IBUPROFEN AND T3 POLYMERIC NPS OPTIMIZED FOR SPINAL INJURY TREATMENT

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SPINAL CORD INJURY (SCI)

Spinal cord injuries (SCI) include traumatic and vascular lesions of the central nervous system, that unfortunately lack any resolutive therapies.
The consequences of suffering an SCI can impact **many areas of the inflicted person's life.** It can compromise **bowel** and **bladder function, mobility** and **autonomic functions,** along with secondary conditions such as **pressure ulcers** and **pain.** For patients who suffer a traumatic SCI, the most important disability is the inability to walk, at least with a velocity that permits normal ambulation [1]. Today, **completely repairing SCI damage is not yet possible** due to the **inability to completely regenerate neural tissue**: for example, a completely severed spine is not repairable. However, it is possible to stimulate the remaining intact tissue or to attempt path regeneration through the use of stem cells. In some cases, depending on the type of injury, these current treatments have led to patients with injured spinal cords renewed ability to flex their toes, ankles and knees.[2].
Decreasing the negative impact of impairments, **promoting full participation in daily life,** and **increasing the welfare of patients with an SCI** is the goal of researchers.

Figure 1: Spinal cord injury [3]

In this contest, **intralesional administration of nanoparticles (NPs) loaded with select drugs** could be **an efficacious tool** to obtain **prolonged effects against spinal cord injuries.**
In fact, nanocarriers, due to their small size, have the potential to improve the site-specific delivery and therapeutic effect of drugs that suffer from poor solubility, poor stability, and unwanted toxicity by changing their tissue distribution and improving their pharmacokinetics.
Moreover, the need to have a **slow drug release modulated over time** instead of a **massive burst release could reduce** the **number of administrations**, **drug toxicity, and improve general treatment outcomes**.
NPs based on FDA approved polymers such **as poly (D,L-lactic acid)(PLA) and poly (D,L-lactic-co-glycolic acid) (PLGA) are promising drug delivery systems.** They widely demonstrate their biocompatibility, biodegradability, and their **suitability for encapsulation and sustained/prolonged release of therapeutics**. They have also been widely proven for the ability to delivery biologically active molecules to the brain [4]
For this reason, **PLGA/PLA NPs were designed for the treatment of spinal cord lesions**, **aiming to impact both the inflammatory state and nerve** regenerative capability by loading various drugs:
1) **Ibuprofen** a small molecule poorly soluble in water with poor biodegradability when administered orally, is **a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic properties**, was used to treat the inflammatory state which characterized the zone around the lesion.
2)**Triiodothyronine**, a small and very lipophilic molecule that **has key remyelination proprieties that performs fundamental roles in the correct growth, development and metabolism of almost all cell types** [5].

PROJECT GOALS

1. **Optimizing and characterizing** control PLGA/PLA NPs and PLGA/PLA T3/Ibuprofen **NPs**
2. **Quantifying drugs encapsulated** in both formulations
3. Performing **NPs drug release studies** in different biologically relevant buffers (pH 6.8 and 7.4)

NANOPRECIPITATION METHOD

Nanoprecipitation: a simple technique used for **encapsulation of both hydrophilic and hydrophobic drugs in NPs**. This method involve the instantaneous formation of NPs, that **can be scaled up**. The present investigation was aimed at developing Ibuprofen and T3-loaded PLA/PLGA-based biodegradable NPs by modifying different parameter involved in the nanoprecipitation methods to obtain a sustained release of the drugs until round 14 days [6].

