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Well-defined nanoparticles from poly(*N*-vinyl pyrrolidone-*b*-dimethylsiloxane) prepared by conventional radical polymerization

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Abstract. Hollow polymer particles with dimensions in the submicrometer region and prepared from amphiphilic block copolymers have attracted grate interest during last decades, because of their tendency to self-assemble in polymeric nanostructures for a number of technological applications. Conventional molecular chemistry requires costly synthetic procedures and only in special cases allows such particles to be prepared with exact control over their size and morphology. We show here one possibility to polymerize 1-vynil-2-pyrrolydon (VP) with conventional radical polymerization by rarely used poly (dimethylsiloxane) macroazoinitiator (MAI). The library of poly(dimethylsiloxane-b-vinyl pyrrolidone) (PDMS-b-PVP) polymers with different chain lengths were synthetized and characterized by numerous methods. The spontaneous formation of vesicles, their morphology, size distribution and size control were investigated. Preliminary cell test was also examined.

Key Words: **poly (dimethylsiloxane) (PDMS), poly (vinyl pyrrolidone) (PVP), macroazoinitiator (MAI), diblock copolymer, DLS, polymeric vesicles**.

I. INTRODUCTION

Vesicles obtained from amphiphilic block copolymers, known as "polymersomes" and used like bioactive molecules carrier have become very important and attractive class of materials. In recent years, one of the priority directions in polymer chemistry progress is related to synthesis of new amphiphilic block copolymers and the obtaining of biocompatible polymeric nanostructures for medical and tissue engineering applications. Amphiphilic block copolymers represent a special class of polymers. They possess many unique properties, both in bulk and in solution [1], which make them widely used in the pharmaceutical sector as drug carriers [2]. Typically, they are composed from one hydrophobic and one hydrophilic part. The chemistry of hydrophilic block will dictate the properties of the vesicles in water, such as adhesiveness in biological environments, the ability to resist against protein adsorption, which is a key property for "stealth" drug delivery systems. Amorphous and rubbery hydrophobic blocks will confer to the membranes the liquid-like character typical for lipid bilayers as well as it will dictate the permeability of solutes in water. One very important polymer which in general is able to give "stealth" properties to polymersomes is poly(ethylene glycol) (PEG). PEG does not trigger an immune response[3] demonstrated also by group of Discher[4]. On the other hand, in the literature are reported many examples of PEGylated biodegradable polymer nanostructures obtained from PCL-*b*-PEG or PLA-*b*-PEG copolymers. The effect of blocks ratio[5,6,7], as well as encapsulation efficiency[5,8,9], leaking kinetics[10,11] and even circulation time[10,8] have been well-investigated.

In last decade, one of the most investigated and bioinert dense polymer material for synthesis and preparation of (amphiphilic) block copolymers is poly (dimethylsiloxane) (PDMS). This polymer has been combined with PEG resulting in material possessing water contact angle lower than conventional PDMS [12] or forming micellar structures [13] and vesicles to multilamellar aggregates [14]. Anionic polymerization, such pretentious and complicate method has been used to prepare conductive copolymers of PDMS-*b*-P2VP [15] and PDMS-*b*-P4VP[16]. Other PDMS based ionomers, synthesized by group transfer polymerization [17, 18] such as PDMS-*b*-PMAA and PDMS-*b*-PAA or synthesized by free radical polymerization [19], are reported. Additionally, block copolymers containing PDMS, considered as "organic-inorganic" elastomer, exhibit unique properties such as hydrophobicity, flexibility, very low glass transition temperature, low surface energy, tension and solubility parameters[20], excellent film forming properties as well as chemical and physiological inertness [21, 22]. These characteristics make polysiloxanes interesting as blocks for the preparation of amphiphilic block copolymers with properties that vary from those of many other organic polymers. On the other hand, one of the hydrophilic block that can be associate to PDMS giving possible "stealth" properties as poly(ethylene glycol) (PEG) is poly(N-vinyl pyrrolidone) (PVP). PVP is well-known water-soluble, biocompatible polymer, which have been initially used as a blood plasma substitute and later in a wide variety of

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applications in medicine, pharmacy, cosmetics and industrial production [23]. PVP has unique property to prevent water from freezing and this has led to the use as cryoprotectant of cells [24], lyoprotectant of proteins [25] or to stabilize them [26] and/or even to prevent DNS degradation in extracellular matrix [27]. Besides, PVP increases the circulation time of peptides as PEG [28[and colloids in vivo[29], as well as increase the water-solubility of some pharmaceutical drugs[30, 31]. Till now in the literature, it has been reported only one study of PVP/PDMS incorporation resulting in ABA-type block copolymer [32], and its properties are poorly studied. No data for AB-type copolymer were found in the literature.

In this paper, we report the synthesis of amphiphilic poly (dimethylsiloxane)-*b*-poly(vinyl pyrrolidone) diblock copolymer by free radical polymerization by means with non-commonly used PDMS macroazoinitiators. A series of block copolymers has been synthesized from PDMS and PVP exhibiting different chain lengths allowed us to evaluate the effect of polymer ratio on the diblock copolymers properties. The structures of block copolymers and thermal properties were characterized by common spectroscopic and thermal methods. Moreover, spontaneous hollow particles formation in water as well as their size, polydispersity and morphology were examined.

II. MATERIALS

Mono (hydroxyalkyl)-terminated poly (dimethylsiloxane) ($PDMS_{4k}$-OH, M_n=4 670 g mol^{-1}), 4,4'-azobis(4-cyanovaleric acid) (ACVA), 4-(Dimethylamino) pyridine (DMAP), and DCC were obtained from SIGMA-Aldrich and used as received. 1-vinyl-2-pyrrolidone was also from SIGMA-Aldrich, but purified by column chromatography using alumina. Mono (carbinol)-terminated poly (dimethylsiloxane) ($PDMS_{10k}$-OH, M_n=10 000 g mol^{-1}, viscosity: 150-250 cSt) was purchased from Gelest Inc. (Morrisville PA, USA). Dichloromethane, chloroform, tetrahydrofuran, methanol and diethyl ether were purchased from Carl Roth and used as received. The purity of all solvents is 99.0 %.

A Synthesis of PDMS macroazoinitiators

PDMS macroazoinitiators were synthesized from PDMS5k-OH and PDMS10k-OH, using modified procedure of Libang Feng et all19. In a 100 ml round-bottom flask was placed 35.5g PDMS4k-OH (7.4 mmol, Mn=4670 g mol-1, d=0.97 g mL-1) or 74.5g PDMS10k-OH (7.4 mmol, Mn=10000 g mol-1) dissolved in 20 mL dichloromethane to which solution was added 1.2 g ACVA (4.4 mmol). The solution was cooled down to 0o C and then 1.5 g (7.4 mmol) of DCC dissolved in 20 mL dichloromethane were added, followed by the addition of 0.14 g of DMAP (0.1 mmol). The mixture was vigorous stirred in the cooling bath for 5 min and followed by mixing for 168 hours at room temperature. The resulting viscous suspension was filtered to remove the dicyclohexylurea (DCU). The PDMS macroazoinitiators were purified by dialysis for 1 week, leading to colorless and viscous products with yield of 87 % for PDMS4k-ACVA and 92 % for PDMS10k-ACVA.

B Synthesis of PDMS-b-PVP amphiphilic block copolymers

To prepare a series of PDMS4k-b-PVP and PDMS10k-b-PVP copolymers was used free radical polymerization initiated by MAI. PDMS macroazoinitiators were placed in presence of 1-vinyl-2-pyrrolidone (VP) monomer, purified by column chromatography, in different ratios without solvent and by considering a total amount of MAI and VP of 3 g, in 10 mL Schlenk flask. The mixtures were bubbled with Ar stream, sealed and after that all tubes were immersed into thermostatically controlled oil bath at 70o C for 12 h. The resulting block copolymers were dissolved in chloroform, slowly precipitated in diethyl ether, repeating the procedure 3 times, washed with ethanol, filtered and the obtained white products were dried in vacuum oven at 35o C for 24 h. The synthesis of PDMS-b-PVP block polymer is presented in Scheme 1.

