P-096 **Formation of Nanocapsules with Emulsion Core and Pegylated Shell by Polyelectrolyte Multilayer Adsorption**

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INTRODUCTION AND OBJECTIVES

The layer-by-layer (LbL) adsorption of polyelectrolytes (PE) is considered as a convenient method to obtain microcapsules' shells on colloidal cores (Shchukin 2003). Solid particles (polystyrene latex, porous silica, $CaCO_3$) are most often used as cores for formation of capsules, which may contain some active ingredient. Alternatively the solid core can be dissolved to leave the hollow shell, which can be then refilled with the desired composition. The disadvantage of that method can be traces of the destructed core trapped in the capsule and low efficiency of loading of the active substance into the hollow shells. Use of emulsions droplets as liquid cores gives the possibility to encapsulate oil soluble active components with control of size and shell properties of obtained capsules. Immobilization of pegylated corona is a one of the available methods to prevent serum protein adsorption and non-specific bindings to cells by the particles. Use of copolymers with PEG chains grafted to a polyelectrolyte backbone is a conventional method to immobilize PEG on polyelectrolytes microcapsules.

The present study describes the development of novel nanocapsules based on a liquid core and with shells prepared by LbL technique using biocompatible polyelectrolyte (PE) with pegylated outermost layer. The process is schematically presented on Figure 1. The resulting nanocapsules are non-toxic and exhibit negligible non specific binding to blood cells. Model drugs like beta-carotene and vitamin A were successfully encapsulated.

Figure 1. Scheme of formation nanocapsules

MATERIAL AND METHODS

The polyelectrolytes: poly-L-lysine hydrobromide PLL (MW 15000-30000), poly(fluorescein isothiocyanate allylamine hydrochloride) FITC-PAH, (MW ~ 70000), Poly-L-glutamic acid sodium salt PGA (MW 15000-50000). All polyelectrolytes, docusate sodium salt AOT ≥99%, sodium chloride, beta-carotene, vitamin A were obtained from Sigma-Aldrich. Chloroform cz.d.a. was purchased from POCH Gliwice. PGA-g-PEG was synthesized in our laboratory according to procedure proposed by Boulmedais (2004). The distilled water used in all experiments was obtained with the three-stage Millipore Direct-Q 3UV purification system.

Capsules were prepared using a modified method proposed by Szczepanowicz (2010). The oil phase for capsules preparation was prepared by dissolution of AOT in chloroform (360g/dm^3). Polyelectrolytes were dissolved in NaCl solutions (0.015 M) at concentration 1 g/dm^3. Emulsion was formed by addition of AOT/chloroform to polycation (PLL) solution during mixing with a magnetic stirrer at 300 rpm. To encapsulate model drugs, beta-carotene or vitamin A were dissolved in chloroform (0,1mg/ml) prior to emulsification with AOT. The multilayer shells were formed by subsequent adsorption of polyelectrolytes from their solutions using saturation method. To create pegylated shell, PLL-terminated nanocapsules with seven polyelectrolytes layers were coated with PGA-*g*-PEG by adding nanocapsules into PGA-g-PEG solution. For preparation of fluorescently labeled nanocapsules the FITC-PAH was used instead first PLL layer.

Size (hydrodynamic diameter) and zeta potential of capsules were determined by DLS (Dynamic Light Scattering) and by the microelectrophoretic method using Malvern Zetasizer Nano ZS apparatus. Nanocapsules were also visualized by SEM. using JEOL JSM-7500F Field Emission Scanning Electron Microscope at the operation voltage of 15 keV. UV-VIS spectrometry was applied to confirm encapsulation of model drugs; spectra were acquired by using an Analytik Jena AG - SPECORD® 40 spectrophotometer. The cytotoxicity of the samples of nanocapsules was tested in a proliferation assay. Cells and nanocapsules were cultured in triplicate wells in round bottomed plates (Costar, Corning Inc. NY, USA). Results are calculated from the mean of triplicates and expressed as percent of ^{3}H incorporation relative to control cultures without nanocapsules. To evaluate the unspecific binding, the fluorescently labeled nanocapsules were incubated with peripheral blood mononuclear cells (PBMC) isolated from the blood of healthy donors. PBMCs were incubated in CellGro DC medium (CellGenix, Freiburg, Germany) with Garamycin (Schering-Plough Labo) in 48 well-plates (Costar, Corning Inc. NY, USA) and nanocapsules samples diluted in the same medium were added to the cells. The cells were incubated with nanocapsules at 37˚C for 3 hours before they were washed once in the medium, and re-suspended in phosphate buffered saline (PBS) for analysis of fluorescence on a BD LSR II flow cytometer (BD, NJ, USA). The results were analyzed with the FlowJo Software (Tree Star, Inc. Oregon, USA).

