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Nano-Encapsulation of Proteins Via Self-Assembly with Lipids and Polymers

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Summary: The solubilization and encapsulation of the weakly soluble protein hemoglobin was investigated at the nanoscale using self-assembly with the branched polymer polyethyleneimine (PEI), the lipid glycerol monooleate (GMO), and two amphiphilic poly(ethylenglycol) monooleate derivatives with molecular weights 2100 g/mol (MO-PEG1) and 860 g/mol (MO-PEG2). The created self-assembly nanovehicles were analyzed by quasi-elastic light scattering (QELS) in order to determine their sizes as well as by circular dichroism in order to characterize the protein presence in the nanoobjects. The cationic polymer PEI formed mixed nano-objects with the protein hemoglobin. The polymer conformation in the nanovehicle was established to be sensitive to dilution, a property that can be essential for the protein release upon administration. The amphiphile MO-PEG1 was a co-surfactant in the dispersion of monoglyceride lipid nanoobjects needed for the hemoglobin encapsulation. The amphiphile MO-PEG2 formed small micelles in the absence of a lipid. The nanoobjects dispersions were studied for their stability on storage and reproducibility.

Keywords: detergents; encapsulation; polymer; protein; self-assembly

Introduction

Many substances of pharmaceutical or biotechnology interest, including proteins, could be insoluble or aggregated in aqueous medium. This fact often hampers their utilization as watery solutions. Therefore, for the development of modern nanomedicines, one should consider the physicochemical properties of the proteins of interest and their capacity for self-assembly with appropriate nanocarriers in aqueous phase. One possibility to improve the solution solubility and homogenization is the self-assembly nanotechnology. It permits to create supramolecular complexes using encapsulation with lipids or polymers as matrix or dispersing agents. Nonionic lipids, such as monoglycerides, and charged branched polymers, such as polyethyleneimine (PEI), here investigated, could ensure a nanostructured medium for encapsulation of weakly soluble proteins and the formation of nanoparticles through self-assembly in excess water phase.

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Encapsulation of Protein in Self-Assembly Nanostructures

Hemoglobin in Glycerol Monooleate/ Polyethylenglycol Monooleate Nanoobjects

The lipid glycerol monooleate (MW 356 g/mol) was mixed with a polyethylenglycol monooleate (MO-PEG1, MW 2100 g/mol) dissolved in phosphate buffer at a concentration of 5×10^{-5} mol/L. The self-assembly and filtration yielded one population of nanoparticles in quasi-elastic light scattering experiments (Nanosizer apparatus).

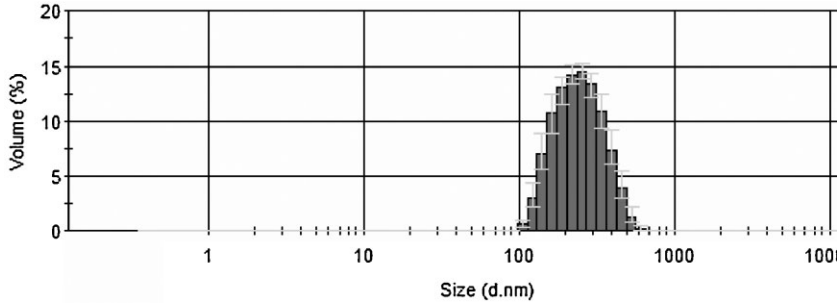

Figure 1.
Quasi-elastic light scattering determination of the nanoparticles sizes in a glycerol monooleate (GMO)/ polyethylenglycol monooleate (MO-PEG1) dispersion.

The mean nanoobject size was 255 nm for the amphiphilic system studied upon 10 times dilution by phosphate buffer (Figure 1). After one week storage, bigger particles with a diameter 531 nm were formed. This indicates that the incubation time leads to growth or aggregation of the nanoobjects in some of the samples.

On the other hand, stable mixed micelles were obtained in self-assembly mixtures of the monoglyceride lipid (GMO) with the amphiphile MO-PEG2 displaying detergent properties. The supramolecular aggregate size, around 18.2 nm, was weakly dependent on the MO-PEG2 concentration, which was varied in the range from 1×10^{-4} to 2×10^{-2} mol/L. The polyethyleneglycol monooleate MO-PEG2 (MW 860 g/mol) formed micelles of a smaller size than those of the MO-PEG1 (MW 2100 g/mol) nanoobjects. The mean size was 5,61 nm. The nanoparticles were found to be stable at an amphiphile concentration 1×10^{-2} mol/L without a tendency to aggregate on storage.

