Design and Synthesis of MicellarThermometric Systems based on Amphiphilic Block Copolymers-Lanthanide Complexes Conjugates

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**Rafael Piñol** 

NanoTBTech Webminar. Friday, 11<sup>th</sup> December.



INSTITUTO DE NANOCIENCIA Y MATERIALES DE ARAGÓN

# **Intracellular Temperature Mapping**

Intensity (arb. units)

540

580

620

Wavelength (nm)

660

Local magnetic hyperthermia



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# Self assembly of amphihilic block copolymer in water



Amphiphilic block copolymers are formed by covalently bonded hydrophobic and hydrophilic blocks that can self assembly in a selective solvent (water) into different morphologies able to encapsulate hydrophobic species improving the stability and solubility in aqueous environments.



**Intrinsic Factor** 

The packing Parameter is influenced by the balance of the hydrophilic and hydrophobic block  $f_{hydrophilic}/f_{hydrophobic}$ 

The packing Parameter increase with the size of the Hydrophic Block

Molecular Weight (Mw) Degree of Polymerization (DP)

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The RAFT process involves conventional free radical polymerization of a substituted monomer in the presence of a <u>suitable</u> chain transfer agent (RAFT agent or CTA)







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#### Control over the molecular architecture

Chains can be extended un addition of further monomers Living Character: Block Copolymers

### Wide Range of Monomers

(meth)acrylates, (meth)acrylamides, styrenes, dienes, vinylmonomer, acrylonitrile CTA-DEPENDENCE





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#### **Commercially available RAFT agents**

cover close to all the monomer classes that undergo radical polymerization



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#### Versatile/ Milder reaction conditions

Applicable to all modes of free radical polymerization, Bulk, Organic or Aqueous Solution, Emulsion, Mini-emulsion, Suspension.



Dithiocarbamate



The RAFT process involves conventional free radical polymerization in the presence of a suitable chain transfer agent (RAFT agent or CTA)

**Chain Transfer Agent** Dithioester

Trithiocarbonate Xanthate

Dithiocarbamate

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(CTA)

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Control over the Molecular weight/Composition

$$Mw_{theo} = \frac{[M]}{[CTA]} \times [Mw_{monomer}] Conv(\%) + Mw_{CTA}$$

**Conv %:** Percentage Monomer Conversion (<sup>1</sup>H-NMR, GS) [M]: Monomer Concentration **[CTA]:** Concentration of CTA agent [**Mw**<sub>monomer</sub>]: Molecular weigth of the Monomer [**Mw**<sub>CTA</sub>]: Molecular weigth of RAFT agent





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Dithioester

**Chain Transfer Agent** (CTA)

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All chains are initiated at the beginning of the process Chains growth at a similar rate Mw/Mn:1.1-1.2



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 $\begin{array}{cccc} S & S & S \\ R-S & R-S & S \\ \end{array} \\ R-S & R-S & R-S & OR' \end{array}$ 

**Chain Transfer Agent** (CTA)

Dithioester Trithiocarbonate Xanthate

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Dead Chains are independent of conversión



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# **HIGH FUNCIONALITY**

Tolerant towards a wide number of functional groups and Solvents -OH, COOH, -NR<sub>2</sub>, -N<sub>3</sub>, -SOH<sub>3</sub>, -CONR<sub>2</sub>

# Functional Groups can be introduce in CTA agent R and Z

#### Hydrophilic Block. Control in the Molecular weight/ Molecular Weight Distribution/Architecture



# **Amphiphilic Block Copolymer**

# Hydrophilic Block. Control in the Molecular weight/ Molecular Weight Distribution/Architecture



70%MOEG(M)A 500 Da

30% OEG(M)A 480 Da

Amphiphilic Block Copolymer

**COMB-LIKE block** 

High density of chains

\*Increase the steric stability of the hydrophilic

corona of the nanoaggregates by interdigitacion of the PEG chains

\*High density of functional groups at the surface



#### **Control in the Molecular weight/ (Composition-Functionalization % of different monomer)**



## **Control in the Molecular weight/ Composition (Hydrophilic/Hydrophobic balance)**



### **Control in the Molecular weight/ Composition (Hydrophilic/Hydrophobic balance)**



# Synthesis of Thermometric Polymer-Lanthanide Complex Conjugates (Eu/Sm)



Improve the stability and Efficiency of Encapsulation vs Physical Encapsulation

Easy Purification and Characterization vs Direct Polymerization (Polymerizable LnL<sub>3</sub>PhenA)

Higher reproducibility in Mn and [In]% vs Direct Polymerization. Independent of reactivity or Solubility (LnL<sub>3</sub>PhenA)

"Library" of Different NT using Different Lanthanide Complexes or Using different Eu/Sm %



# Characterization of the Polymer Lanthanide Conjugates. Coordination of the LnL<sub>3</sub> complex to the Polymer Backbone

# <sup>1</sup>H-NMR&FTIR Confirms the coordination of the LnL<sub>3</sub> complexes



# Characterization of the Polymer Lanthanide Conjugates. Coordination of the LnL<sub>3</sub> complex to the Polymer Backbone



