Catarina Melim [1], Ivana Jarak [1] , Elisiário Tavares da Silva[1,3,4], Fernanda Roleira[1,3,4], Francisco Veiga[1,2] and Ana Figueiras [1,2,*]

[1] Univ. Coimbra, Faculty of Pharmacy, Polo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal
[2] Univ. Coimbra, REQUIMTE/LAQ, Group of Pharmaceutical Technology, Faculty of Pharmacy, Polo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548, Portugal
[3] Univ. Coimbra, Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Polo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548, Coimbra, Portugal
[4] Univ. Coimbra, CIEPQPF, Centre for Chemical Processes Engineering and Forest Products, Coimbra, Portugal
* Correspondence: rfigueiras@ff.uc.pt; Telef : +351 239 488 400

CIEPQPF
LAQV requimte
LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE
1 2 9 0
FACULDADE DE FARMÁCIA UNIVERSIDADE D COIMBRA

Abstract

Osteosarcoma (OS) is a rare, aggressive bone tumor that impacts mostly children and young adults. Despite numerous therapeutic efforts, OS still presents low patient survival rate, high metastasis, and relapse occurrence. To surpass that, polymeric micelles have been researched for the targeted co-delivery of genetic material and drugs. In this work, mixed polymeric micelles with cationic properties containing polyethyleneimine (PEI), Pluronics® F68 and P123 were prepared. Pluronic® F68 was activated by addition of diacrylate groups and conjugated with PEI. Pluronic® P123 was incorporated in the formulation in a ratio of 2:1 regarding the concentration of Pluronic® F68-PEI and Pluronic® P123. The nanosystems were structurally characterized by FTIR and NMR spectroscopy and the morphology was assessed by TEM. Particle size, polydispersity index (PDI) and zeta potential were assessed by Dynamic and Electrophoretic Light Scattering, respectively. Small-sized, irregularly shaped F68-PEI micelles were obtained, with a PDI of 0.346 and zeta potential of 12.59 mV. Incorporating Pluronic® P123 in the formulation lowered particle size and resulted in spherical micelles. Zeta potential decreased due the presence of Pluronic® P123, but remained positive. These results indicate a stable, small-sized nanosystem, characteristics that suggest a capability to surpass multidrug resistance and perform active targeting towards OS.

Keywords: Osteosarcoma; Pluronic F68; Pluronic P123; Polyethyleneimine; Polymeric Micelle.

IECP 2020

Osteosarcoma

Rare bone disease, high incidence rate in children/young adults;

Prognosis practically unchanged...

- Low patient survival rate
  - Worse scenario in younger patients
    - High disease relapse occurrence

IECP 2020

Osteosarcoma

Challenges to treatment

Multiple Drug Resistance

Most common feature in patients suffering from metastatic OS

↓

More aggressive tumor form or type

IECP 2020

Osteosarcoma

Challenges to treatment

Active targeting

Lack of therapeutic agents capable of specific antitumor activity

↓

Drug concentration is lowered to avoid cytotoxicity to normal cells

New therapeutic options are needed!

IECP 2020

Nanomedicine

Nano-sized materials for therapeutic and imaging purposes

Nanosystems researched against cancer include…

Liposomes, metallic nanoparticles, solid lipid nanoparticles, dendrimers, albumin nanoparticles, and **polymeric micelles.**

Core-shell nanostructures between 10-200 nm, composed of a hydrophilic and a hydrophobic segment

IECP 2020

Nanomedicine

Nano-sized materials for therapeutic and imaging purposes

Polymeric Micelles

Advantageous characteristics

- Small size allows for tissue penetration and passive tumor targeting;
- Avoids the RES system and recognition by macrophages, resulting in a higher blood circulation time;
- Higher drug load capacity;
- Benefit from EPR effect;
- Outer hydrophilic block can be tailored by adding ligands.