C Preparation of polymer vesicles

Preparation of polymer vesicles was accomplished by film rehydration, solvent evaporation and extrusion methods. For film rehydration method accompanied with sonication and freeze-thaw cycles, 2 ml of 1mg/mL of each block copolymer in tetrahydrofuran was uniformly coated on the inside wall of test tube Duran® with straight rim (Roth, Germany), followed by evaporation of tetrahydrofuran under stream of gaseous nitrogen for 5 min at room temperature and further dried again for 12 h in a vacuum oven at 35o C. To the dried test tube, 5 mL deionized water was added, left for 30 min at room temperature and then sonicated for 30 min at 60o C into the water bath in a sonicator (Fisher Scientific). In the case of freeze-thaw method, hydrated polymer films were freezed in liquid nitrogen and then defreezed in a water bath at 35o C 10 times. In the solvent evaporation method, 3 mL of 1 mg mL-1 polymer solution in tetrahydrofuran was loaded into flask with micro stir bar and then slowly under stirring (~ 1000 rpm) was added deionized water (1drop per 2 sec) at room temperature. Stirring was continued for 12 h and the suspension was purified by dialysis method (dialysis bags, 3500 Da) for two days against deionized water. Extrusion was performed by stainless steel mini extruder (Avanti Polar Lipids, Inc., US) equipped with different pore size polycarbonate membranes and two micro syringes (Hamilton, US). Vesicles suspension was introduced into a 1.0 mL syringe, pushed through 200, 100, 80 and 50 nm membranes, collected into the second syringe and then pushed again in opposite direction. This was repeated 50

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times. The size distribution of vesicles was measured by dynamic light scattering (DLS).

III. CHARATERIZATION

A.FTIR Analysis

FTIR analysis was measured with a **Tensor 27** FTIR spectrometer (Perkin Elmer) in ATR mode using KBr pellets mixed with 5 mg from each block copolymer. Before preparation of pellets, KBr was dried at 80° C for 12 h and the polymers at 30° C under vacuum for 24 h.

B .^{1}H NMR, ^{13}C NMR

1H NMR and 13C NMR spectra were recorded at room temperature on a 250 MHz BRUKER Avance spectrometer using CDCl3 as a solvent for all block copolymers and DMF-d6 was used as a solvent for ACVA.

GPC Determination

The average molecular weight and molecular weight distribution of all block copolymers were determined by gel permeation chromatography (GPC) instrument HP 1090 chromatograph (Hewlett Packard, USA) equipped with two columns PLGel 500Å and PLGel Mix D 100Å (linear mix, Polymer Laboratory) The calibrations were carried out with P2VP standards with molecular weights between 11,1 kDa and 124 kDa and series of PS standards (Polymer Standards Service, Germany). GPC analysis of all PDMS-b-PVP (PDMS: 4,670 kDa and 10 kDa) copolymers were carried out in tetrahydrofuran as eluent, containing organic modificator (4 vol.% isopropyl alcohol and 0.1 vol.% triethylamine), calibrated with PS and the flow rate was 1 ml/min at 40oC.

C.UV Spectrum

UV analysis of MAI's in toluene solutions at concentrations between 1.0 and 1.5*10-5g.mol-1 was performed with a Lambda 35 spectrophotometer (Perkin Elmer).

D .Dynamic Light Scattering (DLS)

DLS was used for the determination of particle size in water. For this analysis, aqueous suspensions of PDMS-b-PVP copolymer obtained by film rehydration, solvent evaporation and extrusion methods with concentration 1.0 10^{-3} g mL^{-1} were prepared. The suspensions were analyzed with Malvern instruments Autosizer 4700 (Mississauga, Ontario, Canada). Each measurement was carried out 3 times at 25° C at an angle 90° C. The size distribution of particles was recorded.

E. Differential scanning calorimetry (DSC) measurement

DSC measurements were performed with a Netzsch DSC 204 F1 Phoenix apparatus. The sample of mass weight about 3 mg placed in aluminum pan with pierced lid was heated in temperature range from -150 to 200° C at heating rate 10K/min. The system was purged continuously with nitrogen with flow rate at 100ml/min.

F. Thermogravimetry analysis (TGA)

TGA measurements were performed with a Netzsch STA 409 PC Luxx thermo balance with TG-DTA sensor coupled to a Brucker Tensor 27 FTIR spectrometer by a heated transfer line to analyze evolved gases. 10 mg of block copolymer sample was heated in temperature range from about 40 to 800° C and the heating rate was 10K/min under continuous flow of nitrogen at 100 mL min-1.

G. Optical microscopy

Optical microscope model "Nikon" Eclipse (Japan) equipped with digital camera Nikon (Digital Sight D5-2Mv, Japan) was used to observe the self-assembling of PDMS-b-PVP diblock copolymer. The results were manipulated using "NIS elements BR 2.30" software.

H. SEM Observation

Scanning electron microscopy (SEM) Quanta FEG 200 (FEI Europe BV, Branch Belgium) equiped with energy dispersive x-ray (EDX) system for identifying the elemental composition of the specimen, or an area of interest was used. The morphology of the self-assembled block copolymer films was observed at electron acceleration of 7 kV without Au/Pd coating for the samples, and images at various levels of magnification were captured.

IV. BIOLOGICAL INVESTIGASTION

A. Cells

Human fibroblasts were prepared from fresh skin biopsy and used up to the ninth passage. The cells were grown in Dulbecco's minimal essential medium (DMEM) containing 10% fetal bovine serum (Sigma) in a humidified incubator with 5% CO2. For the experiments, the cells were harvested from nearly confluent cultures with 0.05% trypsin/0.6 mM EDTA (Sigma).

B. Cell growth

The cell proliferation was determined via modified lactate dehydrogenase (LDH) assay (Hoffman La Roche, Penzberg, Germany) after 1, 3, and 5 days of incubation. Briefly, at the indicated incubation time, the medium was removed and the cells were lysed with 0.5 mL 1% Triton-X 100 in PBS under shaking for 1 h. The cells lysates were centrifuged at 2000 x g for 5 min. Thereafter, 100 µL of LDH test solution was added to each well, and the samples were incubated for 15 min at room temperature in dark. The reaction was stopped with 50 µL 1M HCl. The absorbance was measured with Spectra Flour Plus

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plate reader (Tecan, Crailsheim, Germany) at 492 nm. The reference wavelength was at 620 nm. Each experiment was quadruplicated.

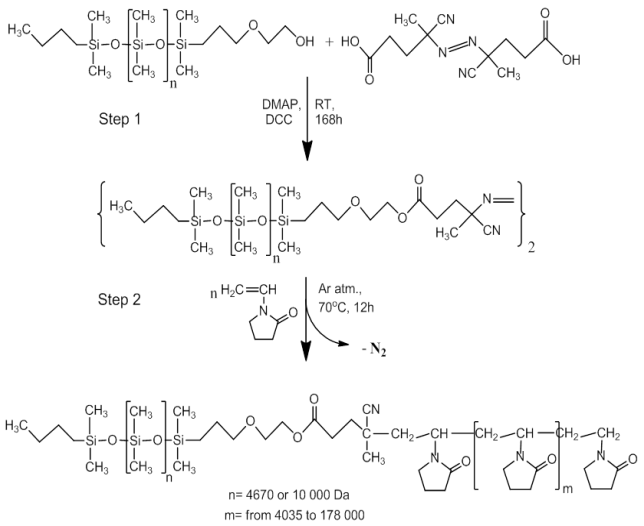
C. *Actin staining*

Overall cell morphology was observed by actin staining. Fibroblasts with density of 1.5x105 cells/ml were cultivated on cover glasses (18x18 mm), placed in 6-well plates for 24h. After the incubation period, the non-adhered cells were removed by triple rinsing with PBS (pH 7.4). The adhered cells were fixed with 1 mL 3% solution of paraformaldehyde (PFA) for 15 minutes at room temperature. The fixed cells were permeabilized using 1 mL 0.5% solution of Triton X-100 for 5 minutes and then incubated with 1 mL 1% solution of serum bovine albumin (BSA) for 15 minutes. The samples were washed three times with PBS (pH 7.4) and incubated for 30 min at room temperature with BODIPY 558/568 phalloidin. Again, the samples were washed three times with PBS and once with distilled water, and then were installed on objective glasses by Mowiol. The samples were analyzed using inverted fluorescent microscope (Leica DMI3000 B, Leica Microsystems GmbH, Germany) with objective HCX PL FLUOTAR 63x/1.25 oil.

