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Synthesis and Properties of a New Carrier for Paclitaxel and Doxorubicin Based on the Amphiphilic Copolymer of N-vinyl-2-pyrrolidone and Acrylic Acid

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This paper deals with the development of polymeric nanocarriers based on amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid of various molecular weights synthesized through the AIBN-initiated radical copolymerization of N-vinyl-2-pyrrolidone and acrylic acid in the presence of *n*-octadecyl mercaptan. The structure of the copolymers was characterized by ^{1}H NMR, ^{13}C NMR, IR and MALDI-TOF MS spectroscopy. It was shown that the length of the hydrophilic block defines the size of the nanoaggregates while impacting the steric stabilization efficiency and the probability of the interchain hydrogen bond formation. The hydrogen bonds formation between the residues of N-vinyl-2-pyrrolidone and acrylic acid was in agreement with the reduction of the ζ-potential of the nanoaggregates and the critical aggregation concentrations upon increasing the molecular weight. The presence of acrylic acid residues in the amphiphilic macromolecules leads to a higher affinity for doxorubicin and slow partial release of doxorubicin bonded with the aggregates' corona, which is helpful for reducing its cardiac toxicity. Nanoaggregates with a paclitaxel-loaded hydrophobic core

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were obtained, showing the possibility of dual loading. The amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid containing an *n*-octadecyl thio end group are thus promising candidates for combination cancer therapy with immobilized anti-cancer drugs, paclitaxel and doxorubicin.

Keywords: amphiphilic copolymers, drug delivery, cancer therapy, paclitaxel, doxorubicin

Introduction

The use of polymer nanoaggregates as carriers for drug delivery revolutionized pharmacology and opened up new possibilities for an extended circulation time, reduced toxicity, optimized release of active substances, and targeted drug delivery[1-3]. Polymer nanoaggregates loaded with anti-cancer drugs has been already successfully used for treatment of various types of cancer[4] and can eventually lead to an effective therapy for his disease which killed nearly 10 million people only in 2020[5]. Doxorubicin[6, 7] and paclitaxel[8, 9] are among the drugs with a broad anti-cancer activity, which, in some cases show a synergetic effect when used together[10-12]. Combined therapy with doxorubicin and paclitaxel can be effective in treatment of aggressive forms of cancer such as glioma[10], osteosarcoma[12] and metastatic breast cancer[13 - 15]. At the same time, paclitaxel in its pure form low bioavailability due to its low solubility in water[16]. Therefore, a number of methods have been developed for loading paclitaxel into polymer nanoaggregates with hydrophilic blocks composed of polyethylene glycol[17-24], polyethylene imine[25] and poly-(2-hydroxy ethyl methacrylate)[26]. From this perspective, poly-(N-vinyl-2- pyrrolidone) (PVP), used as a detox agent to treat poisoning[27], is expected to be superior in binding water-soluble substances. Non-covalent loading of doxorubicin into PVP telehelics has been recently shown to lead to highly water soluble drug carriers[28]. At the same time, however, the release rate of doxorubicin remained high[28].

Noncovalent binding and retension of doxorubicin hydrochloride may presumably be amplified through incorporation of a small amount of the acrylic acid residues into macromolecules along with the N-vinyl-2-pyrrolidone residues. Synthesis of amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymers capable of self-assembly in aqueous solutions with the formation of nanoscale aggregates opens up the possibilities for loading of the hydrophobic cores of the aggregates with paclitaxel as well as the noncovalent attachment of doxorubicin to the hydrophilic

corona of the aggregates. Given the low toxicity of amphiphilic PVP[29, 30], the fact that it is already used in drug delivery[30, 31] and the ease of synthesis, the application of amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid as carriers for co-delivery of paclitaxel and doxorubicin is a promising field. The present paper reports the synthesis and the properties of the amphiphilic N-vinyl-2-pyrrolidone copolymer containing a small amount of acrylic acid residues, as well as its application for simultaneous noncovalent loading and delivery of paclitaxel and doxorubicin.

2. Results and discussion

The radical copolymerization of N-vinyl-2-pyrrolidone and acrylic acid in the presence of *n*-octadecyl mercaptan results in the formation of amphiphilic macromolecules containing a hydrophobic *n*-octadecyl thio end-group and a chain consisting of hydrophilic residues of N-vinyl-2-pyrrolidone and acrylic acid (**Scheme 1**).

The ^{13}C NMR spectrum of the synthesized amphiphilic copolymer confirms the presence of all characteristic signals for the N-vinyl-2-pyrrolidone and acrylic acid residues within the macromolecular chain as well as the n-octadecyl mercapto end-group (**Figure 1**).

