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# Polymeric nanocapsules and nanospheres for encapsulation and long sustained release of hydrophobic cyanine-type photosensitizer

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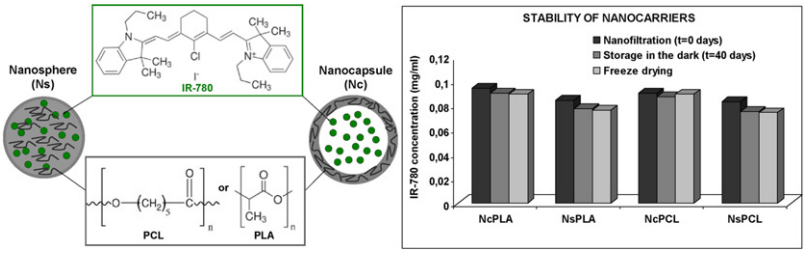
## HIGHLIGHTS

- Colloidally stable polymeric nanocarriers for the IR-780 photosensitizer were obtained by self-assembly of precipitated polymer chains.
- The in vitro release profiles of IR-780 from freshly prepared nanocarriers and from the ones freeze dried were received spectrophotometrically.
- Photobleaching and ROS measurements, of cyanine-dye, both as native and encapsulated in obtained nanocarriers, were successfully performed.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Owing to the nanoprecipitation methodology IR-780 loaded PLA and PCL nanospheres and nanocapsules (stabilized by Cremophor EL and containing, in the case of nanocapsules, coconut oil as the liquid core) were fabricated and their colloidal stability, encapsulated cargo release and photoactivity were assessed. DLS measurements and AFM confirmed, respectively, the obtained nanoparticles diameter below 150 nm as well as their morphology and shape. Doppler electrophoresis provided the $\zeta$-potential of the studied nanoobjects while UV–vis spectroscopy made it possible to determine the encapsulation efficiency (about 90%) of IR-780 and to establish its release characteristics. Nanofiltration and freeze drying approaches prior to storage in the dark provided the best conditions' parameters for the near monodispersed nanocarriers of cyanine. In order to evaluate the photoactivity of IR-780, both as native and encapsulated in the obtained nanocarriers, photobleaching and ROS detection (by means of spectrophotometrical measurements) were successfully performed. The applied methodologies made it possible to obtain successfully stable and long sustained IR-780 loaded monodispersed oil-cored nanocapsules and nanospheres both under physiological conditions, and after freeze drying procedure.



## 1. Introduction

Hydrophobic polymethine cyanine dyes (indocyanines) have been adopted as a non-targeting contrast agent in clinical and experimental NIR imaging and as a second generation photosensitizing agent in photodynamic therapy (PDT) [1–5]. As lipophilic cations with excellent optical properties due to a rigid cyclohexenyl ring in the heptamethine chain with a central chlorine atom (peak excitation at 760–800 nm and peak emission at 790–830 nm) the cyanine-based dyes can make attractive cargo for a variety of nanocarrier platforms for bioimaging and cancer therapy [2,3,5–7]. However, the use of cyanines as photosensitizers may be limited

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by their light-induced decomposition (i.e. photobleaching) resulting in the loss of absorbance, fluorescence and photoactivity, for which the same reactive oxygen species (ROS) may be responsible [8,9]. This phenomenon can be provoked by the high lipophilicity of these compounds and their insolubility under physiological conditions. It should be emphasized that in order to increase the potential of hydrophobic cyanines [1–4] they need to be encapsulated in nanocarriers to avoid any unexpected problems with photoactivity and to improve their dissolution performance by means of encapsulation via template-mediated processes, e.g., interfacial polymerization, subsequent adsorption or interfacial nanoprecipitation [1,4,10,11].

Polymeric nanoparticles have attracted much attention as delivery vehicles in biomedical and cosmetic applications, owing to their enhanced permeation, specific cell targeting and capacity to long sustained release of drugs [12]. Furthermore, polymeric nanocarriers are designed to improve drug performance by utilizing pathophysiological uniqueness of solid tumor, of which conventional low molecular weight drugs are incapable. Nanomedicines show improved tumor-selective targeting. They can deliver pharmaceutics to the target tissue due to enhanced permeability and retention (EPR) effect. The EPR, discovered first by H. Maeda in 1985 [13] is a unique feature which allows drug delivery nanoparticles (cutoff size of < 780 nm) to preferentially accumulate and diffuse in tumor tissues due to the leaky vasculature that allows passage of nanocarriers into the tumor matrix. Long-circulating drug delivery nanoparticles are able to extravasate into tumor tissues, accumulate for a longer duration as tumors have poor lympathic drainage relative to normal tissue, and release the therapeutic drug locally in the extracellular area [10,13].

