Design of RAFT synthesized amphiphilic and stimuli-responsive block copolymers for encapsulation of polyphenols in polymersomes

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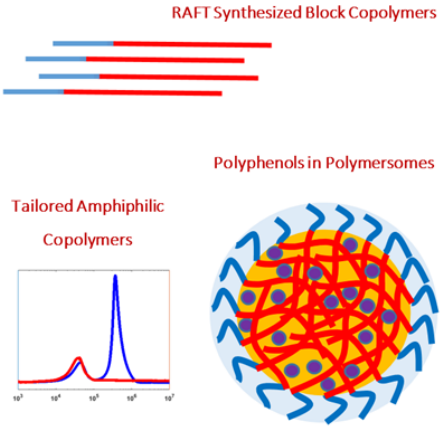

This research is devoted to the design and synthesis of amphiphilic/stimuli-responsive block copolymers for encapsulation of polyphenols in polymersomes. The Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization technique is used to get tailored amphiphilic block copolymers. Multiple combinations between hydrophilic/hydrophobic monomers, RAFT agents and initial compositions are the main design variables of the synthesis runs. Experimental studies are guided by modeling tools to aid in the design of the materials (e.g. to specify the sizes of the two blocks, pH sensitivity). Isolated polyphenols (e.g. resveratrol, quercetin) and more complex extracts from different kinds of plants are afterwards encapsulated in polymersomes, aiming at the development of carriers for protection and controlled release of these bioactive compounds. It is shown that the produced aggregates have a long colloidal stability and are promising vehicles for the stimulated-release of polyphenols.

Introduction

The beneficial effects of polyphenols on human health, namely due to their antioxidant/anti-inflammatory activities and protective actions on cardiovascular system (e.g.) are widely acknowledged by different scientific communities and observed in common populations. However, these bioactive compounds are prone to fast degradation processes (e.g. due to the effect of temperature, pH or light), have a low solubility in biological fluids and their fast metabolism and secretion rates are also commonly observed. Thereafter, the development of vehicles for polyphenols stabilization, protection from degradation and sustained release is a key point to improve their bioavailability and the efficient application in pharmaceutic, biomedical, cosmetic or food industries [1].

Different encapsulation approaches are being considered as possible routes to develop carriers for polyphenols and mitigate the above mentioned issues, namely physical methods (e.g. involving spray drying with a coating agent), physicochemical methods (e.g. based on ionic or hydrophobic interactions, resulting in micelles or liposomes formation) and chemical methods (e.g. in-situ polymerization/crosslinking systems) [1].

In the present research, we explore the Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization technique [2] to design amphiphilic and stimuli-responsive block copolymers for the encapsulation of polyphenols in tailored artificial vesicles (polymersomes [3]). Indeed, among other controlled polymerization techniques, RAFT polymerization offers the possibility to improve the precision concerning the design of special molecular architectures, namely the synthesis of amphiphilic block polymers with segments of tailored size and/or the incorporation of functional groups in polymer structure allowing stimulation (e.g. due to temperature/pH changes).

The work here presented comprises the RAFT synthesis of different kinds of amphiphilic and stimuli-responsive block copolymers, the characterization of the products and the testing of their self-assembly as polymersomes for polyphenols encapsulation. Isolated polyphenols (e.g. resveratrol, rutin, quercetin, etc) and more complex extracts from different kinds of plants (e.g. from chestnut, cherry or olive trees, obtained by hydro-alcoholic or supercritical extraction processes) are considered in these encapsulation studies. The RAFT polymerization synthesis work is accompanied by the development of polymer reaction engineering modeling tools, useful to aid with the design of the block copolymers.

Materials and Methods

The monomers acrylic acid (AA), methacrylic acid (MAA), 2-(dimethylamino)ethyl methacrylate (DMAEMA) and 2-hydroxyethyl methacrylate (HEMA) were used as hydrophilic monomers, leading to the formation of polymer blocks susceptible for stimulation in aqueous environments, namely due to pH changes. Styrene (S) and methyl methacrylate (MMA) were considered to generate hydrophobic moieties in the block copolymers. Azobisisobutyronitrile (AIBN) and 4,4 azobis(cyanovaleric acid) (ACVA) were used as thermal initiators. 2-cyano-2-propyl dodecyl trithiocarbonate (CPDT), 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPA) and 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) were used as RAFT polymerization agents. The RAFT synthesis of the block copolymers was performed in two steps, starting with the formation of the first selected homopolymer in a solution (using dimethylformamide (DMF) or dimethyl sulfoxide (DMSO) as possible solvents) containing the selected amounts of monomer, initiator and RAFT agent. The obtained polymers were purified and dried after stopping the reactions (24 to 48 h are typical reaction times) and the second polymerization step was performed in a homogeneous solution containing the RAFT-macro chain (the first block), the second monomer and a proper amount of initiator (AIBN or ACVA). The block copolymers were obtained after the purification of the products of this second reaction step. The polymerization runs were typically performed at a temperature in the range 60 to 70 °C.