Since the copolymerization was carried out in a large excess of N-vinyl-2-pyrrolidone in order to prevent the formation of the low water-soluble interpolymer complexes, the FT-IR (**Figure 2A**) and ^{1}H NMR spectra (**Figure 2B**) of the obtained copolymer show all the signals characteristic of poly(N-vinyl-2-pyrrolidone)[32 - 34]. A broad intense 3440 cm^{-1} signal can be seen in the FT-IR spectrum of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid (**Figure 2A**), which is related to the stretch of the O-H-bonds in the carboxy groups of the acrylic acid residues that participate in hydrogen bond formation with the amide carbonyl of the N-vinyl-2-pyrrolidone residues. The 2955.7 cm^{-1} and 2925.4 cm^{-1} absorption bands pertain to the C-H-bonds' stretch, while the 1462.7, 1436.7, 1374.1, 1082.1 cm^{-1} and 844.3 cm^{-1} bands correspond to the bending of the C-H-bonds. The 1494.9

cm^{-1} and 1318.0 cm^{-1} signals can be connected to the vibrations in the O=C-N and CH_2-C-N atom systems of the pyrrolidone ring. The most intense 1661.1 cm^{-1} absorption band relates to the amide carbonyl stretch. The 1291 cm^{-1} signal belongs to the C-N-bond stretch, whereas the 1227 cm^{-1} absorption band is apparently due to the stretching of the C-O-bond as part of the carboxy groups of the acrylic acid residues. The signal interpretation of the ^{1}H NMR spectrum of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid is presented in **Figure 2B.**

The MALDI-TOF MS spectrum of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid showed a sequence of signals with the m/z ratio close to 111, which indicates the accumulation of the N-vinyl-2-pyrrolidone residues in the chain (**Figure 3**). The presence of the pair signals with m/z 538.653 and 482.744 as well as 328.778 and 272.794 with the m/z ratio ≈ 56 (**Figure 3**) suggests elimination of a fragment with the molecular weight of 56.

The elimination of the residue with m/z close to 56 was presumably caused by the formation of acrolein following dehydration of the acrylic acid residues and the shift of the C=C double bond along the chain [35] (**Scheme 2**).

The MALDI-TOF MS spectrum obtained in the negative ion mode (**Figure 4**) was in agreement with the polymer chain destruction processes. The m/z ratio of 40 was prevalent in the MALDI-TOF MS spectrum, which corresponds to the loss of the allyl fragment. Therefore, the MALDI-TOF MS spectra confirm the presence of acrylic acid residues in the copolymer among the outweighing number of the N-vinyl-2-pyrrolidone residues.

Increasing the *n*-octadecyl mercaptan concentration in the reaction system leads to a decrease in the number-average molecular weight of the formed amphiphilic copolymers, so a linear dependence in «$10^4 \times \bar{M}_n^{-1}$ - $C(C_{18}H_{37}SH)$» coordinates is observed (**Figure 5**). Therefore, the interaction of the macroradicals with *n*-octadecyl mercaptan causes the growth termination for most of the macroradicals.

The isothermal dependences of the surface tension at water/toluene interface on the concentration of the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid of various molecular weights are shown in **Figure 6**.

The critical aggregation concentration (CAC) values for the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid with an *n*-octadecyl mercapto end-group were shown to have a tendency to decrease with an increase in the number-average molecular weight (**Table 1**). Thus, although the length of the hydrophilic block was growing, the solubility of the synthesized amphiphilic copolymers dropped. Most likely this can be explained by the formation of complexes of N-vinyl-2-pyrrolidone and acrylic acid residues stabilized by hydrogen bonds[36, 37].

The thickness of the adsorbed layer was predictably increasing at first along with the growth of the molecular weights of the amphiphilic copolymers due to an increase in size of the adsorbed macromolecules. Further it began decreasing, which may be explained by the fact that the sufficient chain length was reached allowing for more compact conformations, in which the N-vinyl-2-pyrrolidone and acrylic acid residues interacted through hydrogen bond formation[36, 37]. In this case the negative charge density should increase, which was confirmed by the decrease of the aggregates' ζ-potential with the growth of the number-average molecular weights of the synthesized amphiphilic copolymers (**Figure 7**).

The number size distribution obtained for the aggregates of the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid (**Figure 8A**) demonstrates the existence of an optimal molecular weight of the chain that provides the minimum diameter of the nanoaggregates. It may be expected that a short hydrophilic block does not provide sufficient steric stabilization for the particles. On the contrary, in the case of a too long hydrophilic block the particle aggregation is most likely related to interchain coupling through hydrogen bonds between the residues of acrylic acid and N-vinyl-2-pyrrolidone. The formation of hydrogen bonds between the residues of acrylic acid and N-vinyl-2-pyrrolidone has been described in the literature in relation to obtaining low water-soluble interpolymer complexes of poly(N-vinyl-2-pyrrolidone) and polyacrilic acid[36, 37]. So, in order to ensure sufficient solubility of the amphiphilic macromolecules in water, copolymerization of N-vinyl-2-pyrrolidone was carried out in the presence of as little as 5 mol% of acrylic acid. Therefore, the minimum number average particle diameter was reached for the amphiphilic copolymers ranging in molecular weights from 4000 to 5000 and was determined as 290 – 300 nm (the minimum intensity diameter distribution ca. 480 nm) (**Figure 8 A-C**).