IECP 2020

Nanomedicine

Nano-sized materials for therapeutic and imaging purposes

Pluronics

- Non-ionic surfactants commonly used in PMs.
  - They possess an amphiphilic nature, with hydrophobic PPO blocks and hydrophilic PEO blocks.

IECP 2020

Cationic Polymers

Frequently conjugated with PMs, as they aid nucleic acid transfection

PEI

Most used synthetic cationic polymer

- Spontaneously establishes electrostatic interactions between its protonable **amino** groups and the **phosphate** groups in the nucleic acids.
  - High transfection efficiency in *in vitro* and *in vivo* testing.
    - "Proton sponge" effect helps the endosomal escape.

IECP 2020

Methods

Synthesis of Pluronic® F68 diacrylate

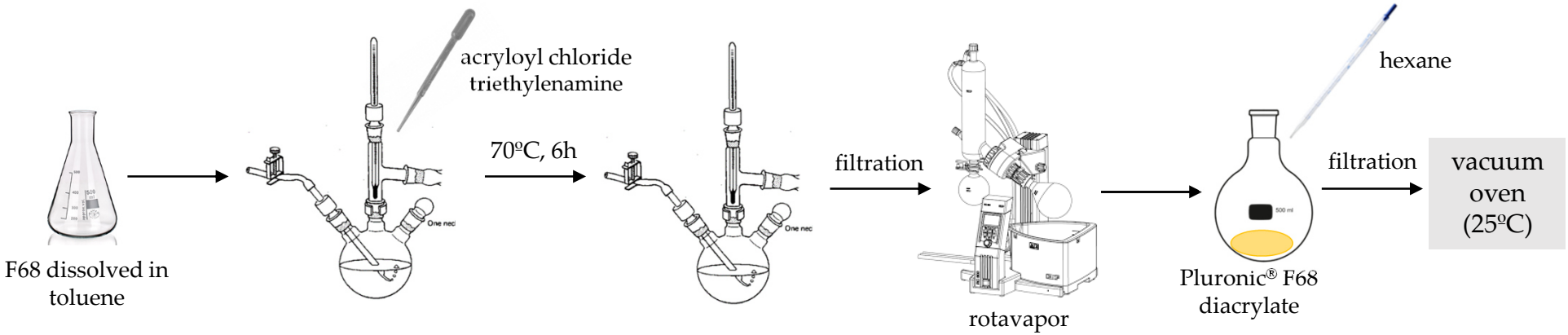

Figure 1 | Schematic representation of the synthesis of Pluronic F68 diacrylate

IECP 2020

Methods

Synthesis of Pluronic® F68 diacrylate complexed with PEI

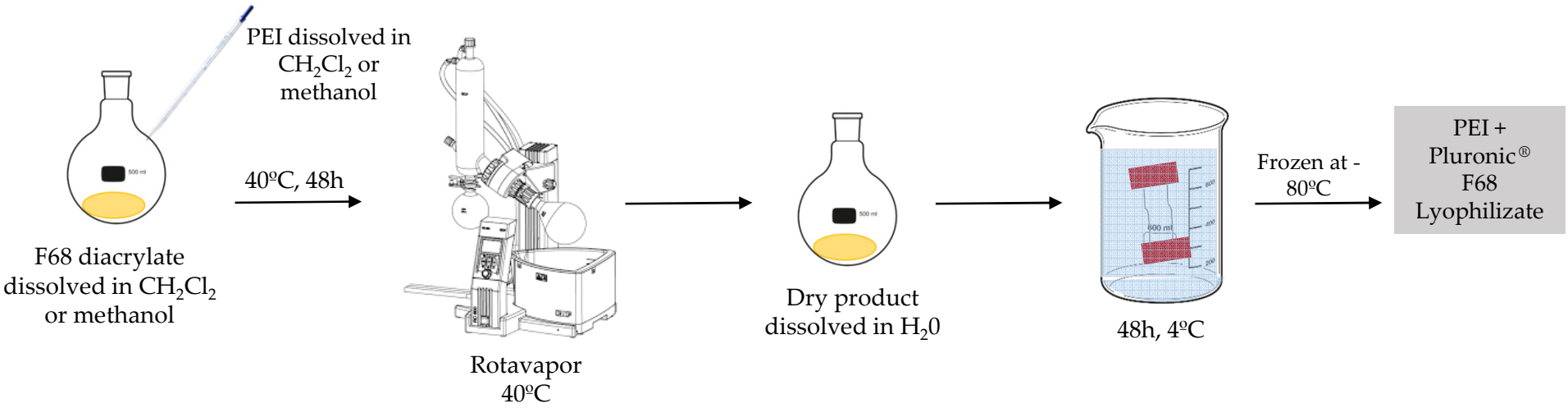

Figure 2 | Schematic representation of the synthesis of Pluronic F68 diacrylate complexed with PEI

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate

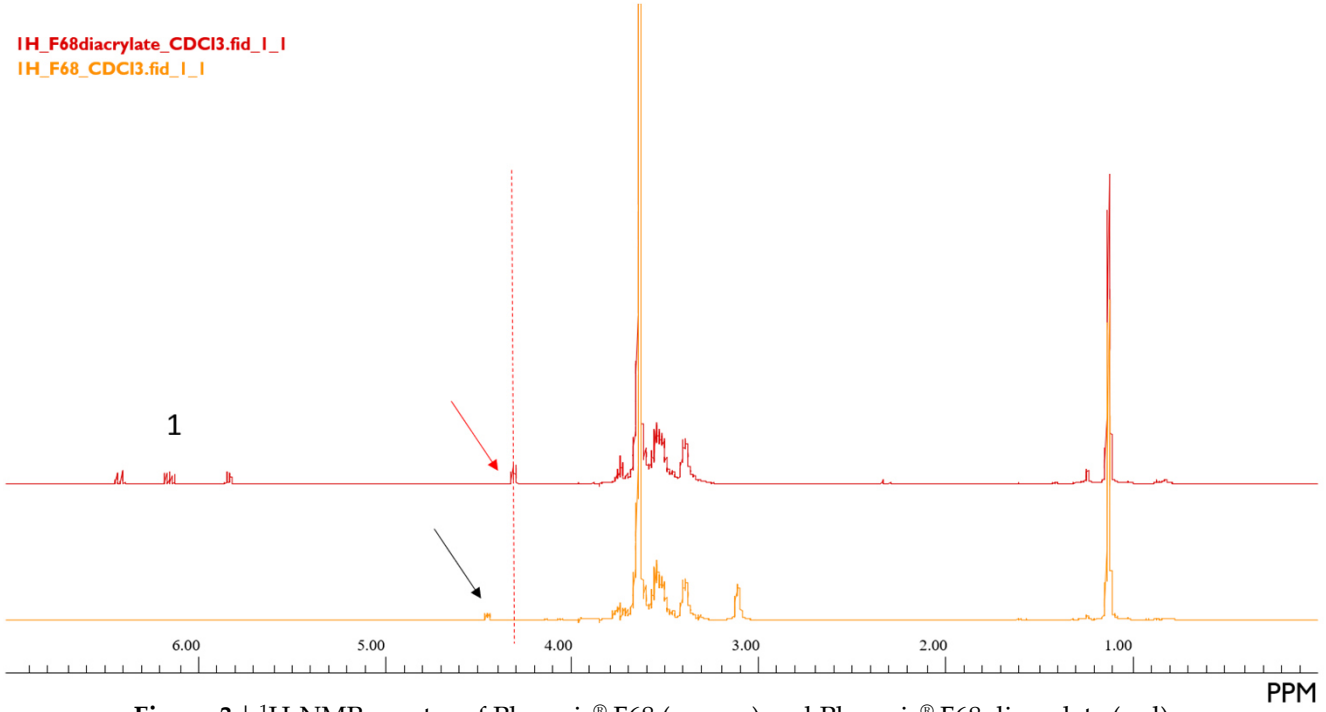

Figure 3 | ^{1}H-NMR spectra of Pluronic® F68 (orange) and Pluronic® F68 diacrylate (red).