V. RESULTS AND DISCUSSION

A Synthesis and Characterization of PDMS macroazoinitiators

In our study, PDMS macroazoinitiators have been prepared by direct reaction of polycondensation of ACVA and mono (hydroxyalkyl)-terminated PDMS with two different molecular weights, using DCC as coupling agent and 4-DMAP as catalyst at room temperature similar to the procedure reported from Libang Feng et all[19], but without using DMF as a solvent for ACVA (Scheme 1, step 1). The reaction time was 168 h at vigorous stirring and the conversion was controlled by GPC. In the first 6 hours the mixture was homogenized very well. After 24 hours the conversion of the two functional polymers $PDMS_{4k}$-OH and $PDMS_{10k}$-OH was about 65% according to the calculation of GPC. However, after additional 144 hours, $PDMS_{4k}$-ACVA, $PDMS_{10k}$-ACVA yielded conversion of about 87 % and 92 %, respectively. This can be explaining with the fact that the viscosity of the polymer solution increases and the active sites are difficult to be reached. Thus, the reaction time increases and the constant conversion was gained after 168 hours. MAI's were characterized by FTIR ^{1}H NMR and ^{13}C NMR spectroscopy. On Figure 1(a) and 1(b) are shown FTIR spectra of mono (hydroxyalkyl)-terminated PDMS-OH and PDMS-ACVA, respectively. Figure 1(b) exhibits a very low intensive bond at 3440 cm^{-1} reflecting the presence of CN bond (cise and trans) coming from 4,4'-azobis(4-cyanovaleric acid) as well as the presence of C=O group through the bond observed at 1743 cm^{-1}, evidencing the efficiency of the polycondensation of PDMS-OH and ACVA. In addition, the PDMS introduction was confirmed by typical vibration bands at 1263 cm^{-1} attributed to Si-CH_3 stretching, a broad band between 1000-1100 cm^{-1} for Si-O-Si asymmetric stretching vibration as well as at 800 cm^{-1} attributed to Si-O stretching (Figure 1(b)).



Scheme 1. ***Different stages of PDMS-b-PVP block copolymer synthesis.***

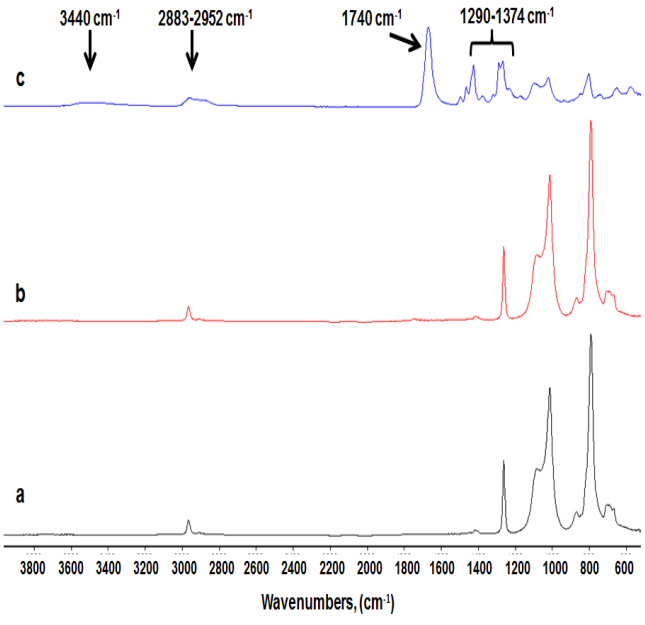


Fig.1. FTIR spectra of (a) mono(hydroxyalkyl)-terminated PDMS10k, (b) PDMS10k –ACVA (macroazoinitiator), (c) PDMS-b-PVP diblock copolymer.

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^{1}H and spectra of $PDMS_{10k}$-OH and $PDMS_{10k}$-ACVA macroazoinitiator as chemical shifts are presented in Fig. 2. Figure 2a shows $PDMS_{10k}$-OH spectrum in which from 3.53 to 3.72 ppm appears methylene protons next to the hydroxyl group as well as chemical shift at 1.97 ppm corresponding to the proton from hydroxyl group and typical shift at 0.05 ppm attributed to the proton from methyl groups bonded to silicon atom. Finally, the chemical shifts at 12.74 ppm for carboxylic protons from ACVA and 1.97 ppm were not observed in $PDMS_{10k}$-ACVA spectrum (Figure 2b) also observed elsewhere[19], which confirm both successful reaction of polycondensation between $PDMS_{10k}$-OH and ACVA and effective purification process. This was also confirmed by ^{13}C NMR with appearing of shift at 170.4 ppm attributed to C=O group from $PDMS_{10k}$-ACVA macroazoinitiator. The results from ^{1}H and ^{13}C NMR spectra for $PDMS_{4k}$-ACVA $PDMS_{4k}$-OH and $PDMS_{4k}$-ACVA and ^{13}C NMR spectrum for $PDMS_{10k}$-ACVA macroazoinitiator are not presented here, but also confirm successful esterification reaction.

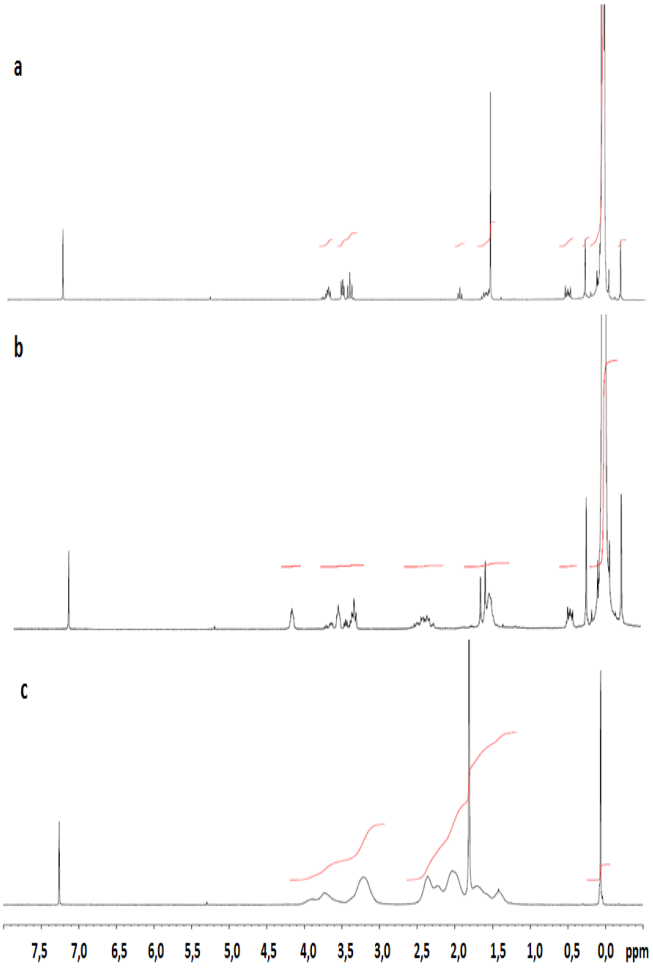

Fig. 2. 1H NMR spectra of (a) mono(hydroxyalkyl)-terminated PDMS-OH, (b) ACVA-azoinitiator, and (c) PDMS-b-PVP copolymer.

The presence **–N=N–** group from $PDMS_{10k}$-ACVA macroazoinitiator was confirmed by UV spectroscopic characterization. For example, in Figure 3 shows the result for $PDMS_{10k}$-ACVA MAI. The maximum of the peak at 335 nm confirms the presence of azo group as it has been observed from Libang Feng et. all[19].