As it can be seen, the vast majority of the particle sizes range from 100 nm to 500 nm (**Figure 8A**), while a considerable amount of the amphiphilic copolymer can be found inside the few large particles above 500 nm in diameter (**Figure 8B**). The size distributions for the particles consisting of the amphiphilic copolymer chains with number average molecular weights ranging from 3500 to 7100 (**Figure 8B**, **curves 2-4**) have a pronounced trimodal nature, which is in agreement with the development of aggregation as a result of both insufficient steric stabilization and hydrogen bond formation. The hydrophilic block length increase boosts the steric stabilization efficiency but also enhances the contribution of the aggregation mechanism related to the interchain hydrogen bond formation. Therefore, in case of the nanoparticles formed through self-assembly of the N-vinyl-2-pyrrolidone and acrylic acid copolymer chains (number average molecular weight 11000), the main contribution to the formation of secondary aggregates comes from the interaction via hydrogen bond formation with bimodal particle size distribution (**Figure 8B**, **curve 1**). On the contrary, for the aggregates of N-vinyl-2-pyrrolidone and acrylic acid copolymer chains with number average molecular weight of 3500 the decisive contribution into the formation of secondary aggregates comes from the insufficient steric stabilization efficiency. At in-between number average molecular weights of the amphiphilic copolymer (4400 and 7100) both mechanisms for the aggregate stability loss contribute to the formation of secondary aggregates, which leads to the highest PDI value (**Figure 8D**). In case of the aggregates of N-vinyl-2-pyrrolidone and acrylic acid copolymer chains with the number average molecular weight of 4400 both mechanisms for the formation of secondary aggregates are implemented, however neither of the two contributes decisively, which ensures the minimal diameter of the particles alongside with the maximum polydispersity (**Figure 8C, D**).

The dilution by four times of the N-vinyl-2-pyrrolidone and acrylic acid amphiphilic copolymer dispersions with various molecular weights lead to the decrease of the number average diameter of the aggregates down to 140 – 145 nm and the intensity diameter distribution of the aggregates to 320 – 335 nm. After dilution the particle size distribution curves are almost unaffected by the molecular weights of the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid; at the same time the fraction of particles exceeding 800 nm in diameter disappears (**Figure 8** and **Figure 9**), which may be related to the destruction of the interchain hydrogen bonds. The typical particle size distribution curves obtained after fourfold dilution of the water dispersion of

the N-vinyl-2-pyrrolidone and acrylic acid amphiphilic copolymer with number average molecular weight 4400 are presented in **Figure 9**.

Comparing the dependences between the S_0 values (**Table 1**), the number average diameters of aggregates (**Figure 8**) and the number-average molecular weights of the amphiphilic copolymers, it can be assumed that at the interface the adsorption of macromolecular associates rather than individual macromolecules takes place. This point together with minor negative ζ-potentials (**Figure 7**) indicates the predominantly steric stabilization mechanism of the nanoparticles.

The formation of nanoparticles during self-assembly of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid macromolecules opens up potential for loading the hydrophobic cores of nanoaggregates with paclitaxel. To ensure the smallest possible particle diameter, the amphiphilic copolymer with $\bar{M}_n = 4400$ was used (**Figure 8C**). The number average particle size decreased from 295 nm to 218 nm upon loading of the nanoscale aggregates with the paclitaxel solution in chloroform (**Figure 10A**). The intensity average diameter also decreased from 468 nm to 319 nm upon loading of the aggregates with the paclitaxel solution in chloroform due to the disappearance of the particles above 750 nm. The stabilization of the aggregates' cores through hydrophobic interactions with the loaded chloroform appears to be the cause for the simultaneous decrease of the particle diameters and PDI from 0.324 down to 0.142. The latter assumption is in agreement with the significant increase of PDI up to 0.460 after removal of chloroform by distillation. The removal of chloroform from the core of the aggregates resulted in the decrease of their number average diameter to 135 nm; however the intensity average size on the contrary increased to 423 nm (**Figure 10**). Therefore, the removal of chloroform by distillation leads to the formation of a large number of small particles alongside with the formation of large aggregates, the weight content of which was significant. It appears that the increase of the share of small particles was related to the loading of the aggregates' cores with the paclitaxel chains that had a stabilizing effect through the hydrophobic interactions with the hydrophobic *n*-octadecyl mercapto end-groups of the amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymer. On the contrary, the fraction of the large diameter particles contained both the secondary chain aggregates and free paclitaxel. The subsequent filtration through a membrane with the 450 nm separation allows removing the large particles and results in the reduction of the number average diameter of the aggregates down to 93 nm and the

intensity average diameter down to 195 nm. The PDI also decreased to 0.213 following filtration (**Figure 10**).