- Appearance of new peaks due to the incorporation of olefinic acryloyl protons, indicative of the acrylate groups end-capped in Pluronic® F68
  - Slight shift in the signal of the terminal CH_2 PEO protons

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate

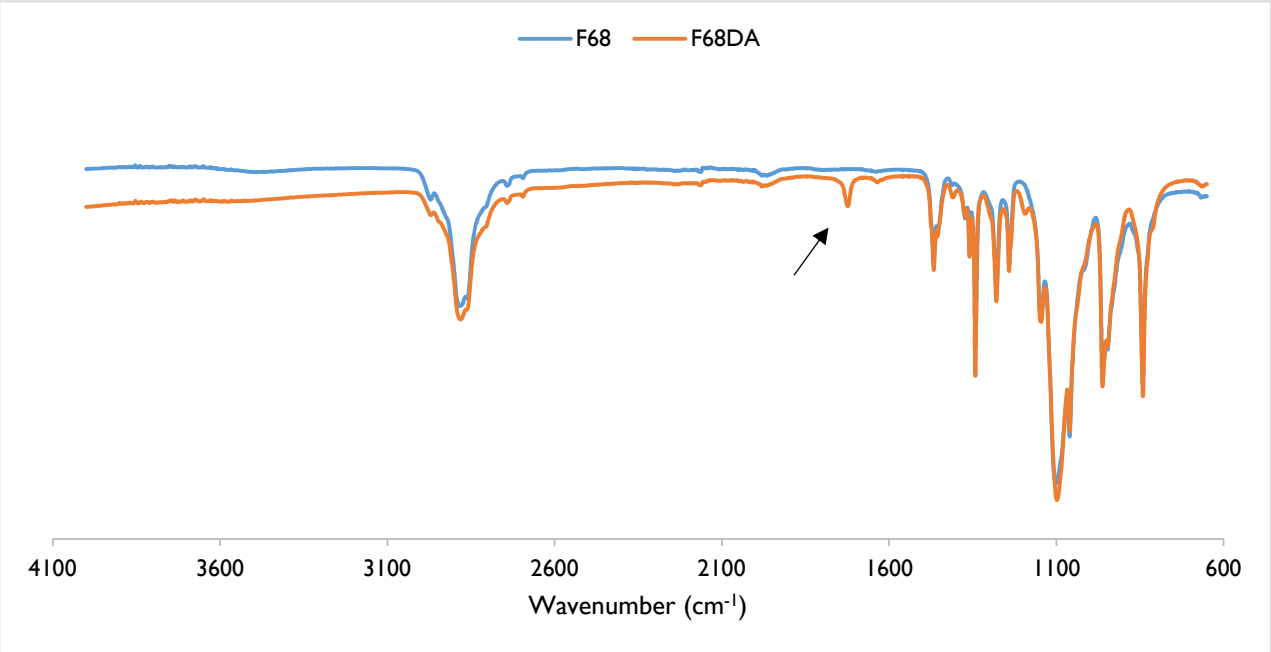

Figure 4 | FTIR spectra of Pluronic® F68 and Pluronic® F68 diacrylate.

A new bond can be detected, representative of the formation of the new ester bond

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate complexed with PEI

Table 1 | Characteristics of the complex formed between Pluronic® F68 diacrylate with PEI in dichloromethane following the lyophilization process, before and after filtration.

Sample	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
F68/PEI before filtration	546.6±44.41	0.549±0.216	--
F68/PEI after filtration	153.3±45.19	0.346±0.092	12.59±6.205

Note: Data are represented as mean ± SD.