The traces of functional PDMS and MAI's were determined and confirmed by gel permeation chromatography (GPC). The results of average molecular weight, molecular weight distribution and their polydispersity indexes are summarized in Table 1.

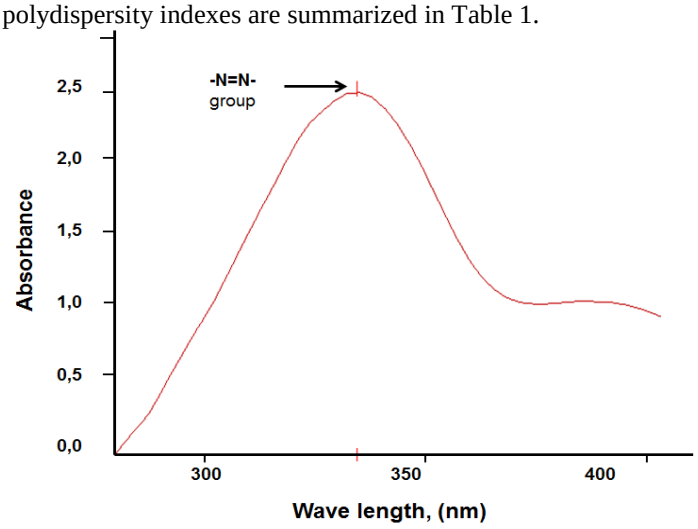

Fig 3. UV spectrum of the successful synthetized $PDMS_{10k}$ - ACVA macroazoinitiator.

TABLE 1. Average molecular weight, molecular weight distribution and polydispersity of functional PDMS and PDMS MAI's.

Samples	M_n [g/mol]	M_w[g/mol]	PDI
$PDMS_{4k}$-OH	4149	4685	1.12
$PDMS_{10k}$-OH	8079	10067	1.24
$PDMS_{4k}$-ACVA	8521	9454	1.10
$PDMS_{10k}$-ACVA	20731	22965	1.11

B. Synthesis and Characterization of PDMS-b-PVP block copolymers

The library of amphiphilic poly(dimethylsiloxane)-*b*-poly(N-vinyl pyrrolidone) copolymers were accomplished by thermal dissociation of rarely used macroazoinitiator (MAI) containing poly(dimethylsiloxane) (PDMS), with molecular weight 4670 and 10000 Da, where synthesized (Scheme 1, step 1) and polymerized by free radical polymerization with different molar ratio 1-vinyl-2-pyrrolydone (Scheme 1, step 2). The ratio between MAI and monomer was varied from 15 wt. % to 80 wt. % in order to study physico-chemical and thermal properties of all block copolymers. On Figure 1(c) is presented FTIR spectra of $PDMS_{4k}$-*b*-PVP, but the spectrum of $PDMS_{10k}$-*b*-PVP is not presented here. It could be seen appearance of the strong

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absorption at 1743 cm^{-1} (Fig. 1(c)) attributed to C=O group from the vinyl pyrrolidone ring, bearing by PVP polymer, which could not be found in PDMS-OH and MAI as well as increased intensity of the absorption bands from 2883-2952 cm^{-1} (Fig. 1(c)) ascribed also to CH_2 group in PVP backbone, indicating successful synthesis of $PDMS_{10k}$-*b*-PVP and $PDMS_{4k}$-*b*-PVP block copolymers. It is also can be seen increasing of the peak between 1424-1494 cm^{-1} related to CH_2, near to C=O group from VP ring and the intensive and broad peak at 3440 cm^{-1} coming from CN band from pyrrolidone ring (See Fig 1(c)). All typical PDMS asymmetric vibrations at 1260 cm^{-1} (Si-CH_3), 1000-1100 cm^{-1} (Si-O-Si) and 800 cm^{-1} (Si-O) were observed in FTIR spectrum.

Figure 2(c) presents ^{1}H NMR and Figure 5 ^{13}C NMR spectra of the PDMS-*b*-PVP block copolymer. As can be seen from proton NMR spectrum (Figure 2(c)) the resonances at 3.21, 2.41 and 2.36 ppm could be attributed to CH_2 groups from vinyl pyrrolidone ring. The strong peak at 1.75 ppm and the resonance at 3.71 ppm (Figure 2(c)) could be ascribed to CH_2 and CH groups of PVP backbone as well as the peak at 0.01 ppm (Figure 2(c)) is attributed to protons from Si-CH_3 in PDMS block.

The carbon NMR spectrum of PDMS-b-PVP copolymer is presented on Figure 5 and supports the data from proton NMR. The peak at 0.00 ppm (Figure 5, f) could be associated with primary carbon from CH_3 group linked to the Si atom from PDMS inorganic backbone. At 174.2 ppm appears signal for C=O group from pyrrolidone ring. On the other hand, the signals at 43.7 ppm (Figure 5, b) corresponding to the carbon from CH_2 in alpha position near to the N atom in PVP ring, 42.5 ppm (Figure 5, c) corresponding to the quaternary carbon (CH group) from PVP backbone linked with N atom from PVP ring, 41.1 ppm (Figure 5, a) corresponding to CH_2 alpha position from PVP backbone, 30.4 ppm (Figure 5, e) (CH_2 group next the C=O group in pyrrolidone ring) and 17.3 ppm (Figure 5, d) (CH_2 group in beta position in the pyrrolidone ring) could be clearly seen, indicating that amphiphilic PDMS-*b*-PVP diblock copolymer was successfully synthesized, using PDMS macroazoinitiator. N. Uyanik and coworkers[32] have been observed for their PVP-*b*-PDMS-*b*-PVP copolymers close to our spectra for FTIR and ^{13}C NMR.

The average molecular weight and molecular weight distribution of all block copolymers and MAI's were determined by gel permeation chromatography (GPC). The results are presented in Figure 4 and their molecular weight and polydispersity index are summarized in Table 1. N. Uyanik and coworkers[32] reported that the molecular weight of their triblock copolymer cannot be measured by GPC, because the copolymers are not completely soluble in tetrahydrofuran. The refractive index dn/dc in common eluents such as tetrahydrofuran; chloroform and dichloromethane tend to zero. The organic modificator was used to change the refractive index and the solubility of the block copolymer solution. Thus, it is possible to visualize correct the chromatograms of the studied products. As can be seen from the Figure 4A, when MAI (4670 Da) with the low molecular weight is used as initiator for free radical polymerization of desired block copolymer, this leads to exponential increasing of the copolymer molecular weight (4A, curve 1) with increasing PVP content and decreasing of PDMS content (under 50 wt.% - 50 wt.% PDMS/PVP ratio). On the other hand, when MAI with molecular weight 10 000 Da is used for free radical polymerization of 1-vinyl-2-pyrrolidone linear dependency is observed (4A, curve 2). Probably, the mechanism of chain growth is different from that one when MAI with low molecular weight is used and second reason could be the higher viscosity of MAI with molecular weight 10 000. This also influences on the PD of the obtained block copolymers – average PD in case of PDMS4670-MAI is lower than this in the case of PDMS10000-MAI. Meanwhile, the molecular weight of all diblock copolymers decreased with increase in the amount of MAI and PDI decreases as well. This fact is consistent with the theory of conventional free radical polymerization.

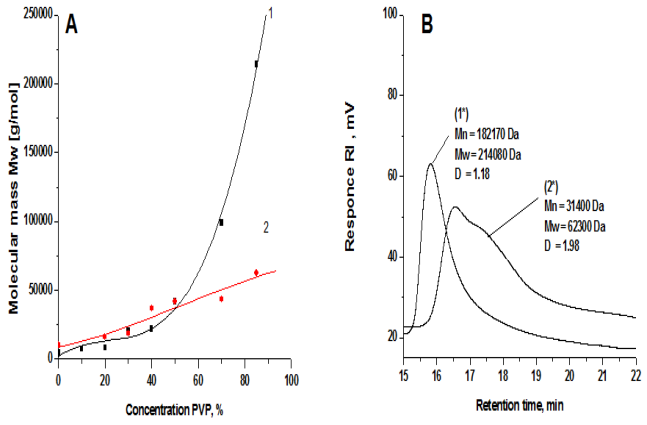

Fig.4. (A) Dependency of Mw from PVP content in PDMS-b-PVP using MAI: $PDMS_{4670}$ (curve 1) and PDMS 10 000 (curve 2) and (B) GPC chromatograms of PDMS-b-PVP at 15 wt.% - 85 wt% ratio: PDMS4670 (curve 1*), PDMS10000 (curve 2*).