The TEM microscopy data point to formation of the aggregates of the amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymer chains with the molecular weight of 4400 and the 300 – 500 nm diameter (**Figure 11A)**, which was qualitatively in agreement with the dynamic laser light scattering data (**Figure 8C)**. After loading with paclitaxel, the diameters of the aggregates went down to 150 – 250 nm (**Figure 11B**), which was also confirmed by the dynamic laser light scattering data (**Figure 10B, curve 4**).

Subtraction of the UV spectrum of the paclitaxel-free aggregates from the UV spectrum of the aggregates loaded with paclitaxel in the 225 nm – 235 nm wavelength range presented a picture typical for absorption of paclitaxel having the molar absorption coefficient of 29800 $l \times mol^{-1} \times cm^{-1}$ at 227 nm[38] (**Figure 12**). Since the maximum paclitaxel concentration upon its complete loading is 5.86×10^{-4} $mol \times l^{-1}$ and the actual concentration of the loaded paclitaxel was 3.93×10^{-4} $mol \times l^{-1}$, this means that the yield of the preparation loading process was 67%. Consequently, the largest share of paclitaxel was loaded into the 500 nm particles, which confirms the assumption regarding the stabilization of the cores of the N-vinyl-2-pyrrolidone and acrylic acid copolymer chains through the hydrophobic interactions with paclitaxel as a reason for the decrease of the number average particle diameter after the removal of chloroform by distillation. The loading of the aggregate cores of the N-vinyl-2-pyrrolidone and acrylic acid amphiphilic copolymer with paclitaxel was followed by the addition of 10 wt.% of doxorubicin hydrochloride from the weight of the initial polymer carrier, which resulted in its electrostatic immobilization as it will be demonstrated below.

Endocytosis studies on the structurally similar aggregates of amphiphilic poly(N-vinyl-2-pyrrolidone) chains loaded with model dyes curcumin[30] and DiI[31] showed fast cellular uptake of the nanoaggregates. On the contrary, the release of paclitaxel from the nanoaggregates by diffusion was rather slow[39, 40]. Given a relatively short half-life of paclitaxel in the body of approx. 7 hours[41], it may be assumed that it is the endocytosis that defines the involvement rate of immobilized paclitaxel into the metabolic processes and its therapeutic performance, rather than its passive release from the nanoparticles. In contrast, the insufficient binding efficacy of the N-vinyl-2-

pyrrolidone homopolymer[28], the slow clearance rate[42] and high cardiac toxicity of doxorubicin[43-45] all point to the importance of the immobilization of doxorubicin to achieve slow release.

Replacing poly(N-vinyl-2-pyrrolidone) for an amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid greatly increases affinity for doxorubicin. As was previously shown, poly(N-vinyl-2-pyrrolidone) only slightly decreases the rate of doxorubicin release[28]. On the other hand, the amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymer binds around 72% of doxorubicin (**Figure 13 A**). It may be expected that loading a polymer chain with the acrylic acid residues that impart a negative charge to the nanoparticles may decrease the rate of their entrapping by the cells due to the negative charge of a cell membrane itself. At the same time the latest research indicates that the negative charge of the nanoparticles does not prevent their internalization by the cells [46] and only slightly increases the contribution of the clathrin- and dynamin-dependent endocytosis vs. macropinocytosis[47] as well as it affects the interaction with the biological environment components such as microscopic biomolecules, cells and the macroscopic environment *in vivo* [48].

The release kinetics for water-soluble substances normally follows a first-order equation[49-51]. Provided maximum conversion, the equation (1) for equilibrium chemical reactions of the first order can be derived[52].

$$\ln(p_\infty - p) = \ln p_\infty - (k_1 + k_2)t \qquad (1)$$

where: p and p_∞ are the actual and the limiting doxorubicin release conversions; k_1 and k_2 are the doxorubicin binding and release rate constants, respectively; t is the time.

As can be seen (**Figure 13A**), the equilibrium release conversion for the pure doxorubicin and of that in the presence of poly(N-vinyl-2-pyrrolidone) were close to 1. Replacing poly(N-vinyl-2-pyrrolidone) with an amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid allowed significantly reducing the equilibrium release conversion down to 0.282 (**Figure 13 A**). At the same time the experimental data for all studied cases displayed linear dependence in the $\ln(p_\infty - p)$ vs. t coordinates (**Figure 13 B**).

