High Encapsulation Efficiency of Proteins using PEG-PPS Block Copolymers

C. O´Neil[†], A. van der Vlies[†], D. Velluto[†], R. Schoenmakers[†], and J. A. Hubbell[† *]
[†] École Polytechnique Fédérale de Lausanne, Lausanne Switzerland
(conlin.oneil@epfl.ch)

Introduction

Typical protein encapsulation efficiencies using block copolymers (polymersomes) is around 5% when prepared using conventional methods such as thin film with extrusion. Here we present a new suite of PEG-PPS bock copolymers which form vesicles from the simple addition of water to the dry polymer, and with a significantly improved encapsulation efficiency.
Triblocks of the type poly(ethylene glycol)-*bl*-poly(propylene sulfide)-*bl*-poly(ethylene glycol) (PEG-PPS-PEG) with short chain PPS form vesicles when prepared by the simple addition of water to the solid polymer at room temperature. These materials can encapsulate >20% of the protein added and form uniform vesicles when FITC-ovalbumin is used as a model protein without the need for thin film hydration (TFH.) Here we compare a typical diblock copolymer using TFH with the new materials and loading method.

Materials and Methods

Block copolymers of PEG-PPS have been synthesized using a new approach to form the intermediate PEG block compared to previously published (Napoli 2001,) and is a similar scheme to work published elsewhere (Bonnans-Plaisance 2003.) Briefly, PEG-Bromine was prepared by first drying the PEG(4750) monomethyl ether in toluene using a Dean-Stark trap. After cooling to room temperature, 2.5 equivalents of thionyl bromine were added, and the mixture was allowed to reflux in toluene for six hours. To purify, the toluene was evaporated and the mix dissolved in a minimum of methylene chloride, and finally precipitated in cold diethyl ether. The precipitate was collected and dried overnight under high vacuum, and analyzed via H'-NMR.

To form the block copolymers, the same basic reaction scheme was followed. A thiol containing monomer would initiate the anionic ring opening polymerization of propylene sulfide under basic conditions. In the case of the triblocks, the polymerization was initiated using ethanedithiol with DBU as a base. After the polymerization, the PPS-EDT-PPS was endcapped with PEG-Br. To form diblocks, the initiating monomer was benzenethiol, and we used the same base (DBU) and PEG(4750)-Br to endcap the reaction. The bromine salts were filtered off using filter cell, and the polymers were then dissolved in methylene chloride and precipitated in cold diethyl ether. The resultant polymers in all cases were analyzed via gel permeation chromatography (GPC) and H'-NMR.

Aggregates of PEG-PPS were produced either by thin film or directly from the addition of an aqueous solution. The diblock copolymer was dissolved in chloroform and slowly evaporated using a rotary evaporator, followed by hydration in 10mm PBS pH 7.4 containing FITC-Ova at 1mg/mL. In contrast, the triblock loaded vesicles were prepared by simply adding the FITC-Ova solution to the dry polymer powder. The aggregates were purified using a column of Sephadex G-200 to remove the free FITC-Ovalbumin and the fractions collected were measured using a fluorescent plate reader.

Samples for sizing (without FITC-Ova) used double distilled water to form the aggragates (ddH2O.) Aggregate size was measured using a dynamic light scattering (DLS) instrument (Malvern, UK.)

Results and Discussion

Gel Permeation Chromatography

Polymer Type	PD	Mp	fPEG
Triblock	1.29	10350	0.92
Triblock	1.22	11181	0.85
Triblock	1.18	12866	0.74
Diblock	1.19	5239	0.91
Diblock	1.16	10720	0.44

Dynamic Light Scattering

PEG(4750)-PPS Block Copolymers

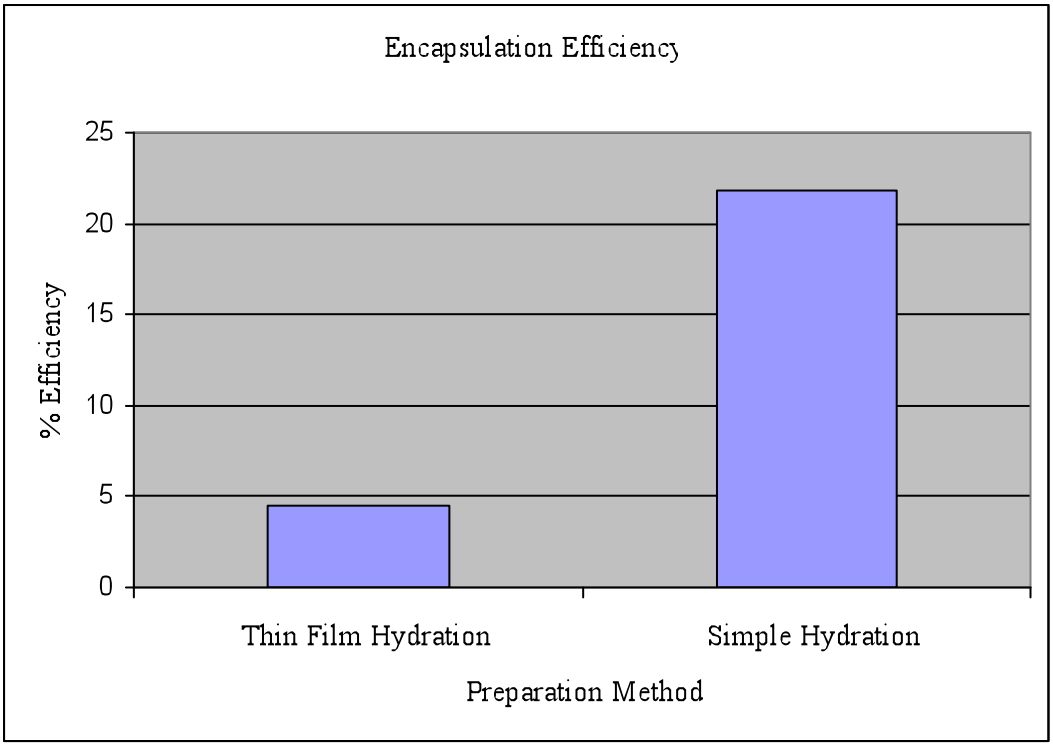
EG(108)-PS(n)

Type	fPEG	Z-Avg	Volume
Diblocks	0.91	55.2	26.4
	0.44	231	249

EG(108)-PS(n)-EG(108)

Triblocks	0.92	148	169
	0.85	304	408
	0.74	355	514

Encapsulation Efficiency of FITC-Ovalbumin



Note: fPEG is defined as: MwPEG/(MwPEG+MwPPS)

Conclusions

As this is a work in progress, any conclusions are tentative. The two main advantages of using these materials are that the efficiency is significantly higher, and that the preparation method does not require the formation of a thin film followed by extrusion to form the uniform loaded vesicles. In this way, the loaded nanocapsules can be formed around sensitive or reactive proteins with minimal processing, and in a short period of time.

References

A. Napoli et al. (2001) *New Synthetic Methodologies for Amphiphilic Multiblock Copolymers of Ethylene Glycol and Propylene Sulfide,* Macromolecules 34, 8913-1917.

C. Bonnans-Plaisance et al. (2003) *New Architectures of poly(ethylene glycol) and poly(methylthiirane) in Block Copolymers,* European Polymer Journal 39, 863-870.