

Loaded nanoparticles with cyanine-type photosensitizers: preparation, characterization and encapsulation

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The aim of the present contribution was to prepare poly(*n*-butyl cyanoacrylate) nanocapsules, loaded with cyanine-type hydrophobic photosensitizers such as IR-768, IR-780 and IR-783 cyanines, by means of interfacial polymerization in oil-in-water (o/w) microemulsions. The microemulsion templates were prepared *in situ* with nonionic surfactants, such as Tween 80 and Brij 96. *Iso*-propylmyristate was used as the photosensitizer solubilizing locus (i.e., the oil phase), whereas *iso*-propanol - as the cosurfactant. The entrapment of the selected photosensitizers - determined indirectly by detecting the concentration of the remaining cyanine in the supernatant following the isolation process of the nanoparticles - was achieved in the range of 60-90% for each studied cyanine. The cyanine-loaded nanoparticles were visualized by images taken with a atomic force microscopy (AFM). They were spherical in shape with a diameter of 111 to 420 nm ± 2.5 nm and highly monodisperse in most cases as revealed by dynamic light scattering (DLS) and AFM.

1. Introduction

Surfactant-oil-water systems may be used as templates to produce nanostructured materials, as delivery vehicles for drugs and food additives, and as solvents in: degreasing, cleaning, bio-separations, polymerization, environmental remediation, and enhanced oil recovery (Date et al., 2008). Among many micellar aggregates and their mediated systems, polymeric nanoparticles have been designed to successfully encapsulate hydrophobic drugs in order to target cells and avoid drug degradation and toxicity as well as to improve drug efficacy (Allemann et al., 1993; Couvreur et al., 2002). Especially, hollow nanocapsules consisting of a liquid core in a thin polymer envelope obtained via interfacial polymerization have received a special interest as one of the most potential polymer - based colloidal drug delivery systems (Guterres et al., 2007). After parenteral administration, the penetration extent and transport range of nanocapsules from the blood to the target tissue depend, inter alia, on the size of these drug carriers. Due to their molecular size, ranging between 100-700 nm, the nanocapsules escape renal clearance and have prolonged serum half-life period. Often

they cannot penetrate the endothelial junctions of normal blood vessels. But vascular endothelium in pathological sites (solid tumors, inflammation tissues and infarcted areas) is discontinuous with large fenestrations of 200-780 nm, which allow the nanoparticle passage (Gaumet et al., 2008). In particular, broad efforts are to be made for the search of effective delivery systems for anticancer drugs which should be selectively retained by malignant tissue. One of the striking examples here is the photodynamic therapy (PDT) - considered as a treatment modality in oncology – it uses a combination of light and photosensitizers to cause cancer cellular and tissue damages (Castano et al., 2004, Crescenzi et al., 2004). In clinical PDT however, the same side effects were observed as a result of dark toxicity of photosensitizers towards cancer and normal cells (Castano et al., 2004). For this reason, the incorporation of such hydrophobic molecules into biocompatible nanocapsules may reduce cytotoxicity of a free photosensitizer and permit to deliver greater amounts of them to the cancer cells. In the present work we continue our studies on searching for the most desirable microemulsion-templated hollow nanostructures for the hydrophobic photosensitizer encapsulation. Previously, we have successfully reported the cyanine IR-768 molecules encapsulated in sugar surfactant microemulsion-templated nanocapsules, which being internalized in MCF-7 breast cancer cells, effectively enhanced cell death after light irradiation (Zielinska, 2008b). At present our interest is focused on the encapsulation of cyanine IR-768, IR-780 and IR-783 dyes in polymeric nanocapsules, prepared in nonionic microemulsion-templated processes to be further studied as potent photosensitizer molecules to the cancer cells in the photodynamic therapy (PDT).

