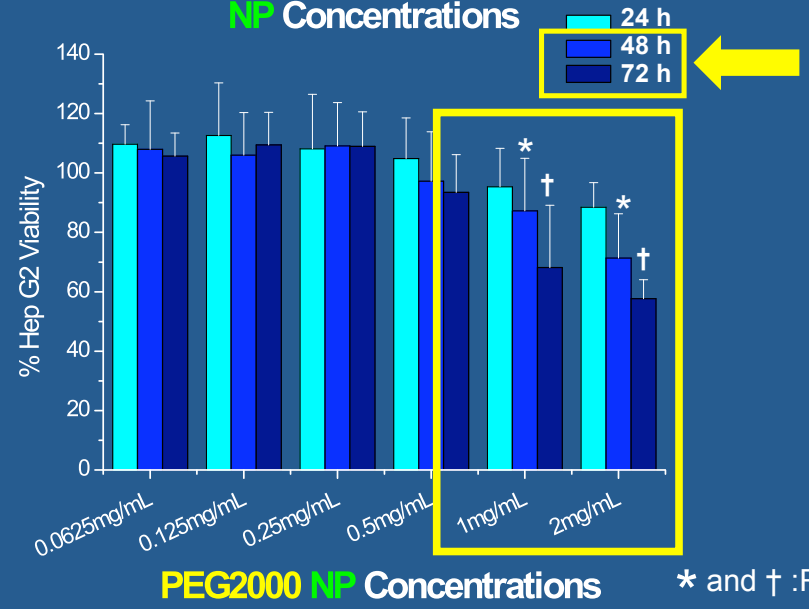

- 30 min, 3 and 24 hours no significant decrease was observed
- 48 and 72 hours a significant cytotoxic effect was observed at the highest concentrations tested (1, 2 mg/mL)

* and † :Results were significant at 0.05 level (Kruskal Wallis-U-Mann Whitney)

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Hep G2 Images

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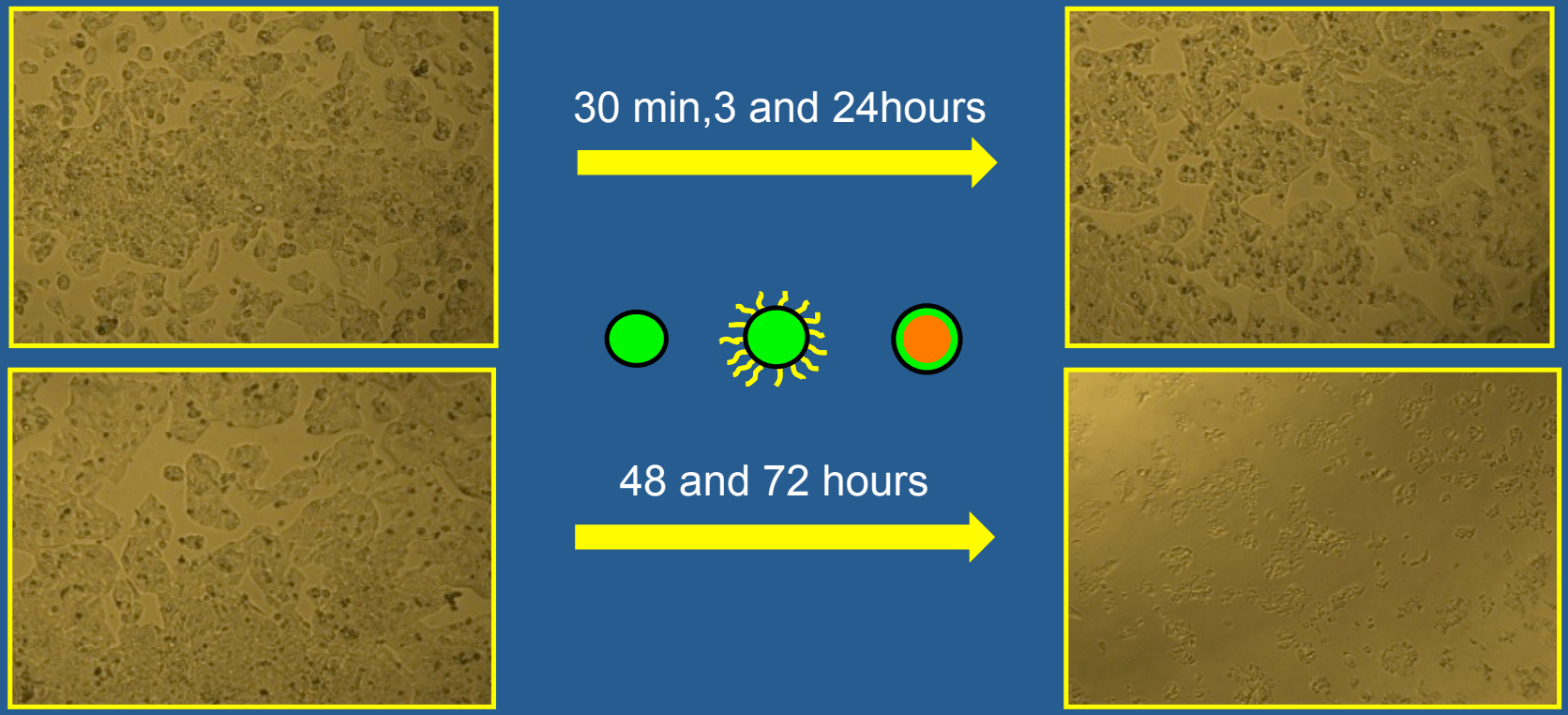

Incubation times of 30 minutes, 3 and 24 hours did not affect the morphology nor the growth of Hep G2 cells.

On the contrary, at 48 and 72 hours, at the highest concentrations tested morphologycal changes and decrease in cell viability were observed.

pH VALUES

INCUBATION TIMES	Poly (anhydride) Gantrez® AN 119 NP	HPβCD NP	PEG2000 NP	DMEM
30 min	6	6.5	7	7
3 h	6	6	6	6.5
24 h	6	6	6	6.5
48 h	6–7.5	7.5	7.5	8
72 h	6–7	7.5	7.5	8

No pH changes were appreciated in culture media as a consequence of the nanoparticles and polymer addition at the different incubation times tested

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Conclusions

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- The nanoparticles were prepared by two methods. The poly(anhydride) nanoparticles displayed a size of approximately 190 nm, a negative surface charge and a spherical shape with a homogeneous size distribution.

- The cytotoxicity studies demonstrated that the poly(anhydride) nanoparticles did not show any toxic effect in Hep G2 cells at 30 minutes, 3 and 24 hours.

- In contrast, at 48 and 72 hours significant cytotoxic effects were observed for 1 and 2 mg/mL nanoparticles concentrations.

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Acknowledgements

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  (Fundación Caja Navarra)

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