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Amino Acid Based Biodegradable Amphiphilic Polymers and Micelles as Drug Delivery Systems: Synthesis and Study

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***Abstract*—**Nanotherapy is an actual newest mode of treatment numerous diseases using nanoparticles (NPs) loading with different pharmaceuticals. NPs of biodegradable polymeric micelles (PMs) are gaining increased attention for their numerous and attractive abilities to be used in a variety of applications in the various fields of medicine. The present paper deals with the synthesis of a class of biodegradable micelle-forming polymers, namely ABA triblock-copolymer in which A-blocks represent amino-poly(ethylene glycol) (H_2N-PEG) and B-block is biodegradable amino acid-based poly(ester amide) constituted of α-amino acid – L-phenylalanine. The obtained copolymer formed micelles of 70±4 nm size at 10 mg/mL concentration.

***Keywords*—**Amino acids, biodegradable poly(ester amide), amphiphilic triblock-copolymer, micelles.

I. Introduction

PHARMACEUTICAL nanotechnology has gained increasing interest in the past few decades as promising therapeutic and imaging tool. Advantages of nanotechnology-based drug carriers (nanocarriers) include the small sizes compatible with intravenous injection and the large surface area per unit volume amenable to modification for targeted delivery. Therefore the application of pharmaceutical nanocarriers has a great potential in the treatment of various diseases. PMs are gaining increased attention for their ability to serve as viable carriers for site specific delivery of vaccines, genes, drugs and other biologicals in the body. They have enhanced biocompability, superior drug/vaccine encapsulation, the capacity for solubilization of water-poorly soluble drugs and pharmaceuticals [1]. The micelles can also be used as vehicles delivering a therapeutic agent to specific tissues or cells with suitable release profiles of the cargo in a sustained fashion, thereby reducing the agent's systemic toxicity. They can also facilitate the penetration of therapeutic agents through biological barriers. Due to the mentioned properties PMs represent an effective delivery system for poorly water-soluble anticancer drugs. PMs with small size (10–100 nm) reveal a long circulation time in the blood and increased cancer accumulation [1], [2].

The therapeutics and chemotherapeutic agents used in treatment of numerous diseases (including cancer) are characterizing by water-insolubility, the short circulation time and toxicity. At the beginning of the 20th century Paul Ehrlich (Nobel Prize winner for Physiology or Medicine, 1908) suggested the innovated approach to create target drug delivery systems to increase their therapeutic outcomes. At the last decades, the scientists working in the fields of nanoengineering and cancer therapy created new generation of nanocarriers constructing by various building blocks and mechanisms such as for example: liposomes and micelles. Nevertheless disadvantages and limitations having the used nanocarriers need to improve the technology of drug and anti-tumor agents delivery and the studies also are still topical [3], [4]. In modern nanomedicine, for the delivery of poorly water-soluble drugs, the most promising are PMs, due to their small size (10-100 nm), which is important and crucial for passive targeting of solid tumors, especially poorly vascularized tumors [5], [6].

Depending on the kind of intermolecular forces block copolymer micelles are: Hydrophobically assembled amphiphilic micelles, polyion-complex micelles, and micelles stemming from metal complexation [7]. The hydrophobically assembled micelles are obtained on the basis of amphiphilic macromolecules building by hydrophobic and hydrophilic domains [8]. The amphiphilic molecules self-assemble into supramolecular core/shell structures in the aqueous medium. Therefore various water-insoluble drugs can be attached to its hydrophobic cores.

Depending on the relative length of hydrophobic/ hydrophilic blocks of micelles and solvent environment, micelles can have different shapes like vesicles, tubules, spheres, rods, lamellae [6], [9]-[11]. The pharmacokinetic properties of micelles depend on their morphology. For example, the circulation time of worm-like filomicelles is ten times longer than the spherical counterpart made of similar material [12].

A variety of PMs drug delivery systems including charged and neutral polymers of both natural and synthetic origin have been developed [1], [13], [14]. However, a few of polymers have the capacity of forming stable micelles, having good solubilising properties, releasing non-toxic and easily metabolized products during biodegradation. Therefore, the search for new and more universal micelle-forming polymers is still topical.

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Poly(ethylene glycol) (PEG) is one of the most used hydrophilic block for obtaining drug delivery micelles. It is characterizing by well water-solubility. In addition, it is not toxic and charged. PEG can form a hydrophilic heart (corona) on the surface of micelles which minimizes the nonspecific interaction with blood particles as well as prolongs the circulation time.

