NANO-SIZED MICELLES FORMED BY SELF-ASSEMBLING OF POLYLACTIDE/POLY(ETHYLENE GLYCOL) BLOCK COPOLYMERS IN AQUEOUS SOLUTIONS

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Introduction

Biodegradable aliphatic polyesters such as polylactide (PLA) and polyglycolide (PGA) have attracted much attention as biomaterials due to the biocompatibility and degradability. These polymers have been used for temporary therapeutic applications such as sutures, osteosynthetic devices, sustained drug delivery devices, and scaffolds in tissue engineering [1-3]. Hydrophilic poly(ethylene glycol) (PEG) blocks have been incorporated into PLA backbones to make copolymers with suitable hydrophilicity and degradability. PEG presents outstanding physicochemical and biological properties, and is able to form a palisade avoiding protein adsorption and subsequent non-specific uptake by the reticuloendothelial system (RES) after intravenous injection [4].

PLA/PEG block copolymers have been widely investigated as drug carriers in the form of microparticles, nanoparticles, and hydrogels [5-8]. The aim of this work was to investigate the micellization properties of water soluble PLA-PEG-PLA triblock copolymers, which should be of great interest for applications in the field of drug delivery.

Materials and methods

PLA-PEG-PLA triblock copolymers were prepared using ring-opening polymerization of L- or D-lactide, in the presence of PEG (M_n=4600) and zinc lactate (0.1 wt%) [9].

PLLA-PEG-PLLA or PDLA-PEG-PDLA copolymers were dissolved in distilled water to yield homogeneous micellar dilute solutions. The two solutions with equal molar concentrations were then mixed to obtain a micellar solution by self-assembling.

Polymeric micelles containing paclitaxel were prepared as follows: polymer (50mg) and paclitaxel (10mg) were dissolved in 1-methyl-2-pyrrolidone (1ml), the solution was dropwise added to 5ml phosphate-buffered saline (PBS, pH 7.4) under supersonic stirring to obtain a microemulsion. The solution was then centrifuged (1000rpm, 30min) to remove the unincorporated paclitaxel.

Paclitaxel-loaded micellar solution was added in a dialysis bag (MWCO=7000) which was then placed in 100ml of PBS. *In vitro* drug release was allowed to proceed at 37°C.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at room temperature with a Bruker spectrometer operating at 250MHz by using DMSO-d_6 as solvent. Differential scanning calorimetry (DSC) thermograms were registered with a Perkin-Elmer DSC6 instrument, the heating rate being 10°C/min. Surface tension of PLA/PEG dilute solutions was determined with a Kruss tensiometer K100. Dynamic light scattering (DLS) was measured using a commercial laser light scattering spectrometer (Malvern Autosizer 4700, Malvern Instrument, Worcs, UK). High-performance liquid chromatography (HPLC) was performed with a LC-10A apparatus (Shimadzu) equipped with a UV detection (SPD-10A, Shimadzu) and a 218MR54 column (4.6×250mm, C_{18}, Vydac, USA). The mobile phase was acetonitrile/water (55:45 v/v) with a flow rate of 1.0 ml/min.

Results and discussion

TABLE 1 presents the molecular and thermal characteristics of the copolymers as determined by ^{1}H NMR and DSC. For the sake of simplicity, triblock copolymers were named as $L_xEO_yL_x$ or $D_xEO_yD_x$ where L, D, and EO represent PLLA, PDLA, and PEG blocks, respectively, x and y representing the number-average degree of polymerization of corresponding blocks.

It is well known that polymeric micelles can be formed only when the polymer concentration is higher than the critical micellar concentration (CMC) which characterizes the micelle stability [14]. The CMC values were obtained from surface tension (γ) measurements of the micellar solutions. FIGURE 1 shows the γ vs. lgC plot of $L_{12}EO_{104}L_{12}$ aqueous solutions. The intersection point at 0.050g/l is estimated to be the CMC of this copolymer. $D_{13}EO_{104}$ D_{13} exhibits the same CMC as $L_{12}EO_{104}L_{12}$. However, the mixed solution of $L_{12}EO_{104}L_{12}$ and $D_{13}EO_{104}D_{13}$ presents a lower CMC = 0.040g/l, thus confirming that mixed micellar solution is more stable than separate ones due to stronger interactions between PLLA and PDLA blocks. These values appeared remarkably lower than those of low molar mass surfactants, indicating that micelles formed from PLA/PEG copolymers as drug carriers are susceptible to retain thermodynamic stability without dissociation even after intravenous injection which induces severe dilution.