Upon mixing of GMO, polyethylenglycol monooleate (MO-PEG2), hemoglobin (MW 66000) and phosphate buffer, self-assembly of small nanoparticles (24–28 nm) was achieved, with a total elimination of insoluble hemoglobin-aggregates after one week of incubation and equilibration. The results demonstrated the effectiveness of the nanoencapsulation of insoluble hemoglobin in PEGylated nanovehicles of a monoglyceride lipid.

Supramolecular Complexes of Hemoglobin and Polyethyleneimine

The branched polymer polyethyleneimine (PEI, 25000 g/mol) has been previously studied as a vehicule for DNA delivery.

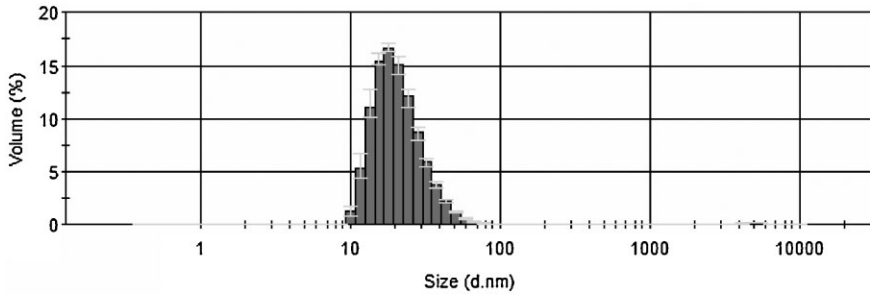

Figure 2.
Quasi-elastic light scattering determination of the nanoparticles sizes in a glycerol monooleate (GMO)/ polyethylenglycol monooleate (MO-PEG2) dispersion.

Table 1.
The obtained nanocapsules show a persistent stability during several weeks.

PEI sample name	Concentration [mg/ml]	Concentration [mol/L]	Effective diameter size [nm]
F 152 P1	100	4×10^{-3} M	3,12
F 153 P1	50	2×10^{-3} M	4,19
F 151 P1	20	8×10^{-4} M	5,61
POLY DIL	1,5	6×10^{-5} M	8,72

We investigated the physical-chemical properties of this cationic polymer (Figure 3) in solution and its capacity to form mixed nanoobjects with a weakly soluble protein macromolecule (hemoglobin).

The effective dimension of the branched PEI nanovehicule was determined as a function of its solution concentration (Table 1). It was found to increase with the decrease in the PEI concentration from 100 to 1.5 mg/ml. This should be due to deploying of the polymer branches in diluted solutions.

The protein nanoencapsulation was established to depend on the polymer/hemoglobin mass ratio. Aggregates of pure hemoglobin directly formed in phosphate buffer aqueous medium due to the limited solubility of the protein macromolecule. In mixed hemoglobin/PEI systems, the progressive addition of the cationic polymer contributed to solubilization of the insoluble protein aggregates as a result of charge interactions. The optimal nanoencapsulation conditions corresponded to a smallest fraction of polymer PEI and a highest fraction of haemoglobin. At a hemoglobin/PEI ratio 2:1, no hemoglobin aggregates were left in the dispersion, despite that the polymer concentration was relatively low. The obtained mixed supramolecular nanoobjects were with an average size 8,7 nm.

The formation of supramolecular complexes hemoglobin/PEI was dependent on the incubation time and the concentration. At high dilutions (Figure 4), longer incubation times were needed to form the self-assembly nanoobjects. Under such dilutions, corresponding to protein administration under physiological conditions, the protein liberated itselves from the polymer nanovehicle.

The presence of the protein hemoglobin in the self-assembly nanocapsules was proven by means of circular dichroism (CD) spectroscopy. The CD peak intensities and widths may indicate whether protein aggregation occurs or not. The protein hemoglobin was detected in the formulation in its non-denaturated state as the corresponding CD spectrum (red plot) was analogous to the graph of the pure hemoglobin. Figure 5 presents typical circular dichroism spectra.