The most applicable nanoprecipitation (also called solvent displacement or interfacial deposition) method – is based on the spontaneous emulsification of the organic internal phase with the dissolved polymer into the aqueous external part in the presence of a surfactant. [11,14,15]. The selected polymer is first dissolved in a water-miscible solvent of intermediate polarity, which results in the precipitation of nanospheres, and then it is injected into a stirred aqueous solution containing a surfactant as a stabilizer. Polymer deposition on the interface between the water and the organic solvent, caused by the fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension. Additionally, when a small volume of nontoxic oil is incorporated into the organic phase, nanocapsule-shaped products can be obtained [12,16]. We can therefore obtain via nanoprecipitation two different types of nanoparticles. First, nanospheres with matrix type of structure (entire mass is solid) are created, when active molecules may be absorbed at the sphere surface or encapsulated within the particle. And the second type, nanocapsules are vesicular systems, acting as a kind of reservoir, in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane (shell). In this case the active substances are usually dissolved in the inner core (either oil or water) but may also be adsorbed to the capsule surface [12]. Generally, as a straightforward and quick methodology, nanoprecipitation does not require high shearing/stirring rates, sonication or very high temperatures and often it enables the production of small nanoparticles (100–200 nm) with narrow unimodal distribution and exhibiting a high drug loading capacity [14,17,18]. Furthermore, as it is widely described in literature [11,14,17,19,20], it has been successfully applied in the encapsulation of cytostatic, antifungal and antiviral drugs, sunscreen agents, proteins and hormones.

As a continuation of our search for new polymeric template-mediated nanoproducts [1,4,5,21,22] we focused our present studies on long sustained nanoprecipitation-based carriers for photodynamic therapy purposes. The main aim of our contribution was to fabricate IR-780 loaded nanocapsules and nanospheres with the most unimodal distribution in size, both exhibiting sufficient chemical and colloidal stability, sustained release characteristics and high cyanine loading efficiency and photoactivity in the encapsulated state. IR-780 was selected for our studies because as a commercially available polymethine cyanine dye, similarly to green-cyanine ICG, it has quite a high value of the molar extinction coefficient in the NIR region, i.e. $2.65 \times 10^5\ M^{-1}\ cm^{-1}$ at $\lambda_{max} = 780$ nm [23]. Due to weak absorption of biomolecules in this spectral region, allowing for deeper tissue penetration, IR-786 is potentially attractive as a photosensitizer [6–8]. Furthermore, it has been proved, that encapsulated IR-780 can reach mitochondria, its excitation with light causing cell death through generation of reactive oxygen species (ROS) – an essential effect for PDT [4]. We selected poly(D,L-lactide), PLA (or PDLLA), and polycaprolactone (PCL) as biocompatible polymers which, especially due to their slow degradation, were found to be well suited for long-term drug delivery systems [11,14]. We used coconut oil as the oil phase and Cremophor EL as the surfactant, both being nontoxic and biocompatible [24]. The present study concentrates on the comparison of PLA and PCL nanocapsules and nanospheres carrying IR-780 as the cargo and on improving the long-term storage stability of the constructed nanocarriers by combining the nanoprecipitation method with the freeze drying procedure.

## 2. Experimental

### 2.1. Chemicals

All the reagents were purchased from Sigma–Aldrich and used as received. IR-780 iodide was used as the cyanine-type photosensitizer. Cremophor EL (polyethoxylated castor oil) and coconut oil were used as the surfactant and the oil phase, respectively. Poly(D,L-lactide) (PLA, $M_w \sim 90{,}000$–$120{,}000$) and polycaprolactone (PCA, $M_w \sim 70{,}000$–$90{,}000$) were employed as biocompatible polymers. Water used in all the experiments was doubly distilled and purified by means of a Millipore (Bedford, MA) Milli-Q purification system.

### 2.2. Preparation of cyanine-loaded nanospheres and nanocapsules by nanoprecipitation method

Nanocapsules (Nc) and nanospheres (Ns) were fabricated by the nanoprecipitation method according to the following procedure. In the case of Nc, first, an organic solution consisting of poly(D,L-lactide), (PLA) or polycaprolactone (PCL) at various concentrations (2–10 mg/ml), and coconut oil (0.1 mg/ml) and IR-780 cyanine dye (0.5 mg/ml) dissolved in acetone was prepared. A constant volume (1 mL) of this organic phase was added dropwise under vigorous magnetic stirring to 5 mL or 3 mL of an aqueous solution containing Cremophor EL in a concentration of 10 mg/ml. The organic solvent was then evaporated at room temperature overnight. The Ns suspension was prepared as described for Nc, but without the coconut oil. In the next step, the respective suspensions were separated via nanofiltration (using cellulose nitrate membranes with a pore size of 200 nm) prior to size, morphology, $\zeta$-potential, sustained release and photokinetic studies. Finally, the colloidal stability of obtained polymeric drug delivery systems after nanofiltration ($t = 0$ days), freeze drying and storage in the dark ($t = 40$ days) was checked.

### 2.3. Characterization

1. *Zeta potential* (*ζ-potential*): ζ-potential of the nanocapsules and the nanospheres suspended in 0.25% (w/v) saline solution (pH 7.4, conductivity 2.0 mS/cm) according to Ref. [15] was measured by the microelectrophoretic method using a Malvern Zetasizer Nano ZS apparatus. All the measurements were performed at

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