The different homopolymers and block copolymers synthesized were characterized using techniques allowing getting information concerning their composition (e.g. FTIR) and the

size of the homo/copolymer chains. SEC with refractive index and multi-angle laser light scattering detection was used with the later purpose, considering tetrahydrofuran (THF) or DMF as eluents. Note, however, that the SEC analysis of such kinds of polymers is cumbersome due to solubility limitations and multiple interactions between the different domains of the chains, the stationary phases of the columns and the eluent. In spite of the use of salts (e.g. LiBr) non-ideal SEC is often observed with possible erroneous determination of the block sizes. Batch static light scattering was thus also considered in the analysis of the homo/copolymers synthesized in this work.
Polymersomes loaded/non-loaded with polyphenols were prepared through the dissolution of the selected block copolymer in THF (or DMF) in the presence/absence of the bioactive molecule (or a more complex plant extract). Afterwards, water (or other aqueous media) was dropwise added, under stirring, to that solution (e.g. at ~ 0.02 mL/min) up to achieve the final desired composition in the two solvents (e.g. THF/water 50/50). The final solution was dialyzed in a cellulose bag membrane (3.5K molecular weight cut off). The loading efficiency and the release of the different polyphenols in the polymersomes were evaluated through HPLC/UV measurements of samples collected in the successive dialysis processes performed or by re-dissolving the purified loaded aggregates in THF/DMF. For comparison purposes, besides the materials produced in this research, commercially available PS/PAA block copolymers (Sigma-Aldrich, average degree of polymerization PS/PAA=275/50, polydispersity index PDI≤1.3) were also used for polymersomes production and polyphenols encapsulation. Static light scattering was used to get information concerning the structural features of the self-assemblies (e.g. radius of gyration and shape from the form factor *P(q)*) and images of the polymersomes were obtained using TEM.

Results

The self-assembly process of amphiphilic block copolymers in polymersomes and the properties of the resulting aggregates (size, shape, etc) is strongly dependent on the molecular architecture of these polymers, namely on the sizes of the hydrophilic and hydrophobic domains. Thus, our research includes the development of modeling tools (see [4] and references therein) to describe and carry out the design the formation of RAFT synthesized block copolymers. Typical results obtained in this context are presented in Figure 1, where the SEC distributions predicted for RAFT synthesized hydrophilic/hydrophobic homopolymers and for the correspondent amphiphilic copolymer are shown. The experimentally measured distribution for a PS/PAA block copolymer it is also presented in Figure 1, highlighting possible non-ideal effects in SEC analysis of amphiphilic block copolymers (static light scattering is being complementary used to get information concerning the structure of these polymers).

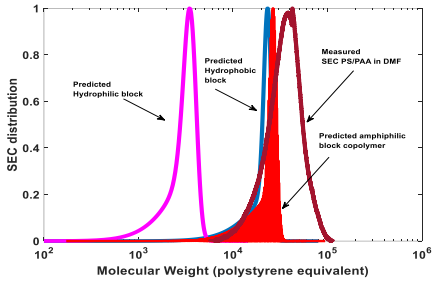

Figure 1. Predicted SEC distributions for RAFT synthesized hydrophilic and hydrophobic polymers and the correspondent block copolymer. The experimentally measured SEC distribution for a PS/PAA block copolymer it is also presented.

In Figure 2(a) are presented photograph images for the final aqueous colloidal suspensions of polymersomes (with/without encapsulated polyphenols) obtained with an amphiphilic block copolymer (PS/PAA). In Figure 2(b) are presented TEM images of synthesized polymersomes showing the possibility to generate spherical aggregates with size below 100 nm. Static light scattering analysis of such colloidal suspensions of polymersomes is also being performed in our research in order to obtain a fast characterization of the aggregates in terms of their radius and shape.
The prepared polymersomes show a colloidal stability of several weeks, confirming their viability for polyphenols encapsulation.

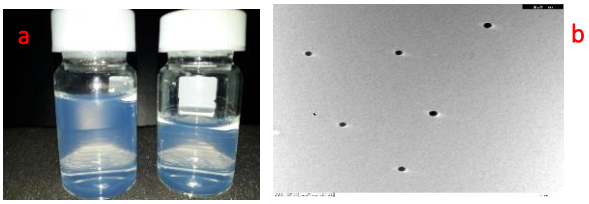

Figure 2. (a) Photograph images for the final aqueous colloidal suspensions of polymersomes with/without encapsulated polyphenol (quercetin). (b) TEM images of synthesized polymersomes showing the formation of spherical aggregates with size below 100 nm.

Conclusion

The usefulness of RAFT synthesized amphiphilic copolymers for polyphenols encapsulation was showed. Their stimulated release from the polymersomes is currently being addressed.

Acknowledgements

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