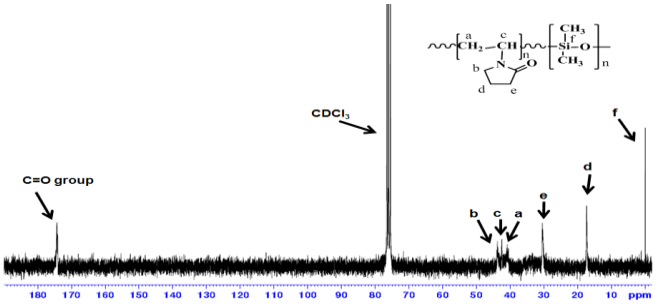

Fig. 5. C NMR spectrum of PDMS-b-PVP diblock copolymer in $CDCl_3$.

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TABLE 2. Composition, average molecular weight, molecular weight distribution and polydispercity of all PDMS-b-PVP block copolymers.

Polymer	MAI/VP ratio		M_n [g/mol]	M_w[g/mol]	PDI
	MAI [wt.%]	VP [wt.%]			
$PDMS_{4k}$-b-PVP	15	85	182170	214081	1.17
$PDMS_{4k}$-b-PVP	30	70	61479	99052	1.61
$PDMS_{4k}$-b-PVP	50	50	36114	41724	1.15
$PDMS_{4k}$-b-PVP	60	40	17734	21693	1.22
$PDMS_{4k}$-b-PVP	70	30	18538	20326	1.09
$PDMS_{4k}$-b-PVP	80	20	5120	6347	1.23
$PDMS_{10k}$-b-PVP	15	85	31423	62258	1.98
$PDMS_{10k}$-b-PVP	30	70	23971	43580	1.81
$PDMS_{10k}$-b-PVP	50	50	32926	41716	1.26
$PDMS_{10k}$-b-PVP	60	40	27779	36523	1.31
$PDMS_{10k}$-b-PVP	70	30	15005	18488	1.23
$PDMS_{10k}$-b-PVP	80	20	15188	15983	1.05

Thermal properties depending on MAI and VP ratio were investigated by differential scanning calorimetry (DSC) and Thermogravimetry analysis (TGA). All copolymers differential scanning calorimetry thermograms displayed one characteristic transition for PDMS phase and two transitions of PVP phase, respectively: $T_{m\ PDMS}$, $T_{g\ PVP}$, $T_{m\ PVP}$ (Summarized in Table 3). The copolymers $PDMS_{4k}$-*b*-PVP and $PDMS_{10k}$-*b*-PVP with 80wt.% PDMS showed melting endotherms of PDMS crystal phase and two weak endotherms at ratio 60 and 70 wt.% in the case of $PDMS_{4k}$-*b*-PVP (Figure 6, A and B). Melting endotherms ($T_{m\ PDMS}$) of PDMS block for all other copolymers were not observed. The typical extremely low T_g (from the literature, -127 ^{o} C), cold crystallization were also not detected, probably because of the low PDMS content in the block copolymers. However, T_g of PVP segment in all block copolymers decreases and T_m increases in compare with homo-PVP values (Table 3), due to the presence of PDMS segment and apparently were affecting as plasticizer, also observed elsewhere32.

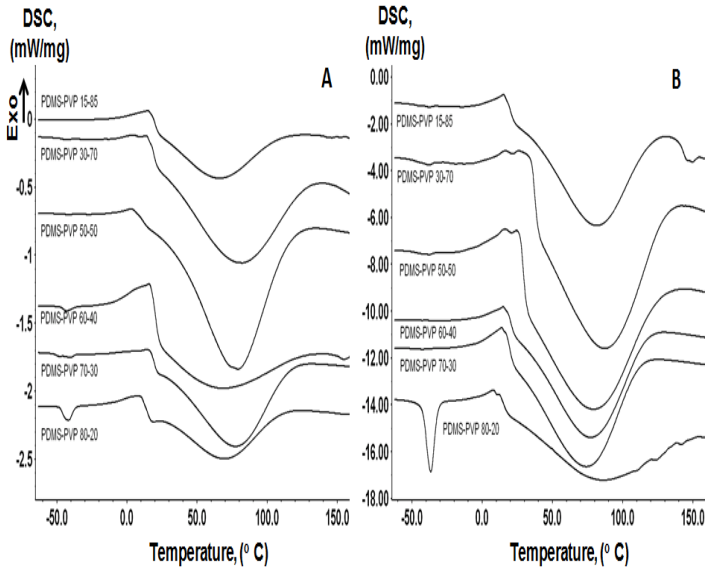

Fig. 6. Differential scanning calorimetry thermograms of PDMS4k-PVP (A) and PDMS10k-PVP (B) block copolymers with different weight percent ratio between PDMS macroazoinitiator and 1-vinyl-2-pyrrolidone monomer.

TABLE 3. *Characterization of the monofunctional homopolymers, MAIs and diblock copolymers*

Polymer	PVP block T_g (^{o}C)	PVP block T_m(^{o}C)
Pure PVP	31.9	66.2
Pure PDMS	- 127 lit.	-
$PDMS_{4k}$-PVP 15-85	24.2	65.9
$PDMS_{4k}$-PVP 30-70	21.4	82.3
$PDMS_{4k}$-PVP 50-50	16.0	79.3
$PDMS_{4k}$-PVP 60-40	29.8	69.4
$PDMS_{4k}$-PVP 70-30	22.1	77.4
$PDMS_{4k}$-PVP 80-20	17.3	69.1
$PDMS_{10k}$-PVP 15-85	24.6	81.5
$PDMS_{10k}$-PVP 30-70	41.2	87.2
$PDMS_{10k}$-PVP 50-50	32.8	79.4
$PDMS_{10k}$-PVP 60-40	23.5	77.1
$PDMS_{10k}$-PVP 70-30	22.7	74.1
$PDMS_{10k}$-PVP 80-20	19.8	86.6

A comparison of Thermogravimetry analysis (TGA) of the $PDMS_{4k}$-*b*-PVP and $PDMS_{10k}$-*b*-PVP block copolymers with those of each homopolymers shows two steps of degradation, first at about 180^{o} C for $PDMS_{4k}$-PVP and 250^{o} C for $PDMS_{10k}$-PVP attributed to PVP degradation and second step of degradation for both PVP and PDMS blocks, starting from 450^{o} C and parallel attended with PDMS backbone degradation at about 650^{o}

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C. Additionally, because of fast swelling of water vapors of all copolymers, which change T_g values, each sample was dried at 40° C for 24 h, to remove traces of water in the block copolymers.

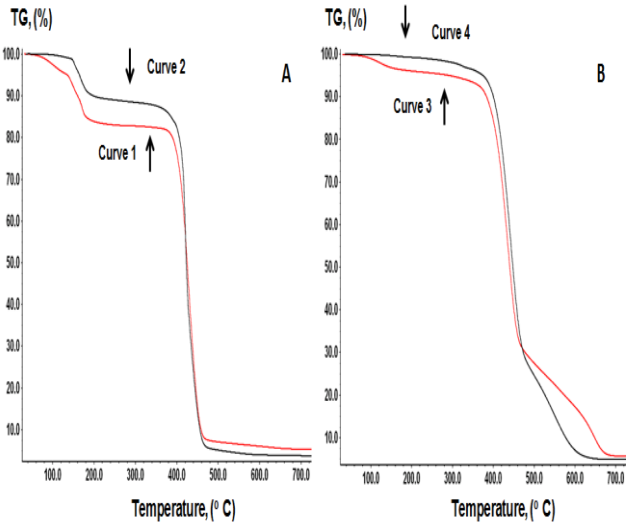

Fig. 7. TGA thermograms of $PDMS_{10k}$-b-PVP15wt.%-85wt% (A, curve 1 - before drying and curve 2 – after drying at 40 °C for 24 h) and $PDMS_{10k}$-PVP 80wt.%-20wt.% (B, curve 1* - before drying and curve 2* –after drying at 40 °C for 24 h) block copolymers.