- Reduction in particle size observed after filtration may be associated with less unbounded PEI in the solution.
- PDI decreased to a more favorable value, indicates a homogeneous and stable solution.
- The zeta potential value is still relevant, despite lower than the admitted value for a stable micelle.

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate complexed with PEI

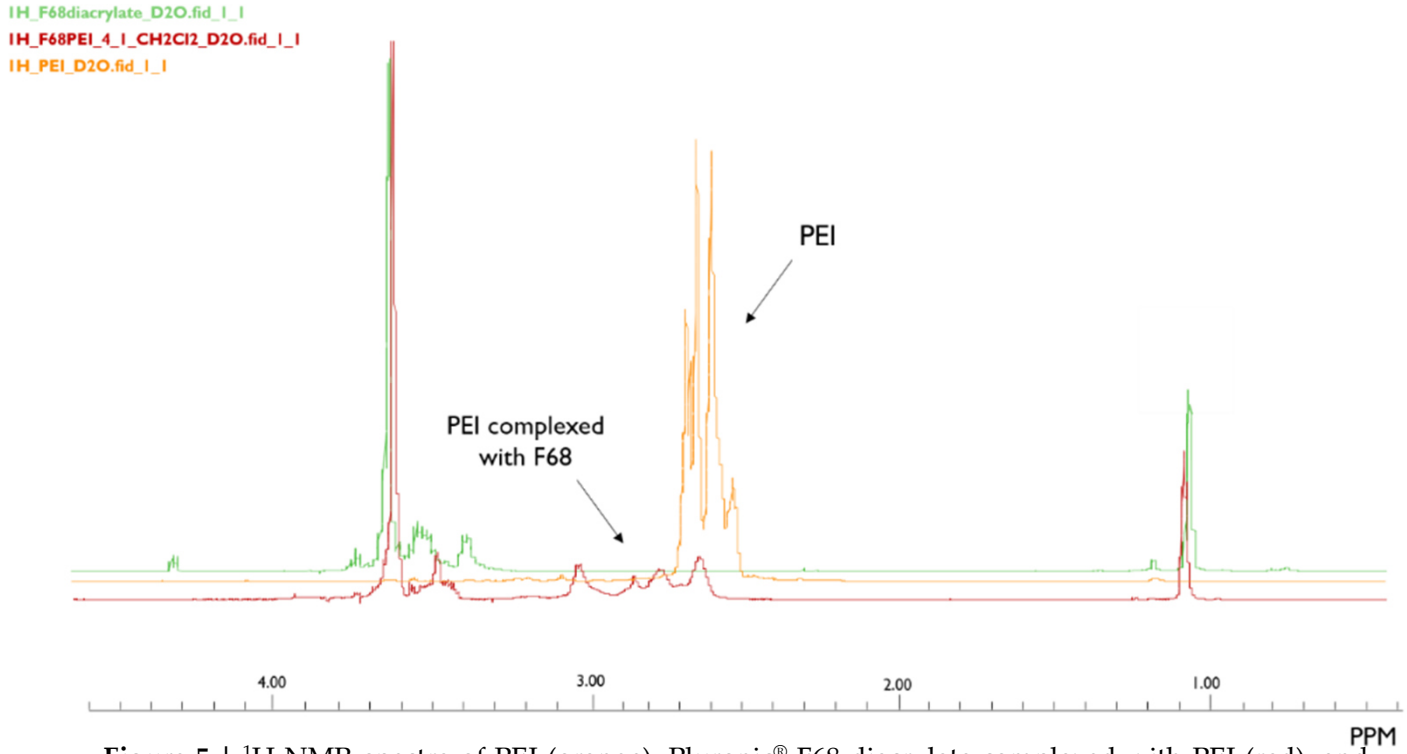

Figure 5 | ^{1}H-NMR spectra of PEI (orange), Pluronic® F68 diacrylate complexed with PEI (red), and Pluronic® F68 diacrylate (green).