The equilibrium release constant for doxorubicin noncovalently bound to the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid (K) can be calculated using equation (2) and was found to be 0.39.

$$K = \frac{k_1}{k_2} = \frac{p_\infty}{1 - p_\infty} = 0.39 \qquad (2)$$

By solving simultaneous equations (1) and (2) the rate constants k_1 and k_2 could be determined, which were equal to 0.21 h^{-1} and 0.55 h^{-1}, respectively. In the presence of poly(N-vinyl-2-pyrrolidone) the k_1 rate constant was equal to 0.57 h^{-1}, while k_2 was negligible, which suggests nearly irreversible release of doxorubicin. Accordingly, replacing poly(N-vinyl-2-pyrrolidone) for an amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid resulted in a decrease of the drug release constant by a factor of 2.7 and a significant increase in the doxorubicin binding rate constant. Therefore, the electrostatic interactions may be used for control of the doxorubicin release rates along with its encapsulation [53] or immobilization through the covalent bonds that are volatile in bio-systems [54, 55].

Consequently, the nanoparticles formed by the chains of the amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymer not only allow for loading of the hydrophobic cores with paclitaxel but can also be used for immobilization of doxorubicin with the possibility of controlled release. The latter opens up new possibilities for combined cancer treatment with paclitaxel and doxorubicin and helps to reduce the cardiac toxicity of doxorubicin.

3. Experimental

3.1. Materials and methods

Acrylic acid, azobisisobutyronitrile (AIBN) and *n*-octadecyl mercaptan were purchased form Sigma-Aldrich. N-vinyl-2-pyrrolidone and 1,4-dioxane were obtained from Acros and additionally purified by distillation under vacuum. Paclitaxel and doxorubicin hydrochloride were sourced from LC Laboratories and Synbias Pharma, respectively, and used without additional purification.

The structure of the obtained amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid was characterized by ^{13}C NMR and ^{1}H NMR spectroscopy in the DMSO-d6 medium using the

Bruker Avance 500 spectrometer (Bruker, Zurich, Switzerland) at the Research Center for New Materials and Technologies at IBCP RAS and by IR-spectroscopy (Nicolet 380) in potassium bromide pellets. MALDI-TOF MS spectra were recorded using an Ultraflex II mass-spectrometer (Bruker, Karlsruhe, Germany) at 25 kV accelerating voltage under desorption with a Nd: YAG laser (355 nm) without matrix. The size and zeta potential of the obtained nanoaggregates were measured with a NANO-flex II nanosizer (Colloid Metrix, Germany). The kinetics of doxorubicin release were determined using the absorbance at 480 nm with a UV-vis Eppendorf BioSpectrometer. TEM micrographs were obtained with the JEM-1011 microscope (Jeol, Tokyo, Japan) after applying the dispersion onto the carbon-coated grids.

3.2. Synthesis of the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid

N-vinyl-2-pyrrolidone **(**9.99 g, 0.09 mol) and acrylic acid (0.325 g, 4.5×10^{-3} mol), as well as AIBN (0.1 g, 6×10^{-4} mol) and a preset amount of *n*-octadecyl mercaptan were dissolved in 1,4-dioxane (45 ml). The copolymerization was carried out at 343 K for 3 hours. After that the reaction mixture was cooled down to room temperature, diluted by fivefold amount of distilled water and followed by rotary evaporation of 1,4-dioxane. The amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid was further purified by dialysis against distilled water for 5 days using a 500 MWCO membrane (Labware supplier store). The dialyzed solutions were then frozen and freeze-dried (Alpha 1-4 LD plus, Martin Christ, Germany). Copolymerization was carried out in the presence of *n*-octadecyl mercaptan (0.5–2.0 mol% with respect to N-vinyl-2-pyrrolidone concentration). The number-average molecular weights of the obtained amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid were determined through the end-group analysis as described earlier[32].

3.3. Release kinetics measurement for noncovalently immobilized doxorubicin

The amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid (0.1 g) synthesized in the presence of *n*-octadecyl mercaptan (1.25 mol%) was dissolved in of distilled water (5 ml). Doxorubicin hydrochloride (0.01 g) was dissolved in distilled water (5 ml). The obtained solutions were mixed and dialyzed against of distilled water (250 ml) in a dialysis bag (Labware supplier store 500 MWCO). The dialysis kinetics was studied at 309 K by recording absorbance values at 480 nm wavelength.

3.4. Loading of paclitaxel into nanoaggregates of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid

The amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid (0.2 g) synthesized in the presence of *n*-octadecyl mercaptan (1.25 mol%) was dissolved in distilled water (10 ml). The amphiphilic copolymer solution was sonicated using a SONOPULS HD4400 (Bandelin, Germany) in a pulse mode with 1 s on 1 s off for two minutes. After the addition of paclitaxel solution in chloroform (1 ml of 5×10^{-3} g·ml^{-1}), the ultrasonic treatment repeated for another 18 minutes. Chloroform from the resulting emulsion was evaporated in a rotary evaporator under vacuum. Unincorporated paclitaxel tends to aggregate and form particles which were removed by filtration through a syringe filter with the pore size of 450 nm.