C. Self-assembling and characterization of diblock copolymer vesicles

Block copolymers in thin films have been particularly well studied because of their ability to spontaneously generate large arrays of structures with dimensions from nanometer to micrometer scale. They are able to mimic lipid amphiphilicity and self-assemble into vesicles in dilute solution, but polymer molecular weights can be orders of magnitude greater than those of lipids. Structural features of vesicles, as well as properties including stability, fluidity, and intermembrane dynamics, are greatly influenced by characteristics of the copolymers. Here we will highlight approaches for controlling block copolymer assembly in thin films to achieve long-range order. In order to confirm our expectation for very easy self-assembling in vesicles of all block copolymers we used well known methods as film rehydration followed by sonication or freeze-thaw cycles, solvent evaporation and extrusion through polycarbonate double supported membranes from mini extruder. The self-assembling was accomplished in deionized water in preliminarily well dried polymer films. Applying these standard methods this led to the formation of vesicles with high polydispersity (D_h, between 150-3000 nm) and very low polydispersity after nano extrusion (D_h ~ 100-200 nm). As prepared PDMS-b-PVP amphiphilic copolymers contain hydrophilic and hydrophobic fragments, at certain concentrations in aqueous media greater than some critical concentration (so-called critical aggregation concentration, (CAC)) they can aggregate with formation of core-shell type polymeric nanoparticle structures. Due to the hydrophobic character of PDMS-chain fragments, these domains will be oriented towards the core of the polymeric nanoparticles while hydrophilic PVP is oriented in an outward direction as an outer shell of the polymeric nanoparticles.

D. Visualization of polymer assemblies

The morphology of self-assembled aggregates formed spontaneously by different amphiphilic polymers in aqueous media is quite diverse. Generally, it is supposed that amphiphilic polymers containing hydrophilic and hydrophobic blocks at concentrations higher than their CAC value produce particles close to spherical form. In the present study, the morphologies obtained from the self-assembly of diblock copolymers were visualized directly by optical microscopy. 10 µL of polymer suspension (polymer concentration 1 mg/mL) obtained by film rehydration or solvent evaporation methods was casted on silicon wafer and observed at different magnifications. Figure 8 (A, B, C and D) show vesicles formed from $PDMS_{4k}$-*b*-PVP (15wt%-85wt%) with spherical form. In all samples were observed very large vesicles (~15 µm) and small one with size ~ 5 µm), calculated by standard software.

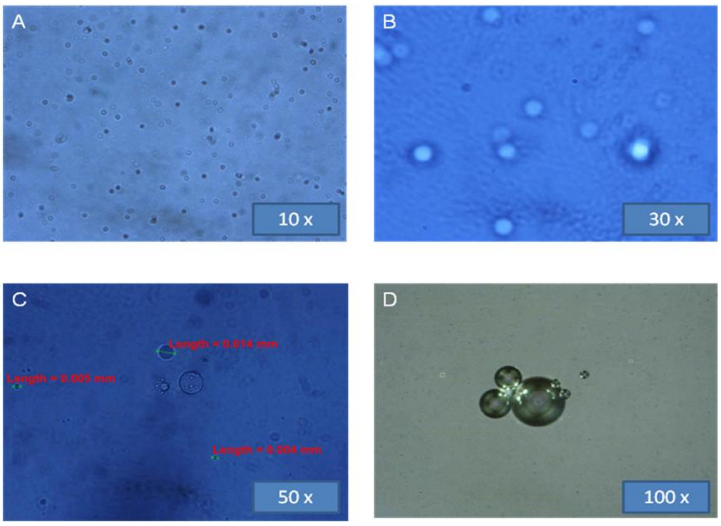

Fig. 8. Optical microscopy images of polymer vesicles prepared from the library of PDMS-b-PVP copolymers by film rehydration or solvent evaporation method.

Detailed structural information was obtained by scanning electron microscopy (SEM). For SEM visualization, after self-assembly 20 µL from each sample was casted onto aluminum holder and dried at room temperature for 24 hours. As can be seen, microscopic images shows vesicles obtained by film rehydration method, after sonication for 30 min at room temperature, followed by vortexing for 1 min (Fig. 9, B and D) and

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freeze-thaw cycles followed again by vortexing (Fig. 9, A and C).

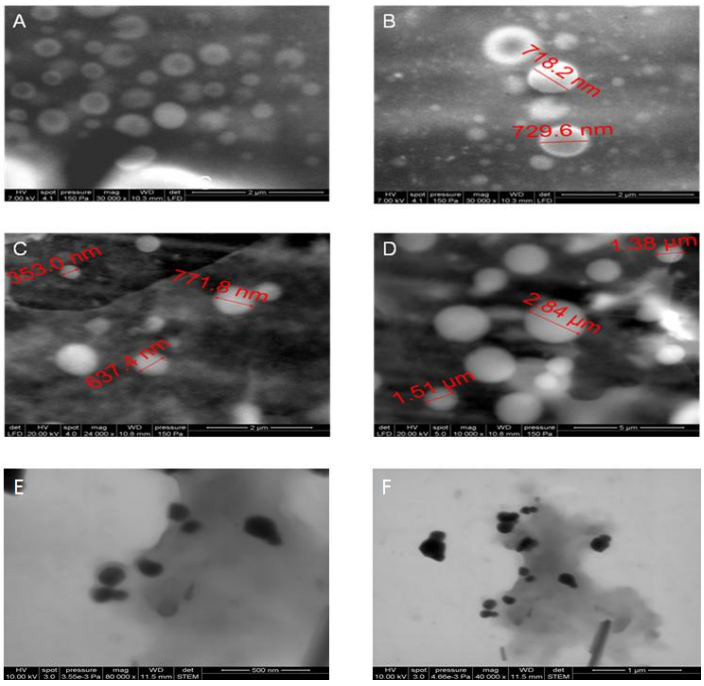

Fig. 9. SEM micrographs of PDMS4k-b-PVP vesicles obtained by film rehydration - B and D sonication for 30 min at room temperature and C vesicles obtained after 10 cycles of freeze-thaw.

In all samples prepared by film rehydration and solvent evaporation methods was observed quite polydispers vesicles and the diameter varied from nano meter scale (350-800 nm) (Fig. 9, B and C) to micro meter scale (1.50-3 µm) (Fig. 9, D). Moreover, the indentations in the centre of the vesicles (Fig. 9, A and B) indicate that they are deflated hollow spherical particles. Similar to the conventional phospholipids these vesicles consist from hydrophobic part (PDMS) and hydrophilic shell made by PVP segment. We consider that the large polydispersity may be due to the relatively high polydispersity of the synthesized block copolymers (Table 2). On the other hand, the differences in diameter in water (~15 µm) and dried state (350-3000 nm) are due to the swelling of water and diffusion and evaporation of water through the PDMS hydrophobic membrane after drying. A close examination of the contours gives a thickness of the hydrophobic wall (measured in STEM mode)of the polymeric vesicles was measured to be approximately 12.5 nm ±0.3 (drying state, at room temperature), which is higher than this in liposomes as recently reported by Discher et al., the vesicles of diblock polymers with a thickness of ca. 8 nm proved to be 5-50 times as tough as the vesicles made of phospholipids and cholesterol[33]. Therefore, we expect that our spherical membranes have good mechanical property. The average hydrodynamic diameter d_h of the formed block copolymer structures was measured with DLS. As it was mentioned above after sonication, freeze–thaw cycling and water addition/solvent evaporation, followed by vortexing, generating vesicles with high polydispersity, DLS measurement indicate that the size distribution is under 1µm (Fig. 10, A and C) in case of freeze-thaw and water addition/solvent evaporation techniques. In order to generate copolymer vesicles with narrow size distribution we have used nano-extruder equipped with polycarbonate membranes with pore size from 50 nm to 200 nm.