RESULTS AND DISCUSSION

For preparation of the suspension of nanocapsules, 0.1 ml AOT in chloroform solution (360 g/dm^3) was added to 200ml of aqueous PLL solution (c=0,1 g/dm^3) during continuous mixing. Chloroform was evaporated from suspensions of nanocapsules after preparation. The average drop size measured by DLS was 70 nm with polydispersity index (PDI) <0,2. Increasing the concentration of PLL solution to 0,5g/dm^3 resulted in the average size of obtained capsules 100nm with PDI<0,2. The zeta potential of the emulsion drops was 77 ± 8mV. The formation of the polyelectrolyte multilayer shells on such prepared cores were performed by subsequent adsorption of polyelectrolytes from their solutions without the intermediate rinsing step. Figure 2 illustrates a typical zigzag dependence of the zeta potential of capsules on the adsorption of layers.

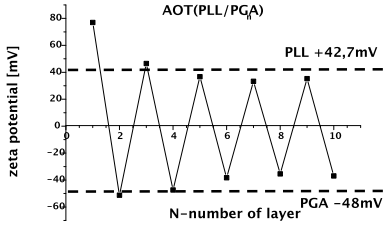

Figure 2. Variations of *zeta*-potential of capsules with the number of PLL/PGA layers.

It provides evidence for formation polyelectrolytes shells. The average size of nanocapsules obtained with five PLL/PGA bilayers, as measured by DLS was 100nm. The example of SEM micrograph of the capsules was demonstrated in Figure 3.

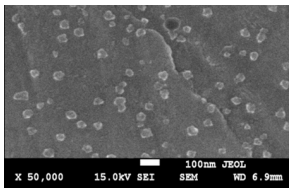

Fig 3. SEM micrograph of AOT(PLL/PGA)$_5$ capsules.

To encapsulate model drugs, beta-carotene or vitamin A was dissolved in chloroform prior to emulsification. Comparison of UV-Vis absorption spectra of capsules contained model drugs with the empty ones provided the evidence of encapsulation. To form pegylated nanocapsules, surface of PLL-terminated nanocapsules with seven polyelectrolytes layers were coated with PGA-*g*-PEG. Grafting of PEG on PGA decreases charge density of the polyanion, therefore, covering capsules with PGA-g-PEG should significantly decrease their zeta potential in comparison with PGA layers. The measured zeta potential of pegylated nanocapsules was close to zero (-3± 4 mV). To determine whether that strong decrease of zeta potential of capsules with pegylated shells does not induce their aggregation, the stability test was performed. Freshly prepared nanocapsules were stored in 0.015M NaCl solution at room temperature for up to 90 days. We did not observe anysignificant changes in size and zeta potentials during that period, that means that PEG corona at the capsule surface provides sufficient steric stabilization.

We tested biocompatibility of the nanocapsules AOT(PLL/PGA)$_{3,5}$PGA-g-PEG in co-culture with a human cell line, which is very sensitive to toxic components such as surfactants and other chemicals used in the synthesis of nanocapsules. The results of this bioassay have shown that the capsules can be used in high concentrations, (dilution 1:10 -1:20) without harming the cells. A second prerequisite for in vivo medical applications of nanocapsules is a requirement for minimal non-specific binding to the cellular elements of a patient blood. We have tested fluorescently labelled capsules AOT(PLL/PGA)$_{3,5}$PGA-g-PEG, for non-specific binding in the model system using human peripheral blood mononuclear cells (PBMC). Our model system was based on fluorescence labelled capsules (by FITC-PAH), which allowed for highly sensitive detection of binding and phagocytosis by flow cytometry after co-culture with cells. No increase in FITC expression was observed in the cells incubated with AOT(PLL/PGA)$_{3,5}$PGA-g-PEG nanocapsules indicating that the nanocapsules were not taken up by the cells and they did not bind to the cell surface. The presented results demonstrate that our nanocapsules show no or minimal evidence of binding to lymphocytes and monocytes, indicating that these cell preparations are rather inert and thus suitable for systemic use.

CONCLUSIONS

The procedure of preparation of emulsion stabilized by complex of ionic surfactant AOT and polycation PLL was proposed. Size of obtained droplets could be tuned in the range 60 – 100 nm. Biocompatible polyelectrolyte shells were successfully built on emulsion droplets. The average size of nanocapsules obtained for five PLL/PGA bilayers were in the range of 80 – 100 nm. The model drugs beta-carotene and vitamin A were successfully encapsulated. PGA-g-PEG was used to create pegylated, outermost layer of the capsules' shell. The nanocapsules appeared non-toxic and with negligible non-specific binding to blood cells. It indicates that that obtained nanocapsules loaded with model drugs, are good candidates for further biological experiments.

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