CMC measurements were performed in 0.1M NaCl aqueous solutions and at 37°C to simulate physiological conditions. As shown in TABLE 2, there is no significant difference between the CMC values of the copolymers in pure water or in NaCl solutions. The insensitivity of the CMC to electrolyte addition is probably due to the non-ionic nature of the polymers, in agreement with literature data [10]. In contrast, the CMC values at 37°C appeared slightly lower than those at 20°C. This finding could be attributed to the increase in hydrophobicity or loss of polarity of PEG at elevated temperatures, thus leading to dehydration of PEG chains and a subsequent decrease in the CMC [11]. On the other hand, chain mobility is improved with increasing temperature, the probability for hydrophobic PLA segments to meet each other and further assemble to form the inner core of micelles is enhanced. Similar findings have been reported in literature [11,12].

DLS measurements were performed to determine the size and size distribution of the micelles. Average diameters of 115.1nm and 108.5nm were obtained for micelles from $L_{12}EO_{104}L_{12}$ and mixed aqueous solutions at a concentration of 1.0 g/l. The polydispersity factors were fairly low (0.2-0.3), indicating a narrow size distribution. It has been reported that micelles less than 200nm can prevent spleen filtering and tend to accumulate at the tumor sites due to the facilitated extravasation [13,14]. The small size of PLA/PEG micelles should enable them to safely achieve the disease site. On the other hand, the micelle size of the mixed solution

Polymer	EO/LA [a]	DP_{PEG} [b]	DP_{PLA} [c]	M_n [d]	T_m(°C) [f]	H_m(J/g) [f]	T_g(°C) [g]	T_c(°C) [g]
$L_{12}EO_{104}L_{12}$	4.2(3.0) [e]	104	24	6330	55.7	96.7	-46.8	-26.2
$D_{13}EO_{104}D_{13}$	4.1(3.0)	104	26	6470	54.2	97.8	-47.1	-32.5
PEG4600	-	104	-	4600	67.5	170.8	-	-

[a] Calculated from the integration of NMR bands belonging to PEG blocks at 3.6 ppm and to PLA blocks at 5.2 ppm. [b] $DP_{PEG} = M_{nPEG}/44$. [c] $DP_{PLA} = DP_{PEG}/(EO/LA)$. [d] $M_n = DP_{PEG}\cdot 44 + DP_{PLA}\cdot 72$. [e] Data in parentheses corresponding to EO/LA ratios in feed. [f] Obtained from the first heating. [g] Obtained from the second heating.

TABLE 1. Molecular Characteristics and Thermal Properties of PLA/PEG Block Copolymers.

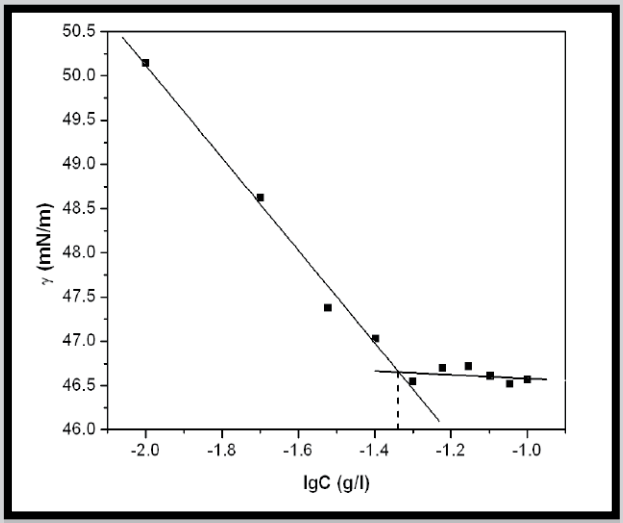

FIG.1. Surface tension changes of $L_{12}EO_{104}L_{12}$ solutions as a function of concentration.