2. Experimental

2.1. Materials

All reagents were of analytical grade and used as provided. Most chemicals were purchased from Sigma-Aldrich, i.e., the polyoxyethylenated nonionics: Tween 80 (Polysorbate 80) - polyoxyethylene 20 sorbitan mono-oleate and Brij 96 - polyoxyethylene 10 oleyl ether, chloroform, ethanol, dimethylsulfoxide (DMSO), Dulbecco's Modified Eagle medium (DMEM), cyanine IR-768, IR-780 and IR-783, *iso*-propanol IP, *iso*-Propylmyristate IPM (from Fluka). *n*-Butyl cyanoacrylate was kindly donated by Tong Shen Ent. Co., (Taiwan). Water used for all experiments was doubly distilled and purified by means of Millipore (Bedford, MA) Milli-Q purification system.

2.2. Preparation of microemulsion-based templates

All oil-in-water microemulsions were constructed at a 1:1 surfactant-to-cosurfactant weight ratio according to directions given in (Zielinska et al., 2008a). The samples were prepared by diluting surfactant:cosurfactant/oil and cyanine mixtures with water. After each addition of water fraction samples were vigorously shaken and left for 24 h to attain equilibrium. Samples were checked by both visual observations, and by inspection with a polarizing light microscope (Olympus CX 41, Japan) to identify the non-birefringent system.

2.2. Preparation of loaded nanocapsules

n-Butyl cyanoacrylate (BCA) monomer (1.4 mg/mL, 3.6 mg/mL or 7.2 mg/mL concentrations) dissolved in chloroform (20 – 90 μ l) was slowly added to 4 ml of selected microemulsion containing IR-768, IR-780 or IR-783. Interfacial polymerization was performed at 4°C and the system was stirred for at least 4 hours to

complete the synthesis. Metertech SP8001 spectrophotometer with 1 cm path length quartz thermostated cell was used to determine the amount of the photosensitizer encapsulation in nanocapsules by detecting the concentration of the remaining cyanine in the supernatant following the isolation process of the nanoparticles (centrifugation at 12 000 rpm for 1h at 25°C). All measurements were performed in triplicate. Cyanine IR-768 was detected at 775 nm, IR-780 at 786 nm and IR-783 at 789 nm.

2.3. Dynamic light scattering (DLS)

The particle size and distribution of both the o/w microemulsions, and the PBCA nanocapsules were determined by DLS (Zetanosizer Nano series ZS, Malvern Instruments Ltd.) according to directions given in (Zielinska et al., 2008a; Zielinska et al., 2008b). The droplet's mean diameter was computed by the Stokes–Einstein's law: $R_H = k_B T / (6\pi\eta D)$, where R_H , k_B , T , η , and D are the hydrodynamic radius of the droplet, Boltzmann's constant, temperature in Kelvin, viscosity and diffusion constant, respectively. The DTS (Nano) program was applied for the data evaluation. In the case of the nanocapsules, residual oil and surfactant were removed by repeated washing in ethanol, centrifugation, and then dry nanocapsules were dispersed in propylene glycol.

2.4. Atomic force microscopy (AFM)

A total of 20 μL of ethanol suspension of the nanoparticles was deposited on a freshly cleaved mica surface, which corresponded to an approximate surface concentration of about 10 molecules/ μm^2 . The samples were then dried overnight. Imaging was carried out using ultra-low amplitude tapping mode on Veeco NanoScope Dimension V AFM, with a with a RT ESP Veeco tube scanner. Scanning speed was 0.5 Hz, and a low resonance frequency pyramidal silicon cantilever resonating at 250-331 kHz was used (force constant = 20-80 N/m).

2.5. Absorption spectroscopy

Absorption measurements of cyanines dissolved in tetrahydrofuran:water (THF:water 1:1, v/v) as well as empty and loaded PBCA nanocapsules dispersed in THF:water were made on a Metertech SP8001 SP 2101 UV–Vis spectrophotometer with 1 cm path length quartz thermostated cell.

3. Results and discussion

Chemical structures of the studied cyanine-type photosensitizers along with their abbreviations are placed in Figure 1.

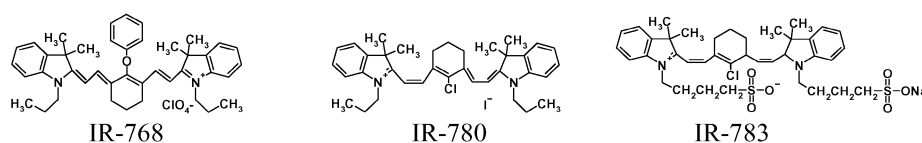


Fig. 1. The selected cyanines as photosensitizers.