While the PEG still represents the most popular biocompatible hydrophilic block, various hydrophobic polymeric blocks can be attached to it. Among these hydrophobic blocks, the most promising are nontoxic and biodegradable polymers. Biocompatibility and biodegradability are two important prerequisites in designing these micellar carriers for clinical application. The biodegradation of the block-copolymers is highly desirable to provide the clearance of nano-carriers from the body after their function is fulfilled.

In the polymeric materials, polyesters (like poly(lactic acid) (PLA), poly(glycolic acid), poly(lactic-co-glycolic acid), etc.) are the hydrophobic domains. Polyesters undergo enzyme-catalyzed hydrolysis *in vivo* and thus are considered biodegradable. But they have some important limitations [15] such as:

- Releasing acidic products (glycolic and lactic acids having pK_a 3.83 and 3.86, accordingly) upon biodegradation which exhibit some undesirable side-effects at cellular level;
- low affinity to living tissues (due to the lack of hydrophilic CO-NH bonds in the backbones);
- short shelf-life.

Among different studies carried out with the aim of creating biodegradable bio-materials one of the best approaches was developed by Katsarava et al. at the Research Center of Medical Polymers and Biomaterials of Georgian Technical University. Georgian researchers created a large variety of Amino Acid Based Biodegradable polymers (AABBPs): like poly(ester amide)s, PEAs, poly(ester urethane)s, PEURs, and poly(ester urea)s, PEUs. The polymers are tested as carriers and matrices for covalent immobilization/impregnation of medications, enzymes and other bio-chemicals. They are used as substrates in *in vitro* enzymes' catalyzed hydrolysis (biodegradation) and for producing both porous scaffolds and micro- or nano-containers for drug delivery, etc. [16].

One of the most important classes of AABBPs are PEAs. These polymers combine all advantages of aliphatic polyesters and polyamides. There are: biodegradability (PEAs), an affinity to tissues causing a good compatibility with them, and a wide range of mechanical properties. The scopes of applications of PEAs can substantially be expanded by their functionalization through the incorporation of chemically active and/or hydrophobic groups into the backbones or lateral chains. Functional PEAs can be attached to various bioactive compounds by covalent or non-covalent bonds [17].

Considering above the NPs (like micelles) designed from mentioned AABBPs were expected to have an enhanced bioavailability. Therefore we exploited one of the AABBPs in our research.

In our previous study [18], we reported about micelle-forming ABA triblock-copolymer consisting with the biodegradable poly(ester amide) (PEA) on the basis of α-amino acid L-leucine, 1,6-hexane diol and sebacic acid (M_w *ca*. 4000 Da) as a hydrophobic block "B" and H_2N-PEG (M_w 2000 Da) as a hydrophilic block "A". The present paper deals with micelle-forming ABA triblock copolymer in which biodegradable PEA composed of more hydrophobic α-amino acid L-phenylalanine, 1,6-hexane diol and sebacic acid is used as a block "B", and H_2N-PEG-2000 as a block "A". We assumed that increased hydrophobicity of the block "B" (i.e. more hydrophobic core of the micelles) can improve the solubilizing potential of the nanoparticales. It has to be mentioned that PEAs composed of the hydrophobic amino acids, fatty diols and dicarboxylic acids showed reasonable biodegradation rates and excellent biocompatibility [16], [17].

II. Experimental Details

A. Materials and Procedures

L-phenylalanine (F), 1,6-hexanediol, sebacoylcloride, p-nitrophenol, p-toluenesulfonic acid monohydrate, and solvent - diethyl ether were purchased from Sigma-Aldrich. H_2N-PEG (M_w 2000) (amino-PEG-2000) was purchased from Laysan Bio, Inc.. All of listed materials were used without further purification. Solvents, Dimethysulfoxide (DMSO) and triethylamine (TEA) (from Sigma-Aldrich), were dried and purified according to standard procedures prior the use.

For the synthesis of micelle-forming triblock amphiphilic copolymers, we applied the one pot/two-step method which was found the best among the strategic approaches we have examined [18].