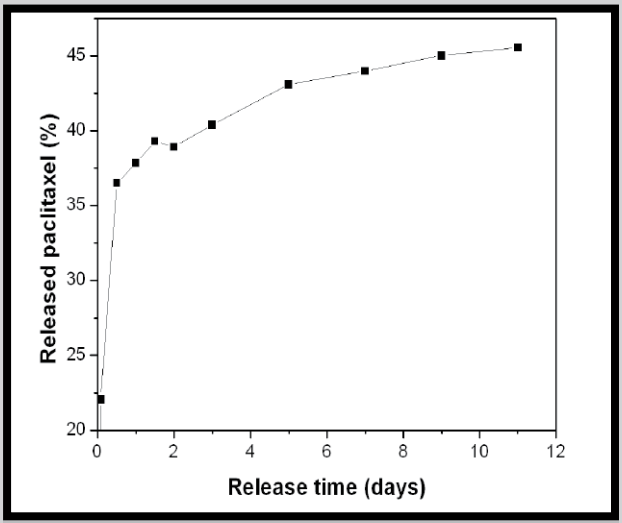

FIG.2. Paclitaxel release profile from polymeric micelles of L12EO104L12 aqueous solutions.

appeared smaller than that of the separated one, which is assigned to the more compact structure due to stronger interactions between PLLA and PDLA blocks.

Paclitaxel is regarded as one of the most successful anticancer drugs. It has been widely applied to treat various cancers, especially breast and ovarian cancer. FIGURE 2 shows the cumulative release curve of paclitaxel from the micelles in vitro. A biphasic release profile is observed. In the first 12 hours, 35% of paclitaxel were rapidly released. Afterwards, the release rate slowed down and nearly 45%

Copolymer	EO/LA	CMC(g/l)		
		Water (20°C)	0.1M NaCl (20°C)	Water (37°C)
$L_{12}EO_{104}L_{12}$	4.2	0.050	0.046	0.045
$D_{13}EO_{104}D_{13}$	4.1	0.050	0.052	0.045
mixed[a]		0.040	0.048	0.037

[a]mixed micellar solution of PLLA/PEG and PDLA/PEG copolymers

TABLE 2. CMC Values of PLA/PEG Triblock Copolymers and Mixed Solution at 20°C, in 0.1M NaCl aqueous solutions and at 37°C.

of paclitaxel were released within 12 days. Compared with the release profile of paclitaxel from PLA-PEG-PLA nanoparticles obtained by solvent extraction/evaporation method in which a total of 49.6% paclitaxel was released within 1 month,15 the micelles in our work exhibited faster release rate. This facilitated release can be ascribed to the less compact structure of the dynamic micelles prepared by self-assembly in aqueous solutions.

Conclusion

Bioresorbable polymeric micelles were prepared from aqueous solutions of PLA-PEG-PLA triblock copolymers. The micellar solutions exhibited very lower CMC values, the mixed micellar solution of PLLA/PEG and PDLA/PEG copolymers appearing more stable than separate ones due to stronger interactions between PLLA and PDLA blocks. CMC measurements in the presence of salt and at 37°C indicated that the polymeric micelles could keep good stability under physiological environment. The size of micelles was around 100nm with a narrow size distribution. The release profile of paclitaxel from the micelles shows a biphasic pattern with 45% of drug released within 12 days. Therefore, PLA/PEG micelles are of great interest as injectable drug carriers because of the advantages as compared to most drug-delivery systems, especially easier formulation and absence of toxic organic solvents. Further studies are underway to investigate the degradation properties of the micelles and the effects of copolymer composition on the drug encapsulation and drug release.

Acknowledgements

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HIGH-HYDROPHILIC MEMBRANES FOR DIALYSIS AND HEMODIALYSIS

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Researches of high-hydrophilic and tromboresistive dialysis membranes have been carried out and possibility of their creation using polyvinylpyrrolidone has been confirmed.

Development of hemodialysis membranes, cardiovascular implants and other artificial organs put forward the problem of thromboresistive materials creation [1]. One of the effective ways of thromboresistance increase is immobilization of heparin, which is blood natural anticoagulant, over material surface. The main problem of heparin immobilization by polymeric membranes is its permanent minimal desorption at a contact with blood.