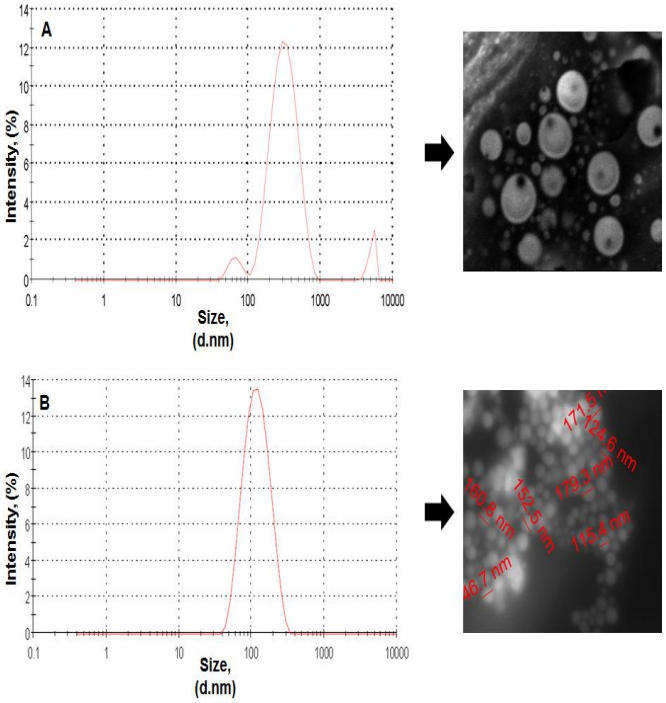

Fig. 10. Average size distribution graphs of PDMS-b-PVP vesicles obtained by film rehydration or solvent evaporation method (A) and (B) vesicles obtained after 50 times extrusion through 100 nm polycarbonate membrane, supported by SEM.

TABLE 4 Average size of PDMS-b-PVP vesicles before and after extrusion.

Pore size of membrane	$PDMS_{4k}$-*b*-PVP (70wt%-30wt%)		$PDMS_{10k}$-*b*-PVP (70wt%-30wt%)	
	average D_h, (nm)	PDI	average D_h, (nm)	PDI
Without extrusion	546.3	0.383	208.0	0.338
200 nm	225.9	0.075	155.2	0.169
100 nm	114.4	0.134	121.5	0.193
80 nm	105.8	0.150	103.0	0.174
50 nm	100.9	0.160	108.3	0.227

After extrusion of all copolymer suspensions through the membranes, it were generated comparatively small, monodisperse vesicles just over 100 nm and close to 200 nm in size, as verified by dynamic light scattering (Fig 10, B and D) and SEM images obtained in STEM mode (Figure 9, E and F). Stepwise extrusion, first through a

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200 nm membrane and then through a 100 nm, 80 nm and 50 nm membrane, eliminates any enhanced viscous resistance to extrusion due to increases in polymer molecular weight. Finally, $PDMS_{10k}$-*b*-PVP and $PDMS_{4k}$-*b*-PVP with approximately equal PDMS chain and PVP chain, increased the hydrophobic nature of PDMS-*b*-PVP copolymers provided poor solubility in water, and that is why we failed to obtain stable suspension of nanoparticles for $PDMS_{4760}$-*b*-PVP_{5120} and $PDMS_{10000}$-*b*-PVP_{15188} (See Table 2) copolymers. The DLS supporting data of average diameters before extrusion and after extrusion are summarized in Table 4.

Finally, preliminary investigation in cell growth and proliferation onto thin layer of the block copolymers shows that the samples are with very good potential for drug delivery or tissue engineering. It is known that the shape and the size of the biomaterial particles as well as surface roughness of thin layers also influence the cell recognition ability and interaction [34]. Because of these reasons, we have investigated the influence of our copolymers on the cell proliferation.

The capability of different PDMS-*b*-PVP samples to support the cell growth over a period of 7 days was studied using LDH assay. As illustrated in Figure 11, the $PDMS_{4k}$-*b*-$PVP_{56.8}$ tends to increase the cell growth. The last one is significantly higher for the 3rd and 7th day. This effect depends maybe of the PVP chain length and different topography of the thin layer in all samples which is important for cell proliferation.

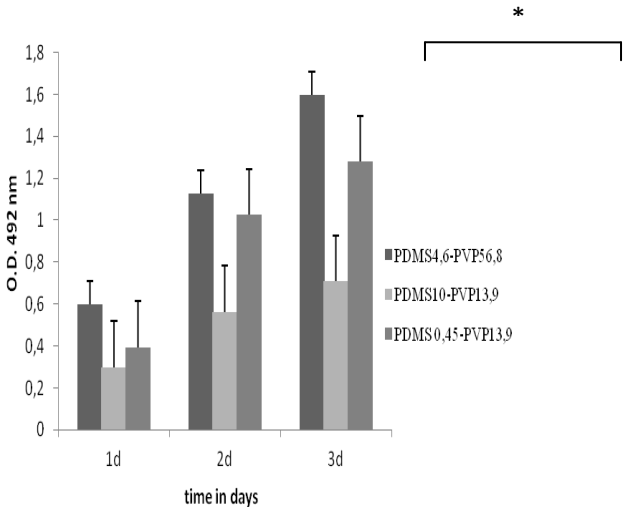

Fig. 11. LDH test with human fibroblast cells onto PDMS4k b-PVP samples.

It is evident that the fibroblast number on all three PDMS-b-PVP samples is more then, the control silicon wafer (Fig. 12 a). In addition, the fibroblasts on the $PDMS_{10k}$-b-PVP_{045K} and $PDMS_{4,6K}$-b-$PVP_{19,9}$ surfaces represent similar to round shape, the same as control and again stress fibers are not expressed, and actin is defuse (see arrow), in a contrast with Fig. 12 b. Here the cells are more than the other samples (12 a, c and d) with long distal stress fibers (see arrow) and very good cell growth and spreading especially on $PDMS_{4,6k}$-b-$PVP_{56,7}$. This could be explained by the high hydrophilic nature of PVP block and longer PVP chain in this material (Fig. 12,b). It is well known (10) that the strong hydrophobic surfaces as PDMS, for example, do not support the initial interaction with living cells (Fig. 12 a). On the other hand, PDMS-*b*-PVP in water self-assembled in soft polymeric particles with size from nanometer to micrometric scale. This could explain that all PDMS-*b*-PVP thin layers have different surface topography. Is is well known that cells prefer rough surfaces. Thus, we observed good biological effect on the samples with longer PVP chain length than the block copolymer with shorter PVP chain length.

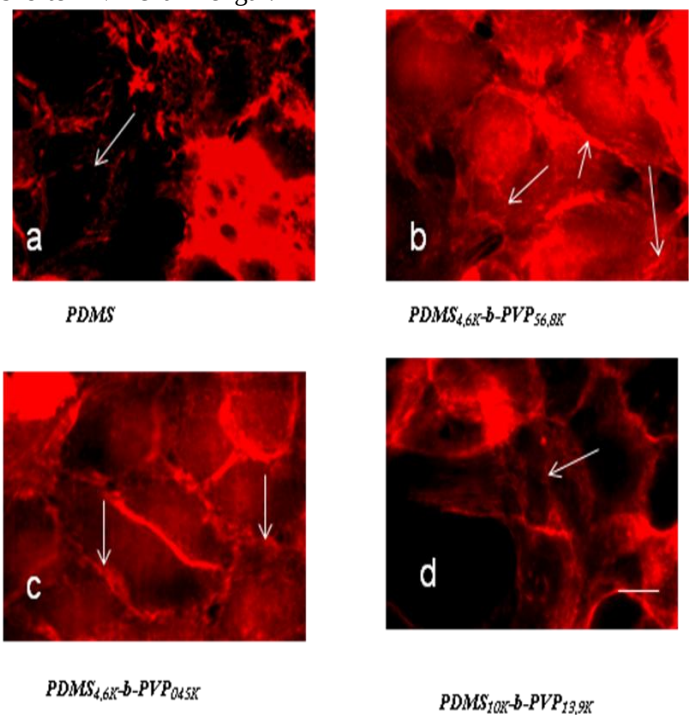

Fig. 12 Fluorescent microscopic images of actin staining of fibroblasts interaction with PDMS and different PDMS-b-PVP fiber scaffolds: (a) PDMS, (b) $PDMS_{4,6K}$-b-$PVP_{56,8K}$, (c) $PDMS_{4,6K}$-b-PVP_{045K}, (d) $PDMS_{10K}$-b-$PVP_{13,9K}$. 63x