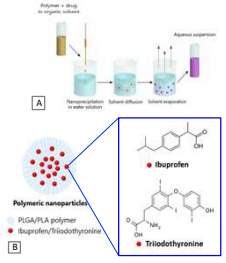


Figure 2: Nanoprecipitation method (A) NPs loaded with Ibuprofen/T3 (B)

FORMULATION OF IBUPROFEN PLGA/PLA NPs

NPs based on PLGA and PLA containing different doses of **Ibuprofen** were prepared using the nanoprecipitation technique to obtain the best polymer/drug ratio.
The method required two different solvents (one organic and the other aqueous) that are miscible with each other and result in the spontaneous formation of NPs.
As previously stated, the low solubility and molecular weight of Ibuprofen is a major obstacle to achieving high encapsulations and prolonged release of the drug. For this reason we optimized the nanoprecipitation modifying several parameters:

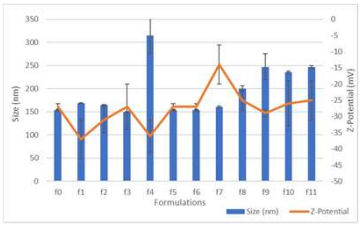
1. Polymer composition
2. Organic Phase
3. Surfactant type
4. Surfactant Percentage
5. Volume Ratio between Organic and Aqueous Phase
6. Aqueous Phase Viscosity
7. pH Aqueous Phase

Samples	Organic solution (OS) Containing 10 mg Ibu			Water Solution (WS)	
	PLGA (PLA) (mg)	Solvent	Volume OS (ml)	Surfactant (w/v)	Volume WS (ml)
f0	50	acetone	4	Pluronic 3%	12.5
f1	50	acetone	4	Pluronic 3% pH 3.5	12.5
f2	50	acetone	4	Pluronic 3%	8
f3	50	acetone/DMSO	2.5/1.5	Pluronic 3%	12.5
f4	50	acetone	4	Tween 80 2%	12.5
f5	50	acetonitrile	4	Pluronic 3%	12.5
f6	50	acetone	4	Pluronic 3% + sucrose 2%	12.5
f7	50	acetone	4	PVA 1%	12.5
f8	25 (25 PLA)	acetone	4	Pluronic 3%	12.5
f9	10 (40 PLA)	acetone	4	Pluronic 3%	12.5
f10	(50 PLA)	acetone	4	Pluronic 3%	12.5
f10 *Dialysis	(50 PLA)	acetone	4	Pluronic 3%	12.5

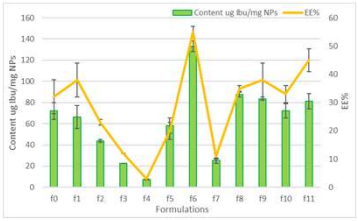
Scheme 1: Modified formulation parameters of Ibuprofen NPs

NPs CHEMICO-PHISICAL AND PHARMACEUTICAL CHARACTERIZATION

All **19 NP formulations were characterized** in terms of size, size distribution, Z-potential, Ibuprofen content (µg/mg NPs) and encapsulation efficacy (EE%).



Scheme 2: Chemico-physical characterization of Ibuprofen NP formulations



Scheme 3: Pharmaceutical characterization of NP formulations

Results
In *scheme 2* was observed that all the formulations presented the **hydrodynamic diameters** (size) ranged from **150-300 nm** and **narrow size distribution (PDI<0.3)**, showing to be homogeneous and monodisperse, favorable for a local administration.
NPs showed **comparable surface charge**, ranged from **-35 to -15 mV,** due to the exposure of the carboxylic group of PLGA.
Regarding content and encapsulation efficiency, in *scheme 3 it* was observed that the formulations with the higher EE% are: **f1** (pH 3.5), **f6** (Sucrose 2%), **f9** (PLGA 20:PLA 80), **f11** (PLA 100 dialysis).

Release studies were performed, for all the formulations, in **sink conditions** and in **PBS buffer** at two different pH **7.4** and **6.8.** The acid pH was established to **simulate the biological environment** that characterized an inflamed area.
However, the NP formulations prepared show a rapid release of the Ibuprofen entrapped in the NPs (from 15 minutes to 1 day), which does not satisfy the objective in charge to obtain a prolonged release over time (10-14 days).