The peak corresponding to the CH_2 groups of PEI, and the appearance of new peaks in F68/PEI spectrum in the same ppm zone and its absence in the diacrylate spectrum indicates a **successful conjugation** of F68 with PEI

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate complexed with PEI

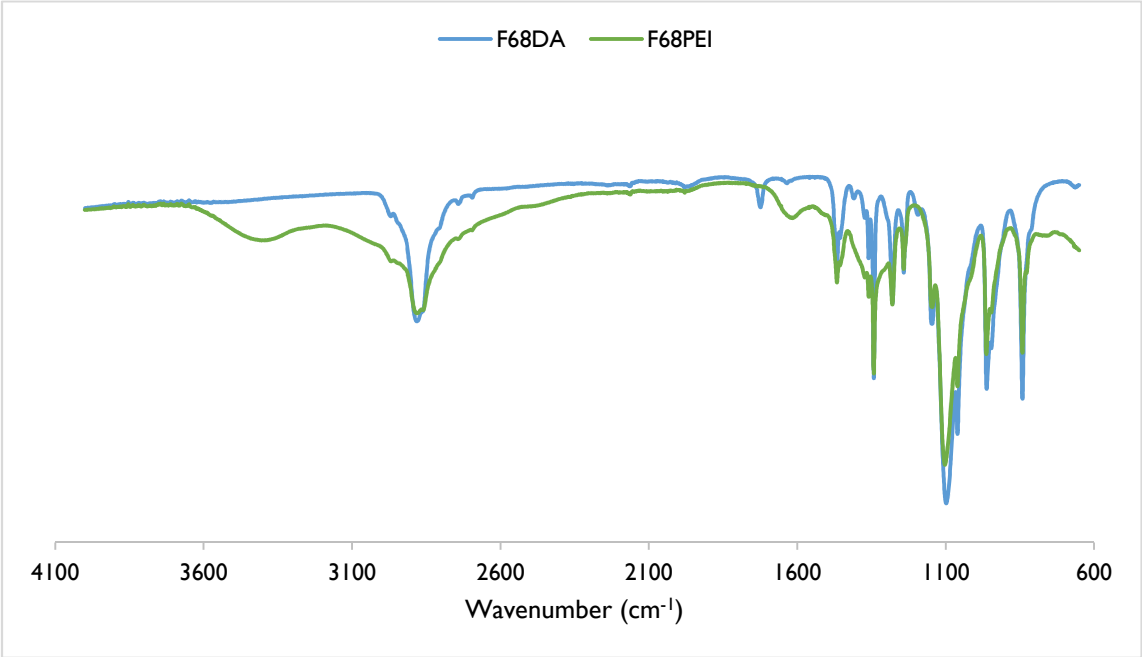

Figure 6 | FTIR spectra of Pluronic® F68 diacrylate and Pluronic® F68 diacrylate complexed with PEI. The PEI band is identified at 3400 cm^{-1} (amine N-H vibrations).

New band appeared at 3400 cm^{-1}, due to the N-H bond vibration, proving the presence of the conjugated PEI in the formulation

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate complexed with PEI and Pluronic ® P123

Table 2 | Characteristics of the complexes formed between Pluronic® F68/PEI with the addition of Pluronic® P123 at different ratios, after filtration.

Ratios	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
1:1	83.36±1.273	0.337±0.033	7.747±1.256
1:2	108.6±7.546	0.437±0.006	3.833±0.847
2:1	119.6±1.973	0.442±0.019	7.287±2.277

Note: Data are represented as mean ± SD.

- Addition of Pluronic® P123 was able to lower the particle size, at any given ratio, to below 200 nm, and even when compared with the F68/PEI particle size measurement after filtration.
- Lower concentrations of Pluronic® P123, in the 1:1 and 2:1 ratios, led to a higher value of zeta potential.