It has to be emphasized as the first priority that the photosensitizer, which is absorbed by all cells and selectively retained by malignant tissue after light exposition is promoted to an excited state and induces local release of cytotoxic reactive oxygen species (ROS). Depending on the experimental conditions and cell sensitivity, the

cytotoxic molecular species resulting from PDT may trigger cell apoptosis or induce necrosis (Almeida et al., 2004). In clinical PDT the some side effects were observed as a result of the dark toxicity of photosensitizers towards normal tissues. Low dark toxicity is one of the important criteria for assessing the usefulness of photosensitizers (Castano et al., 2004). Characteristics of poly(*n*-butyl cyanoacrylate) hollow nanoparticles (PBCA) prepared in microemulsion-templated polymerizations are placed in Tables 1 were S denotes the selected surfactant, Co – the cosurfactant, Ol – the oil phase.

Table 1. Characteristics of poly(*n*-butyl cyanoacrylate) loaded hollow nanoparticles prepared by o/w microemulsion-templated processes

System*	microemulsions			loaded nanocapsules								
	S	Co	Ol	IR-768			IR-780			IR-783		
				R _H [nm]	PdI	E [%]	R _H [nm]	PdI	E [%]	R _H [nm]	PdI	E [%]
1a	Tween 80	IP	IPM	194.7	0.19	89.4	184.6	0.37	61.1	158.1	0.18	74.8
1b				171.7	0.27	89.5	384.5	0.62	60.9	221.3	0.39	75.0
1c				274.6	0.43	88.5	420.0	0.65	65.8	351.3	0.62	77.3
2a	Brij 96	-	IPM	124.6	0.25	73.7	111.2	0.19	80.1	134.3	0.19	87.9
2b				159.7	0.46	79.1	136.1	0.30	86.9	148.6	0.37	88.5
2c				202.5	0.39	82.3	180.9	0.35	86.5	195.5	0.45	90.1

* the monomer concentrations for 1a and 2a was 1.4 mg/mL; for 1b and 2b - 3.6 mg/mL; for 1c and 2c - 7.2 mg/mL

Selected o/w microemulsions (R_H equalled to 9.5 and 12.8 nm, respectively for Brij 96 and Tween 80 microemulsions) were applied in our studies for the transcriptive synthesis of PBCA nanocapsules by interfacial polymerization using *n*-butyl cyanoacrylate as the reactive monomer at 1.4 mg/mL, 3.6 mg/mL or 7.2 mg/mL concentrations. Size distribution studies and PdI values indicated some differences in the homogeneity of the reported oil-cored nanoparticle population, but nanocapsules prepared from the Tween 80/IP/IPM/water and Brij 96/IPM/water templates were highly monodispersed. The diameter of the obtained nanocapsules was dependent on the template applied and the monomer amount used for the polymerization. The particle size ranged from 111 to 420 nm ± 2.5 nm and did not differ for unloaded and cyanine – IR-768, IR-780, IR-783-loaded nanoparticles. The size of the nanocapsules was the most desirable and our results corresponded well with other reports (Gaumet et al., 2008; Krauel et al., 2005). The investigated cyanines diluted in THF:water exhibit a strong absorbance in the red region as in (Kassab, 2002), with a maximum wavelength at 775, 786 and 789 nm for IR-768, IR-780 and IR-783 respectively. The results presented in Figure 2 show that selected photosensitizers loaded in nanocapsules do not suffer changing in photophysical properties after the encapsulation process, as it has also been described for nanoparticles containing phthalocyanine (Ricci-Júnior et al., 2006). Additionally nanocapsules from both ternary (Brij 96/IPM/water) and pseudoternary (Tween 80/IP/IPM/water) o/w microemulsion templates have high encapsulation efficiency (in the range of 60-90%, Table 1) of all used cyanines.

The obtained cyanine loaded nanocapsules are spherical in shape when viewed with AFM (see some examples in Figure 3). Moreover, no differences in their morphology were visualized for particles performed from various types of microemulsions.

