**ENCAPSULATION OF DOCETAXEL WITH ELASTIN LIKE RECOMBINAMERS BY SUPERCRITICAL CO2 FOR ADVANCED ANTICANCER APPLICATIONS**

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**Highlights**

* Set-up of the SAS process with ELRs was done reaching high process yields.
* Physico-chemical characterization shows particle size of 40 nm in aqueous solution.
* Drug delivery profiles were found to fit to the equation of Peppas-Sahlin.
* Breast cancer cells were more affected and showed diminished cell viability.

**1. Introduction**

The controlled release of drugs from biodegradable polymer nanoparticles is having a great medical impact in a wide range of therapeutic areas. Elastin Like Recombinamers (ELRs) [1] whose sequence is inspired in natural elastin and consist in repeats of the sequence (VPGXG)n, where X can be any amino acid except proline, are unique in this field thanks to their recombinant nature. ELR sequence can be altered by adding specific functionalities such as cell adhesion domains and their natural capacity of self-assembly and their stimuli responsiveness allow intelligent processes [2]. The encapsulation of Docetaxel (DTX) has been the subject of study by many researchers, implementing long and complex methods, which also involve the use of toxic organic solvents and post-processes. Therefore, a very powerful idea would be to coat the DTX avoiding toxic organic solvents and in one-step process. In this work, Supercritical AntiSolvent (SAS) [3] technique has been proposed to co-precipitate both compounds, due to, it serves as a completely clean separation agent since it uses mild temperatures in the process that do not harm the product, it is a non-flammable, non-corrosive, non-toxic, non-carcinogenic element, has a large selective capacity and does not generate waste [4].

**2. Methods**

Nanoparticles of DTX coated with ELRs, (EI)2 and (EI)2RGD, were obtained by Supercritical Antisolvent technology (SAS) studying process parameters to achieve the best operational conditions. The proportions of both compounds were determined by NMR. The morphology of the dry particles from SAS process was study by Scanning Electron Microscopy (SEM). Furthermore, Surface charge of the particles (ζ-Potential) and particle size in aqueous solution were measured by Dynamic light scattering (DLS) using a Zetasizer Nano ZS at 37oC and neutral pH. Particle morphology and amphiphilic behavior of the particles in aqueous media, was observed by TEM at neutral pH and at room temperature. Drug delivery experiment was performed in triplicate using dialysis method at 37oC under sink conditions and determined by UV-vis spectrometer and fitted with mathematical models. After characterization of nanoparticles, the effect of ELR-based nanoparticles was measured *in vitro* in endothelial (HUVEC) and breast cancer (MDA-MB-231) cells.

**3. Results and discussion**

The setting-up of the SAS pilot plant were performed with (EI)2 biopolymer and DMSO as a solvent, using a new coaxial nozzle designed in this work to get the best operational conditions. Results achieved with this conditions shows a drastic reduction in the solvent residues and a high process yield. The particles analyzed by SEM did not present a remarkable aggregation and has a smooth surface with many small particles stuck to them that provides a wide particle size distribution. Microparticles of (EI)2+DTX and (EI)2RGD+DTX obtained after the process can be disaggregate in aqueous solution forming a nanoparticulate delivery system keeping the drug inside with PDI below 0.2 and with ζ-Potential of around -30 mV, which clearly indicates that is monodisperse and stable in time. This results were confirmed by TEM where same size and behavior were found. Drug release study of the (EI)2+DTX particles shows a clear delay in the delivery of the DTX and the Peppas-Sahlin equation used to fit the profiles shows that the process is governed by the Fickian diffusion mechanism. In the cellular assays carried out, it has been proven that thanks to the incorporation of the RGD sequence in the ELR, the treated breast cancer cells were more affected and showed a lower cellular viability than cells treated with free DTX. This effect was not seen in HUVEC cells, which could be explained by the fact that cancerous cells have enhanced ability to internalize due to their enhanced metabolic rate and higher expression of integrins.

**4. Conclusions**

In this work, we have been able to set up the operational conditions in the Supercritical Antisolvent pilot plant with CO2 to make both microparticles of Elastin Like Recombinamers and co-precipitated Docetaxel-ELRs in one step process. Microparticles after the SAS process are able to disaggregate in aqueous media forming stable nanoparticles with low PDI which shows a controlled DTX release profile following Fick diffusion mechanisms. In addition, we may emphasize that it has been possible to increase the solubility of this highly hydrophobic anti-tumoral drug in aqueous solution by 5 orders of magnitude. Moreover, in the cellular assays carried out, it has been proven that thanks to the incorporation of the RGD sequence in the ELR, the treated breast cancer cells were more affected and cell proliferation was completely abolished, therefore, the strategy developed in this work opens the way to new systems of controlled release, more precise than non-selective chemotherapeutic drugs and with a very promising potential.

**References**

1. M. Santos, S.S.-D., J. Gonzalez-Valdivieso, R. Vallejo, A. Girotti, P. Cuadrado, F. J., Arias, Current Medicinal Chemistry, 25 (2018).
2. J.C. Rodríguez-Cabello, FJ. Arias, M. Alonso and A. Girotti, Advanced Drug Delivery Reviews 97, (2016) 85-100
3. A. Natolino, C. Da Porto, S. Rodriguez-Rojo, T. Moreno, M.J. Cocero. The Journal of Supercritical Fluids, 118 (2016) 54-63.
4. M.J. Cocero, A. Martín, F. Mattea, S. Varona, The Journal of Supercritical Fluids,47 (2009) 546-555