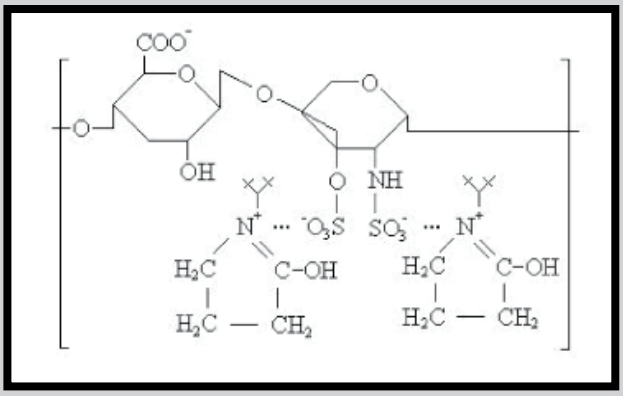
Researches concerning medical polymers syntheses and application are carried out at the Department of Chemical Technology of Plastics Processing of Lviv Polytechnic National University. These researches are directed mainly on the synthesis of new and modification of already existent polymers. Polyvinylpyrrolidone (PVP) was chosen as a base initial product after the protracted approbations. Originality of PVP properties and application is stipulated by its structure and physico-chemical properties. The presence of carbamate group favors high selective-sorption properties, complexation with iodine and other compounds and the formation of macromolecules ionic form in aqueous medium [2]. In addition to the foreseen PVP physiology activity and functional ability, it positively affects the kind of polymerization reaction at the synthesis of its copolymers.

Netted copolymers of oxyalkylenmethacrylates with polyvinylpyrrolidone [3] are perspective compounds for the production of dialysis membranes. The presence of PVP ionogenic groups in the composition of mentioned copolymers assumes the expansion of biochemical and sorption characteristics and obtaining of membranes with additional functions on their basis.

Hydrogel membranes were obtained by graft polymerization of 2-hydroxyethyl methacrylate (HEMA) over PVP (molecular mass was $10\text{-}50\cdot10^3$) in aqueous medium, what allowed to combine the synthesis stage and membrane swelling. Before the researches membranes were washed with the distilled water during 48 hours for the removal of unreacted products. The permeability of the synthesized hydrogel membranes in the dialysis process for the aqueous solutions of sodium chloride was determined at the special dialyzer with peristaltic pump. The saturation of membranes with heparin was realized in glycerin buffer solution (1M glycerin solution, pH=2,7), which contained 250000 units of heparin in 1 l. The amount of sorbed and desorbed heparin was determined by photocolorimetry, based on quantitative determination of heparin and methylene blue complex.

Synthesized hydrogel membranes with PVP links have advanced immobilization ability relative to heparin (TAB.1).

Increased content of heparin on membranes with PVP is assigned, to our opinion, by the formation of ionic connections between heparin and PVP macromolecules. Also it should be taken into account that PVP link may exist as ketoform or in the form that contains nitrogen cationic atom [2]:



In spite of the fact that part of cationic form is insignificant [2], mentioned links connect heparin anions efficiently. As a result, PVP-heparin complex is so strong, that heparin does not precipitated for 24 hours (see TABLE 1) at membranes keeping in solutions with different pH (glycin buffer solution with pH=2,7, physical solution with pH=7 and solution of sodium tetraboric acid with pH=9,1). Here the selective-transport characteristics of membranes are changed insignificantly. As for membranes based of polyHEMA and modified cellulose, there is an insignificant precipitation of anticoagulant in acid and neutral media, while in alkaline medium it grows to 80...95%.

We have established that the presence of –OH and N–C=O hydrophilic groups in the composition of membrane copolymers increases their sorption ability which is characterized with water content (TABLE 2). The increase of PVP content multiplies dialysis permeability (KNaCl) of hydrogel membranes based on HEMA-PVP, but their strength falls down (TABLE 2). Hence, changing hydrogel chemical structure it is possible to change permeability of membranes on the basis of HEMA-PVP copolymers.

High-hydrophilic membranes synthesized on the basis of HEMA-PVP copolymers sorb plenty of water and form polymeric hydrogels, possessing high elasticity. All these factors also create additional preconditions of successful coexistence with biological tissues similar to the physical