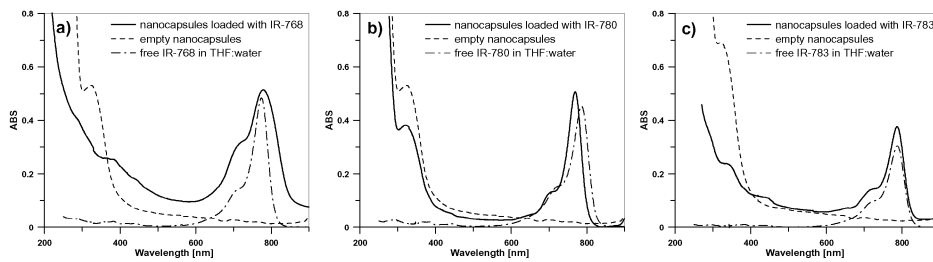


Fig. 2. Absorption spectra of Brij 96/IPM/water nanocapsules in a THF:water solution in relation to respective cyanines.

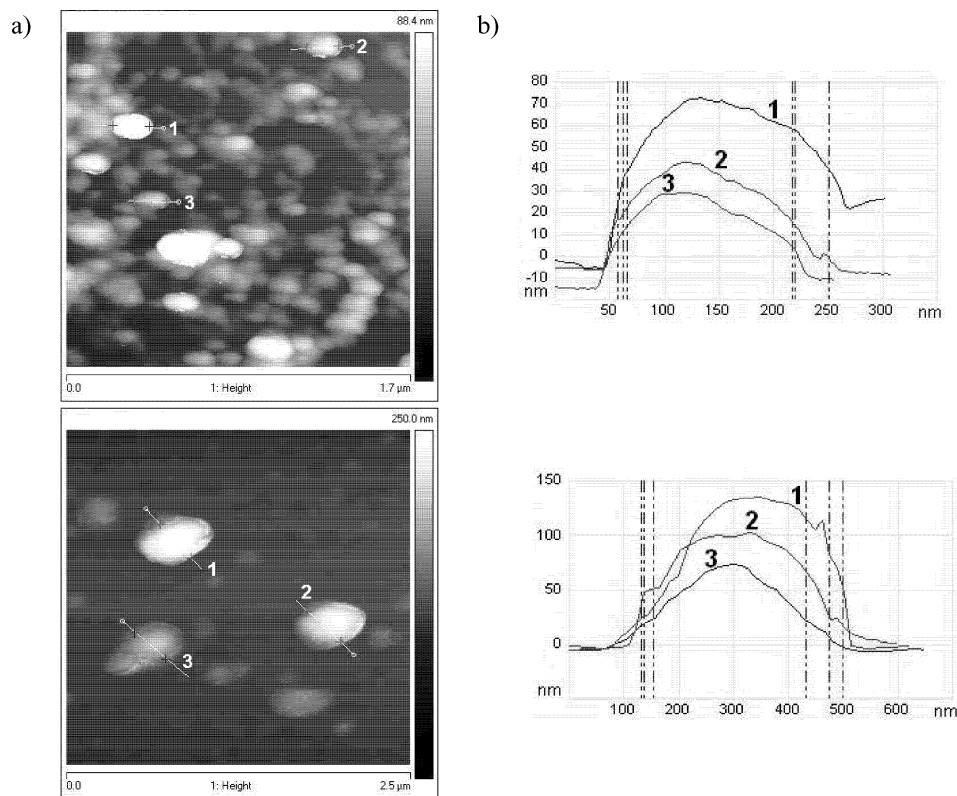


Fig. 3. Morphology of Brij 96/IPM/water nanocapsules (according to system 1b in Table 1) – upper part and Tween 80/IP/IPM/water nanocapsules (according to system 2b in Table 1) – lower data. (a) AFM images, (b) cross sections.

4. Conclusions

The results obtained will be useful for our further investigations on biodegradable nanocapsules and their release characteristics as useful photosensitizer delivery systems

in the PDT of some cancers. No differences in their morphology were visualized for the loaded nanoparticles formed from various types of templates. The size of the studied nanocapsules was found to be a function of the microemulsion type and ratio of the monomer mass used for the polymerization process (thickness of the polymeric wall).

Acknowledgements

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