3.5. Measurement of surface tension isotherms

The dependences of the surface tension at water/toluene interface on the concentrations of the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid of various molecular weights were determined by the pendant drop method using the KRUSS DSA30 automated drop shape analyzer at 296 K. The obtained dependences were used for determination of the CAC, as well as for calculation of the effective area occupied by the chain fragments of the amphiphilic copolymers in the adsorbed layer (S) and the thickness of the adsorbed layer (δ) according to equation (3) and (4), respectively.

$$S = \frac{1}{\Gamma_{max} N_A} \quad (3)$$

$$\delta = \frac{\Gamma_{max} M}{\rho} \quad (4)$$

where: Γ_{max} – max Gibbs adsorption value, N_A – Avogadro's number, M, ρ – molecular weight and density of the copolymers.

For the calculation purposes it was assumed that the excessive Gibbs adsorption was close to the Helmholtz adsorption and thus the max Gibbs adsorption value (Γ_{max}) was determined as a reciprocal slope of the linear dependences in the "$\frac{C}{\Gamma}$ vs. C" coordinates.

Conclusion

The amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid containing a hydrophobic *n*-octadecyl mercapto end-group were synthesized. The size of the aggregates is defined by the interplay of steric stabilization and the interchain coupling through hydrogen bonding. It was found that increasing the length of the hydrophilic block leads to an increase in ζ-potential, related to an increase in the chain packing density, as well as a decrease in the CAC. This is in agreement with the hydrogen bond formation between the N-vinyl-2-pyrrolidone and acrylic acid residues. Consequently, the size of the chain aggregates and the critical aggregation concentration are defined by the molecular weight of the amphiphilic macromolecules, which is controlled by the amount of *n*-octadecyl mercaptan in the reaction system.

The aggregates of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid can be used for simultaneous loading of the hydrophobic core with paclitaxel and immobilization of doxorubicin on the corona. The latter is electrostatic by nature and related to the presence of the acrylic acid residues in the chain. While poly(N-vinyl-2-pyrrolidone) only mildly slows down the release of doxorubicin during dialysis, the introduction of acrylic acid residues into the chain leads to incomplete release, a significant drop in the release rate constant and an increase of the doxorubicin binding rate constant.

The obtained polymer nanocarriers have a potential for combination cancer therapies and may be promising for increasing bioavailability of paclitaxel and decreasing cardiac toxicity of doxorubicin. In all likelihood, the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid may be used for immobilization of any drug tandems, in which one is sufficiently hydrophobic for core loading and the other contains an amino group that provides binding with the corona.

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Conflict of Interest. The authors declare no conflict of interest.

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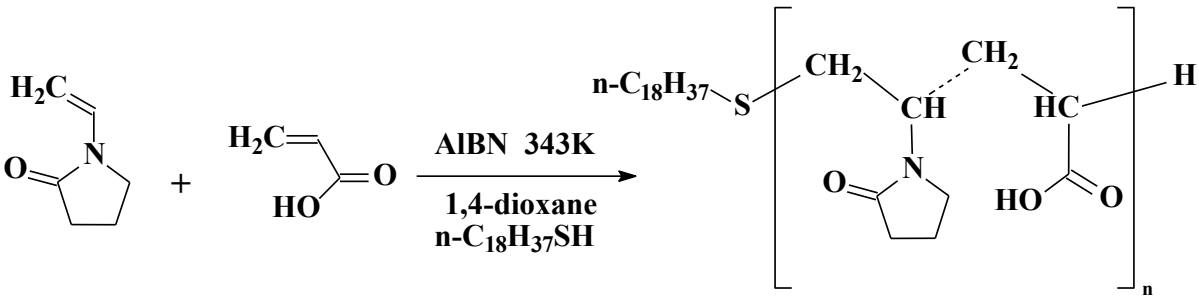
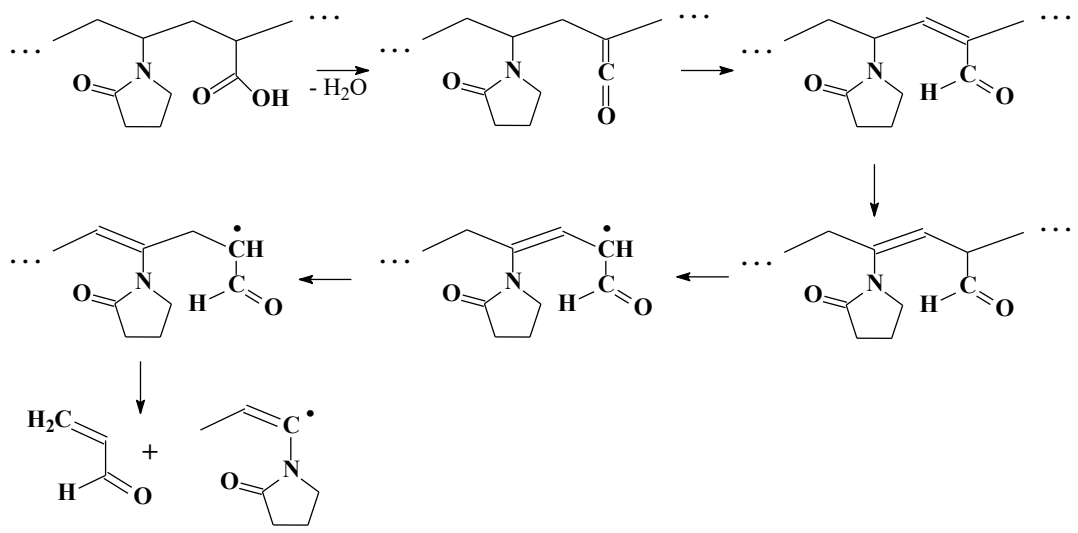