# Influence of the Method and The Processing Parameters on the Size of Polymeric Micelles





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# Influence of the Method and The Processing Parameters on the Size of Polymeric Micelles



Mn, Molecular Weight of the Polymer **f** Hydrophobic/Hydrophilic Balance Stifnness, Glass Transition Temperature (Tg) **Hydrophobicity** [Pol]<sub>o</sub> Initial Polymer Concentration Viscosity and Polarity of the Organic Solvent [Vol%] Final Concentration of Organic Solvent (v: ml/min) Rate of Addition (rpm) Rate mixing Stirring (T<sup>a</sup>) Temperature Universidad Zaragoza **署CSI** 

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# Synthesis of the Thermometric Luminiscent Micelles by Cosolvent method. Solvent Effect





Solvents with lower viscosity mix faster with water, which causes more uniform supersaturation, leading to smaller micelles.

CONTRACTOR CONTRACTOR

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# Synthesis of the Thermometric Luminiscent Micelles by Cosolvent method. Addition Rate





#### **Control In the size of the Micelles**

# Micelles Characterization STEM (MA/DNPD)

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#### Micellar Colloidal Stability after 6 months. Chemical Stability



Good Steric Stabilization (Z(mV):-2mV) NaCl 10 mM)



FTIR Detachment of the complexes was not observed



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Micelles Was diluted in different buffers (1:10) and incubated at RT for 24 h

Monomodal distribution histograms . Aggregation was not observed



### Micellar Colloidal Stabilty vs T<sup>a</sup> of MA/DNPD based Polymeric Micelles





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#### **Photoluminescence studies**

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Thermometric analysis was performed in the RT-55 C temperature range based on a change in the intensity of the  ${}^{5}D_{0}-{}^{7}F_{2}$  (Eu<sup>3+</sup>) and  ${}^{4}G_{5/2}-{}^{6}H_{5/2}$  (Sm<sup>3+</sup>) transitions



## Which is the best NTH?



	BTFA	DNPD
Quantum Yield (%)	3.5	0.2
Max Relative Sensitivity (%K <sup>-1</sup> )	1.5% K <sup>-1</sup> (at 328 K) <sup>1</sup>	1.7 % K <sup>-1</sup> (at 328 K)
δ Temp.	6 mK	0.20 K
uncertainty	(at 328 K)	(at 305 K)
Brightness (M <sup>-1</sup> cm <sup>-1</sup> )	17-740	0.40-32



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#### **Optical properties of DNPD based polymeric micelles in different Media**



Micelles were diluted in different buffers (1:10) and incubated at RT for 24 h

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# **Micelles Characterization Dilution Effect (DLS-Emission)-CMC**





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# Cell Viability and Cell Uptake Studies (MA/DNDP)



#### MDA-MB-468 breast cancer cell line



DMEM 24 h at 37°C



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# Cell Viability and Cell Uptake Studies (MA/DNDP)



The percentage of internalized micelles was estimated by ICP-OE, analyzing the concentration of lanthanides present in the recovered cell culture medium (10-15%)

#### Flow Citometry-Lanthanide emission



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#### **Real-time intracellular temperature MA/DNPD**



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# **Acknowledgements**

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# Intracellular Temperature

Local magnetic hyperthermia



#### **Cell Viability and Internalization Studies**



Low Toxicity Effect and similar internalización rates was obserbed for different micellar compositions



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#### Synthesis of the Second Generation of MNH-LNT for Cell Internalization





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![](_page_46_Figure_0.jpeg)

#### Synthesis of the Second Generation of MNH-LNT for Cell Internalization

![](_page_47_Figure_1.jpeg)

### Local MNH temperature gradients in aqueous suspensions/ in cell magnetic Hyperthermia

Measurement of Local Temperature on the surface of magnetic cores with high temporal resolution.

![](_page_48_Figure_2.jpeg)

![](_page_48_Figure_3.jpeg)

MNH-LNT preserve their thermometric properties in cell culture medium

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![](_page_48_Figure_5.jpeg)

Temperature gradients (5°C) in the MNH-LNT in the interior of MDA-MB468 cancer cells under magnetic hyperthermia

![](_page_49_Picture_1.jpeg)

Amphiphilic block copolymers aggregate in water by association of the Hydrophobic blocks (Hydrophobic effect). This process can lead to a range of morphologies: mostly spheres, rods, sheets or vesicles

![](_page_49_Figure_3.jpeg)

#### **Intrinsic Factors**

Amphiphilic block copolymers can self assembly in a selective solvent (water) and this process can lead to a range of different morphologies mostly micelles, vesicles and planar bilayers

![](_page_50_Figure_2.jpeg)

#### **Intrinsic Factors**

#### **Thermometric analysis**

![](_page_51_Figure_1.jpeg)

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# **Temperature Cycling experiments / Reproducibility.**

![](_page_52_Figure_1.jpeg)

#Cycle

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