HIGHLIGHTS
Ibuprofen NPs based on PLGA/PLA were **successfully prepared** with the nanoprecipitation technique. All the formulation result **monodisperse, homogeneous** and **stable** over the time but the EE% and the Ibuprofen released need to be improved. For that reasons, **optimizations are still being performed** to enhance kinetic release profile.

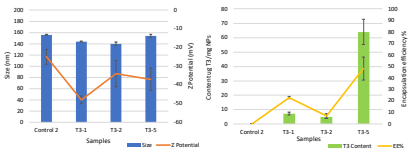
FORMULATION OF T3 PLGA NPs

Because of the **high lipophilicity of T3**, several solubility tests were performed to identify the suitable solvent to dissolve the drug and nanoprecipitate it with the polymer PLGA using the **nanoprecipitation technique**.
Control NPs were prepared testing **different ratio between the organic solvents acetone and dimethyl sulfoxide (DMSO)** to obtain the best formulation. DMSO was selected because it is both solvent of the drug and miscible with water and acetone.
The method was optimized, and the best conditions were selected and applied to obtain the best formulation of T3 NPs, in term of chemico-physical characteristic and stability over the time.
All **the NP formulations were characterized** in terms of **size**, **size distribution**, **Z-potential**, **T3 content** (µg/mg NPs) and **encapsulation efficacy** (EE%).

Samples	Organic solution (OS) Containing 50 mg PLGA		Water Solution (WS)	
	T3 (mg)	Solvent (ml)	Surfactant % (w/v)	(ml)
Control 2	0	3 acetone 1 DMSO	Pluronic 3	12.5
T3-5	5			
T3-2	2			
T3-1	1			

Scheme 4: Formulation parameters of different doses of T3 NPs

NPs CHEMICO-PHISICAL AND PHARMACEUTICAL CHARACTERIZATION

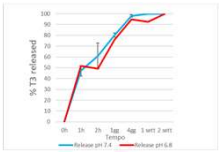


Scheme 5: Chemico-physical characterization of T3 NPs (left) and pharmaceutical characterization of T3 NPs (right)

Results
In *scheme 5* was observed that all the formulations presented the **hydrodynamic diameters** (size) ranged from **144-155 nm** and narrow **size distribution (PDI<0.2)**, showing to be homogeneous and monodisperse, favorable for a local administration.
NPs showed **comparable surface charge**, ranged from **-48 to -25 mV,** due to the exposure of the carboxylic group of PLGA.
Regarding **content and encapsulation efficiency (EE%)**, only NPs with 5 mg of T3 gives a good percentage of EE% around 48%.

RELEASE STUDY OF T3 NPs IN ACSF BUFFER

The release study was **conducted in sink condition** in the **artificial cerebrospinal fluid (ACSF) at pH 7.4 and 6.8** to simulate physiological and pathological conditions respectively. **The release of T3 is modulated and prolonged over time up to 4 days.**



Scheme 6: Release study of T3 NPs in buffer ACSF pH 7.4 and 6.8

Results
The *scheme 5* highlights that there are no particular differences in the two studies conducted at different pH probably due to the very low solubility of T3 in the media.
Release pH 6.8: 50% over 1 hour, 95% over 4 days
Release pH 7.4: 50% over 1 hour, 100% over 4 days

HIGHLIGHTS
NPs based on PLGA and **loaded with 5 mg T3** were **successfully prepared** with the **nanoprecipitation technique** using as organic solution a mixture of Acetone/DMSO (ratio 3:1). The formulation show an **initial burst release around 50%** of the total drug entrapped. Then, the **T3 released is modulated over the time until 4 days.**

CONCLUSION AND FUTURE DEVELOPMENT

- Strategies **for stabilizing and increasing Ibuprofen controlled release** maybe using a hybrid drug of Ibuprofen.
- Full **chemico-physical**, **morphological** and **technological characterization** of both the nanosystem.
- **Quantify the T3 released** in ACSF using **Elisa method**.
- **Cell differentiation studies** before proceeding with ***in vivo*** **studies with T3 NPs**

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Asse 1 – Ricerca e innovazione, Azione 1.2.2.

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