Scheme 1. Radical copolymerization of N-vinyl-2-pyrrolidone and acrylic acid in the presence of *n*-octadecyl mercaptan.



Scheme 2. The suggested chain destruction mechanism for the N-vinyl-2-pyrrolidone and acrylic acid copolymer, involving formation of acrolein under laser desorption.

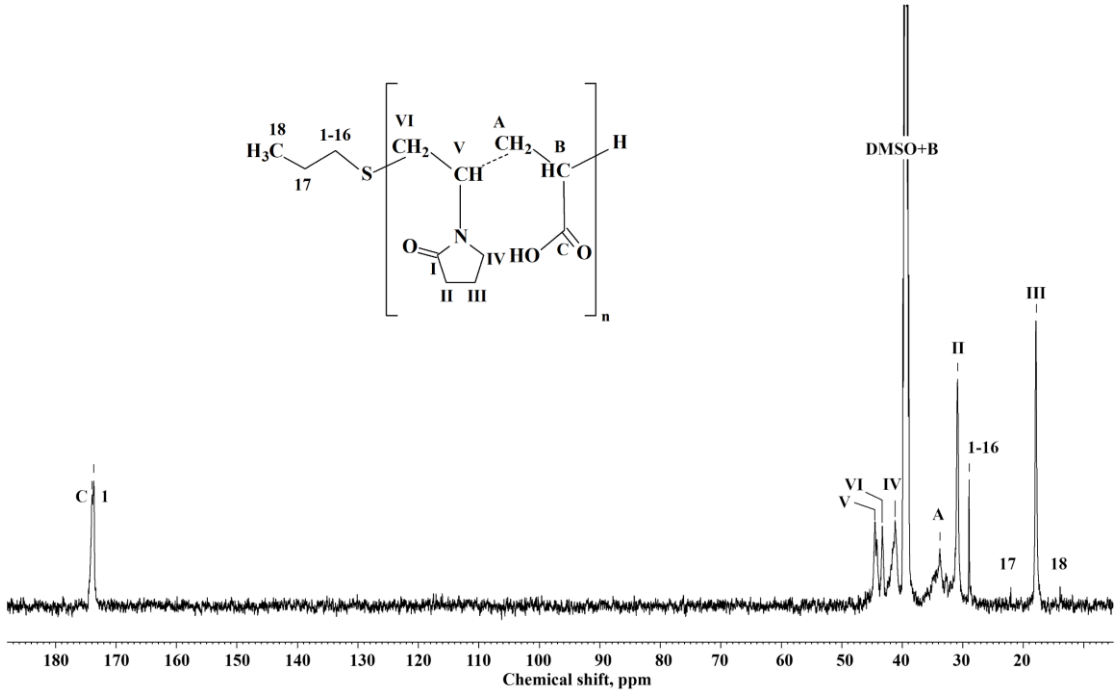


Figure 1. The ^{13}C NMR spectrum of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid in the DMSO-d6 solution.