VI. CONCLUSION

A library of amphiphilic diblock copolymers based on PDMS-*b*-PVP were successfully synthesized by conventional radical polymerization of 1-vynil-2-pyrrolidone, using poly(dimethylsiloxane) macroazoinitiator. The copolymers were characterized in detail as well as molecular weight distribution and polydispersity were measured successfully by GPC, using organic modificator. It was found that these copolymers

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self-assemble in soft polymeric particles with high polydispersity and size distribution from nanometer scale to micrometer scale. We demonstrated also that is possible to generate monodisperse particles with size distribution between 100 and 200 nm by stepwise extrusion through polycarbonate membranes with different pore size, investigated by DLS. The morphology of the obtained particles was observed in detail by optical microscopy, SEM and SEM in STEM mode as well. It was found that these polymeric structures are hollow soft particles. This conformation lead us to conclusion that they could be loaded with biomolecules or even could exhibit "stealth" properties as it is with PEG copolymers, reported in the literature. Additionally, our preliminary biocompatibility tests showed that the both copolymers $PDMS_{4k}$-*b*-PVP and $PDMS_{10k}$-*b*-PVP are promising materials for medical application. The results of the preliminary biological tests indicate better biocompatibility effect for the copolymers $PDMS_{4K}$-*b*-PVP with longer PVP chain comparing to the copolymer with shorter PVP chains and polymers with $PDMs_{10k}$-bPVP. This was expected because since PVP is with hydrophilic nature and this copolymer surface is very attractive substrate for the cells growth and proliferation. Additionally, the copolymer surfaces probably possess different surface roughness and this makes preferable the sample with longer PVP chain length like better biological surface. Finally, we believe that these polymer nanocapsules possess great potential for encapsulation and controlled release of guest molecules in/from their interior, especially since the controlled formation of these structures can be achieved rather easily and the composition of the underlying diblock copolymer. This would allow adapting, for example, the permeability of the nanocapsules to the desired application. Nevertheless, in this context, clearly more information is needed about the properties of these polymer nanocapsules, which are beyond the goal of this paper and, hence, will be reported in the future.

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REFERENCES

[1] Hamley, I. W., Ed. Developments in Block Copolymers Science and Technology; John Wiley and Sons: Hoboken, New Jersey, 2004.

[2] Solovskij, M. V., Gudaitis, A. A.Boimirzaev, A.S., Nesterov, V.V., Panarin, E.F.: Iran. Polym. J., 10, 347, 2001.

[3] Sinha V.R., Aggarwal A., Trehan A., Am. J Drug Delivery, , 2, 157-168 , 2004.

[4] Lee J.C.M., Bermudez H., Discher B.M., Sheehan M.A., Won Y.Y., Bates F.S., Discher D.E., Biotechnol. Bioeng. 73, 135, 2001.

[5] Arifin D.R., Palmer A.F., Biomacromolecules. 6, 2172, 2006.

[6] Lee Y., Chang J.B., Kim H.K., Park T.G., Macromol. Res., 14, 359, 2006.

[7] Ghoroghchian P.P., Frail P.R., Li G.Z., Zupancich J.A., Bates F.S., Hammer D.A., Therien M.J., Chem. Mater., 19, 1309 , 2007.

[8] Rameez S., Alosta H., Palmer A.F., Bioconjugate Chem., 19, 1025, 2008.

[9] Wittemann A., Azzam T., Eisenberg A., Langmuir., 23, 2224, 2007.

[10] Pang Z.Q., Lu W., Gao H.L., Hu K.L., Chen J., Zhang C.L., Gao X.L., Jiang X.G., Zhu C.Q., J. Controlled Release., 128, 120 , 2008.

[11] Ghoroghchian P.P., Li G.Z., Levine D.H., Davis K.P., Bates F.S., Hammer D.A., Therien M.J., Macromolecules. 39, 1673-1685, 2006.

[12] Klasner S.A., Metto E.C., Roman G.T. and Culbertson Ch.T. Langmuir, , 25 (17), 10390-10396, 2009.

[13] Rheingans O., Hugenberg N., Harris J.R., Fischer K. and Maskos M., Macromolecules, 33, 4780-4790, 2000.

[14] Kickelbick G., Bauer J., Hüsing N., Andersson M. and Palmqvist A., Langmuir, 19 (8), 3198-3201 ,2003.

[15] Nugaz N., Kücükzavuz Z. and Kücükzavuz S., Polymer, 34 (22) 4649-4654 , 1993.

[16] Lee J. and Hogen-Esch Th. E., Macromolecules. 34 (9) 2805-2811, 2001.

[17] Lim K.T. and Webber, Macromolecules, , 32 (9) 2811-2815 , 1999.

[18] Lim K.T. and Webber, Ind. Eng. Chem. Res., 39 (8) 2655-2664 , 2000.

[19] Libang Feng, Hongxia Fang, Shuxue Zhou, Limin Wu, Macromol. Chem. And Physics, 207, 1575-1583, 2006.

[20] Pouget E., Tonnar J., Lucas P., Lacroix-Desmazes P., Ganachaud F., Boutevin B., Chem. Rev., 110, 1233-1277, 2010.

[21] Noll W., Chemistry and Technology of Silicones. Academic Press: New York , 1968.

[22] Graiver D, Farminer K. W, Narayan R. J Polym. Environ.,11, 129, 2003.

[23] Fischer, Frank; Bauer, Stephan: "Polyvinylpyrrolidon. Ein Tausendsassa in der Chemie". Chemie in unserer Zeit, 43 (6): 376–383 , 2009.

[24] G. F. Doebbler. Cryoprotective compounds. Cryobiology .3:2–11, 1966.

[25] M. Townsend and P. P. DeLuca., J. Parent. Sci. Technol., 37:190–199, 1988.

[26] W. R. Gombotz, S. C. Pankey, R. Phan, R. Drager, K. Donaldson, K. P. Antonsen, A. S. Hoffman, and H. V. Raff., Pharm. Res., 11:624–632, 1994.

[27] R. J. Mumper, J. G. Duguid, K. Anwer, M. K. Barron, H. Nitta, and A. P. Rolland., Pharm. Res., 13:701–709, 1996.

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 7, January 2014

[28] H. Kamada, Y. Tsutsumi, S. Tsunoda, T. Kihira, Y. Kaneda, Y. Yamamoto, S. Nakagawa, Y. Horisawa, and T. Mayumi., Biochem. Biophys. Res. Comm., 257:448–453 (1999).

[29] V. P. Torchilin. J. Microencapsulation., , 15:1–19 ,1998.

[30] G. P. Bettinetti, P. Mura, A. Liguori, G. Bramanti, and F. Giordano., Farmaco., , 43: 331–343, 1988.

[31] M. C. Tros de llarduya, C. Martin, M. M. Goni, and M. C. Martinez-Oharriz., Drug Dev. Ind. Pharm., , 24:295–300 , 1998.

[32] Nurseli Nyanik, Berrin Köker, Yalcin Yildiz, J of Appl. Polym. Sci., 71, 1915-1922, 1999.

[33] B. M.Discher, Y. Won, D. S. Ege, J. C-M. Lee, F. S. Bates, D. E. Discher, D. A. Hammer, Science 284, 1143, 1999.

[34] J. Champion, "UROs, tacos, worms and surfboards: what they teach about material-cell interactions," in Proceedings of the NATO ARW Nano-Engineered Systems for Regenerative Medicine, Varna, Bulgaria, September , 2007.

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