B. Measurements and Techniques

The resulting ABA triblock-copolymer was characterized by FTIR spectroscopy. The spectrum was recorded at the spectrometer "FTIR THERMO NICOLET" Avatar 360, Multi-Bounce Flat Plate 45 degree Ge, Diapason 400-4000 cm^{1}. For the determination of the Critical Micelle-forming Concentration (CMC) and sizes of the obtained micelles, we used Dynamic Light Scattering (Malvern zetasizer Nano ZS ZEN3600, malvern, UK, equipped with "red" laser (633 nm)).

C. Synthesis

At the first stage, the key monomers were obtained. In this study was used two monomers: di-*p*-toluenesulfonic acid salt of bis-(L-phenylalanine)-1,8-hexylene diester, F8, and di-p-nirtophenylsebacate, *p*NFS.

Synthesis of di-p-toluenesulfonic acid salt of bis-(L-phenylalanine)-1,8-hexylene diester (F6) was carried out according to Fig. 1.

The monomer was synthesized by direct condensation of 0.44 Mole of L-phenylalanine (F) with 0.2 Mole of 1.6-hexanediol in the presence of 0.44 Mole p-toluenesulfonic acid monohydrate (TosOH) in 600 mL of refluxed benzene during 16-18 h using Dinn-Stark trap to collect the liberated water. The reaction proceeded heterogeneously. After cooling to room temperature the obtained solid product was filtered

off, washed with water, and recrystallized from water. Yield 97%, m.p. 213-215 °C coincides with previously reported data in [16].

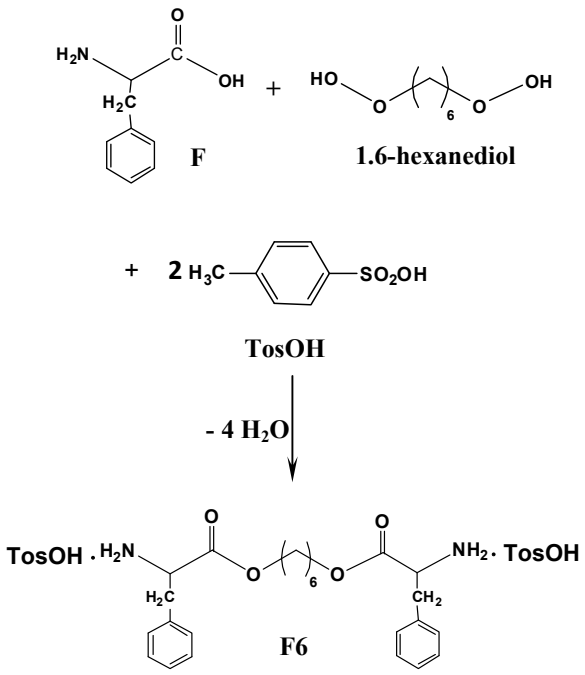

Fig. 1 The synthesis of F6

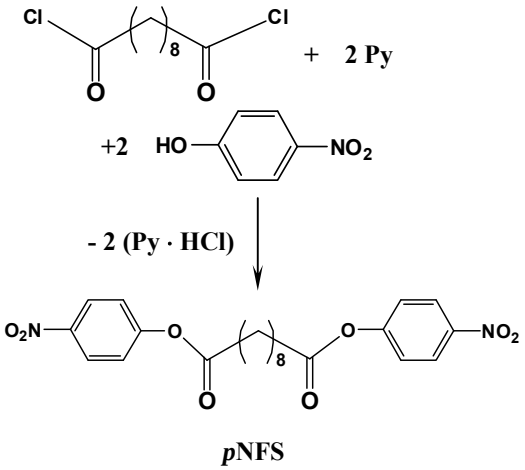

Fig. 2 The synthesis of ***p*NFS**

Synthesis of activated di-p-nirtophenylsebacate (pNFS) was carried out by acceptor-catalytic method. To a chilled (to 0-5 °C) and stirred mixture of p-nitrophenol (2.1 Moles), pyridine (Py) (2.1 Moles) and ethyl acetate (200 mL) a predetermined volume of sebacoyl chloride (1 Mole) in ethyl acetate (100 mL) was added drop-wise. The reaction mixture was detained for 1 hour, then was filtered off, washed with acidic water (HCl) and dried. The resulting product was recrystallized from ethyl acetate. Yield 82 %, m.p. 106-108 °C coincides with previously reported data in [16].

The synthesis of activated di-p-nirtophenylsebacate is given in Fig. 2.