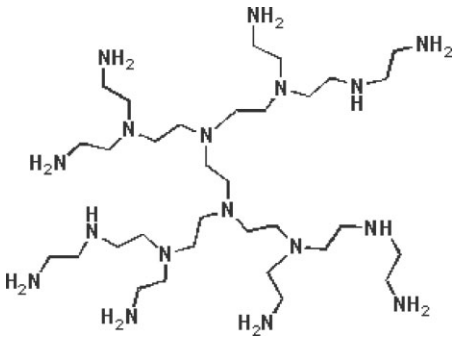

Figure 3.
Scheme of branched polyethylenimine (PEI, MW25000 g/mol).

Conclusion

Both amphiphilic polyethyleneglycol monooleate derivatives and the polymer PEI show a tendency for formation of nanoparticles for vectorisation of proteins. MO-PEG1 self-assembled with the lipid glycerol monooleate in stable nanocapsules. The detergent MO-PEG2 has a propensity to form micellar capsules. The

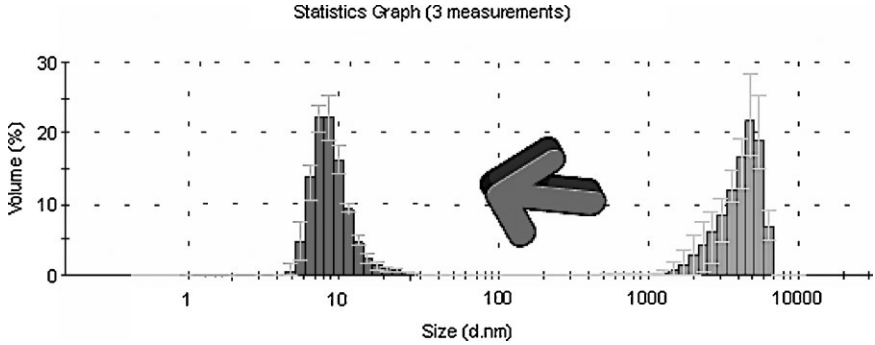

Figure 4.
In hemoglobin/PEI systems, the incubation time of 3 days leads to total solubilization of the macromolecular aggregates (with sizes of several micrometers) to PEI-entrapped protein nanoensapsulation (8.7 nm mean vector size).

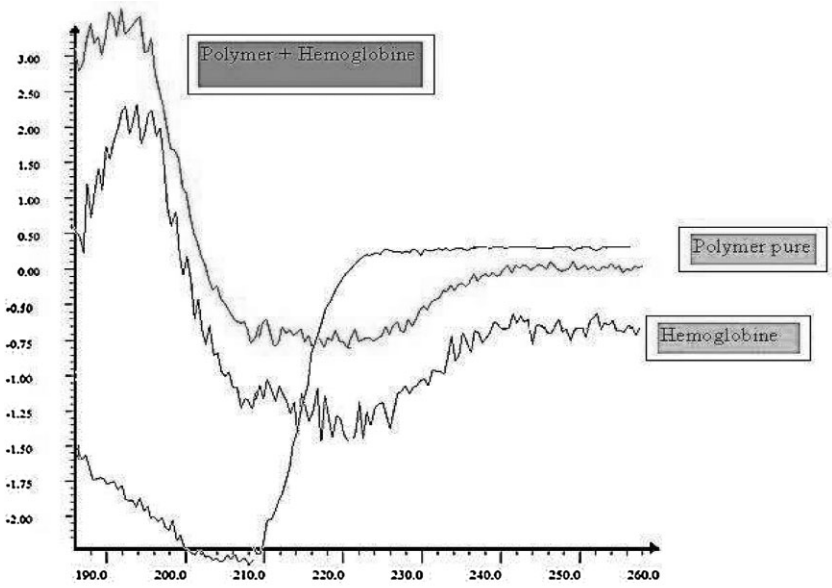

Figure 5.
Spectra of circular dichroism *versus* wavelength for the protein hemoglobin (red line), the polymer PEI and the nanocapsule constituted by PEI and hemoglobin.

resulting nanoobjects of MO-PEG2 are effectively smaller than the nano-objects of PEG1. The cationic polymer PEI changes its effective size, as a function of the concentration, by deploying its branches. It is capable to essentially improve the solubility of an almost insoluble protein, such as hemoglobin, by forming supramolecular nanoobjects. The optimal polymer/protein mass ratio, permitting homogeneous nanoencapsulation, was established. At high dilutions, the self-association depends on the incubation time. The polymer vehicle liberates the protein by itself upon dilution. The obtained nanocapsules show a persistent stability during several weeks.

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