IECP 2020

Results and Discussion

Synthesis of Pluronic® F68 diacrylate complexed with PEI and Pluronic ® P123

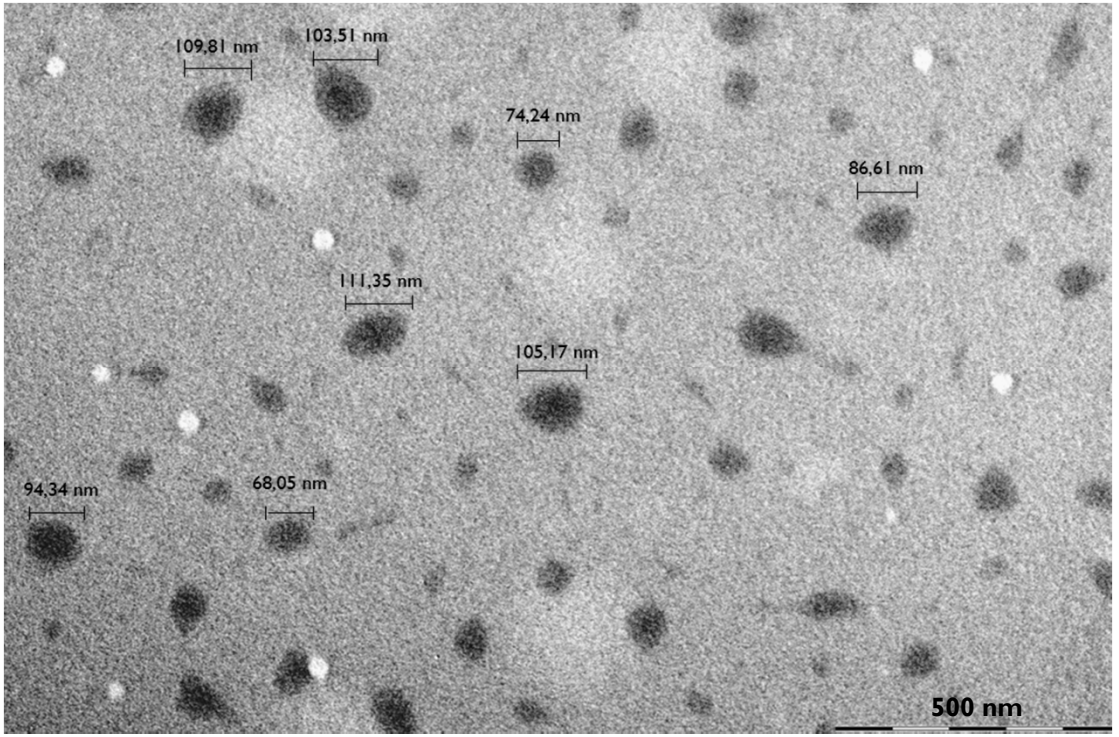

Figure 7 | TEM image of Pluronic®F68/PEI + Pluronic® P123 (2:1) with measurements of particle size.

Nanoparticle size is similar to mean results obtained through DLS, indicating the sample is uniform and presented low aggregation.

The particles were spherical and presented a darker center and a lighter shell. The latter could be due to the hydrophilic chains of PEI.

IECP 2020

Conclusions

Pluronic® F68 diacrylate was synthesized and characterized by ^{1}H-NMR and FTIR spectroscopy.

The complexation of PEI with the diacrylate was performed and the particle size measurements were favorable (20-200nm), but a slightly high PDI and a low zeta potential was obtained.

The incorporation of Pluronic® P123, at different ratios, to the Pluronic® F68 reaction with PEI did ameliorate the polydispersity index and originated smaller particles, but zeta potential remained lower than favorable.

TEM results showed nanoparticles with a micellar structure and in the presence of PEI, a lighter hydrophilic shell was observed.

IECP 2020

Acknowledgments

Cofinanciado por:

UNIÃO EUROPEIA
Fundo Europeu
de Desenvolvimento Regional