The synthesis of the goal triblock-copolymers: Our synthetic strategy is based on the preparation of oligomeric poly(ester amide)s (PEAs), terminated with activated ester groups as an intermadiate electopholic building block for constructing block-copolymers, with subsequent covalent attachment of hydrophilic PEG-block *via* the amide bond using amino-PEGs. A high stability of the activated ester groups in polar aprotic solvents [19] is a guarantee of the successful synthesis of electrophilie-terminated oligomers ("living oligomers") and manipulation with them in solution without substantial complications.

The synthesis of micelle-forming block-copolymers *via* the one pot/two-step method was carried out as follows: at the first step the intermediate telechelic PEA containing activated ester end-groups (Step 1, Fig. 3) was synthesized by solution polycondensation (DMSO, 25°C, 18 h, TEA as an acid acceptor) of F8 with *p*NFS, at a mole ratio F8/*p*NFS = 7/8 that led to the telechelic PEA with an average M_w of 4233.75 Da; at the second step the telechelic PEA was interacted *in situ* (25 °C, 24 h) with 2 moles of amino-PEG-2000 (Step 3 in Fig. 1) resulting in the goal ABA triblock-copolymer depicted in Fig. 3.

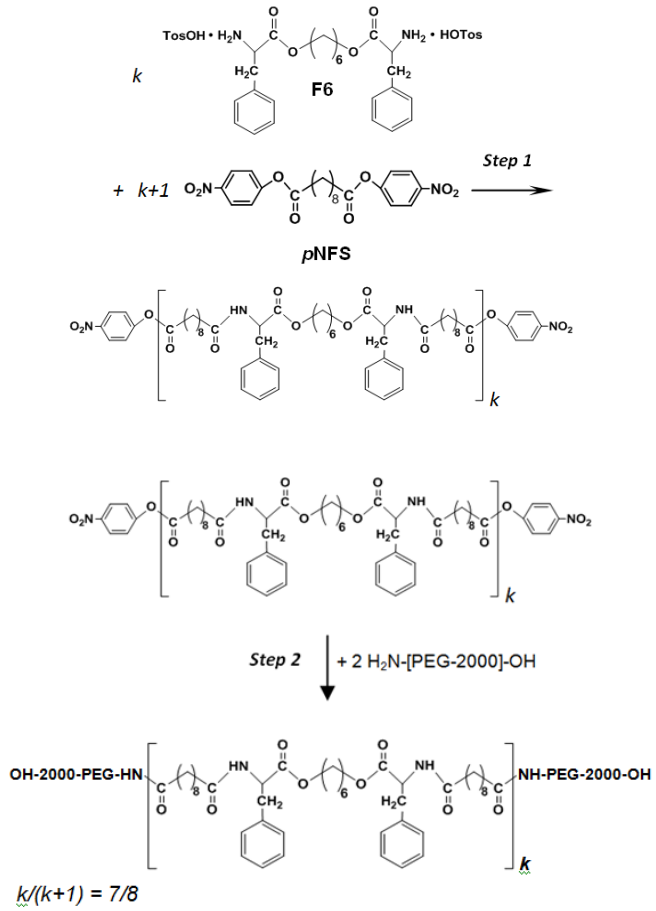

Fig. 3 The synthesis of micelle-forming biodegradable ABA triblock-copolymer

D. Results and Discussion

The ABA triblock-copolymer obtained was tested for the micelle-forming property. This study showed that obtained polymer can form 70±4 nm stable micelles within a concentration range 1-10 mg/mL which is considered as an optimal range for pharmaceutical micelles [2].

We assume that the micelles from obtained ABA triblock-copolymer are formed by the folded molecules as it is shown in Fig. 4.

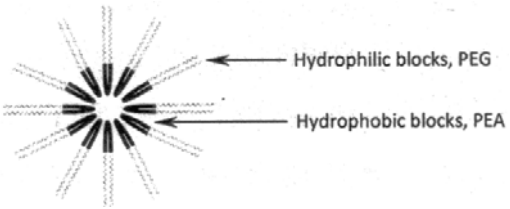

Fig. 4 Schematic representation of the micelle formed by folded molecules

III. Conclusion

Because of their distinct advantages such as small size, high solubility, and controlled release of drugs, PMs seem to be the prototype of an ideal carrier for poorly water soluble drugs. The obtained preliminary data allow concluding that the ABA triblock-copolymer we have obtained is promising for constructing biodegradable micellar nanocarriers suitable for delivering poorly water-soluble drugs. More detailed studies of the obtained micelles including the interaction with hydrophobic drugs are in progress now.

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