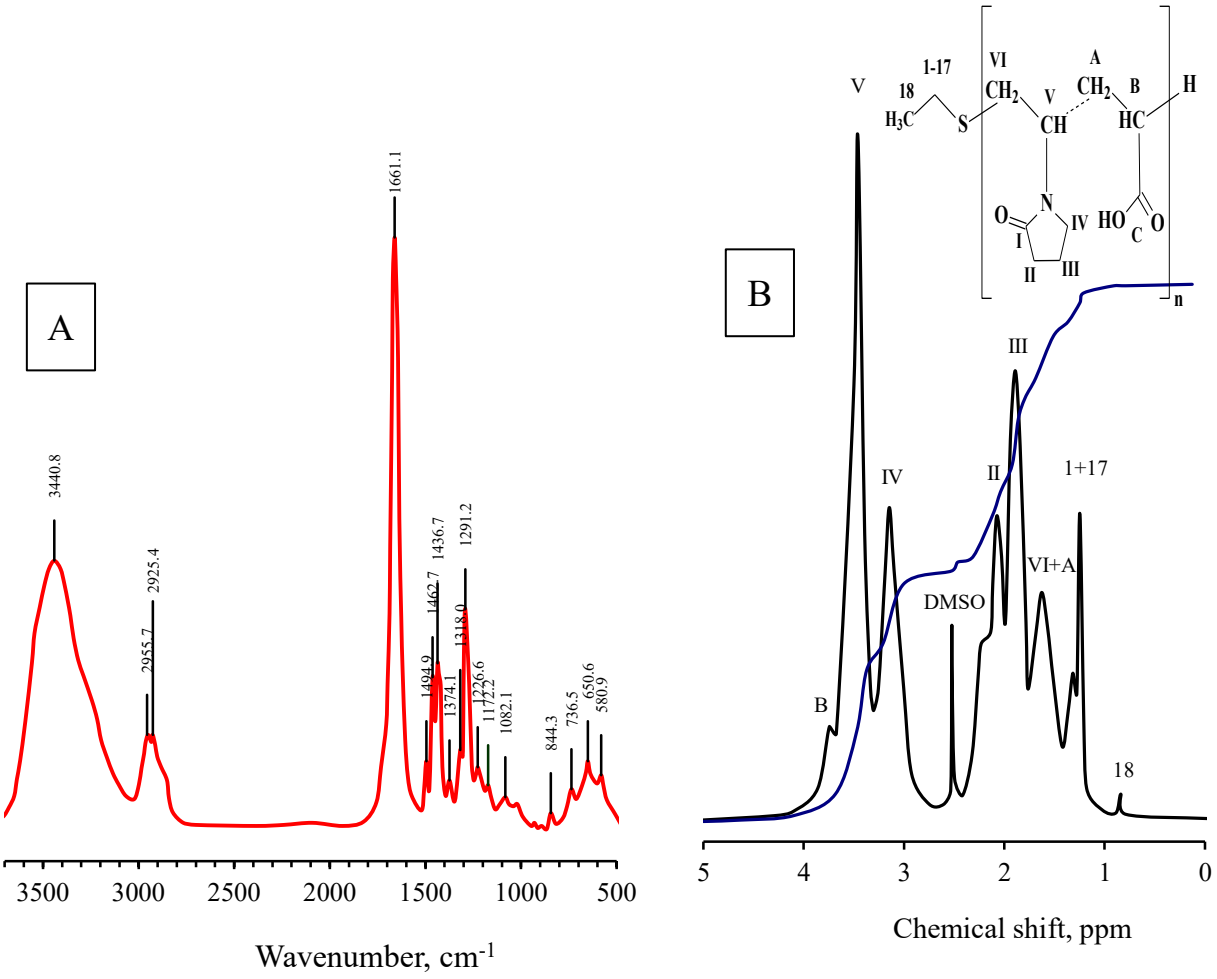


Figure 2. (A) FT-IR (in potassium bromide pellets) and (B) ^{1}H NMR spectra (in DMSO-d6 solution) of the N-vinyl-2-pyrrolidone and acrylic acid copolymer with an *n*-octadecyl end-group.

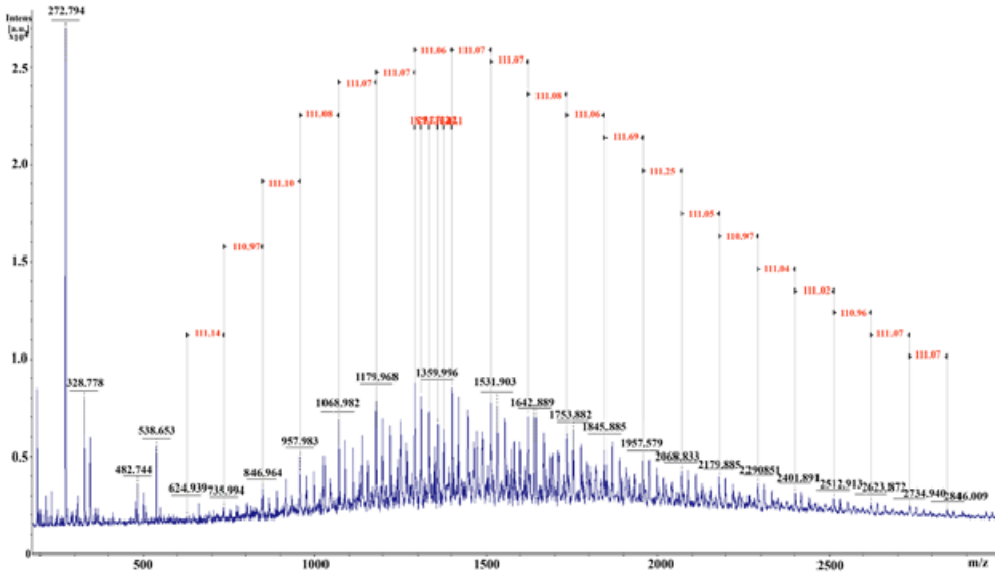


Figure 3. MALDI-TOF MS spectrum of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid in the positive ion mode.

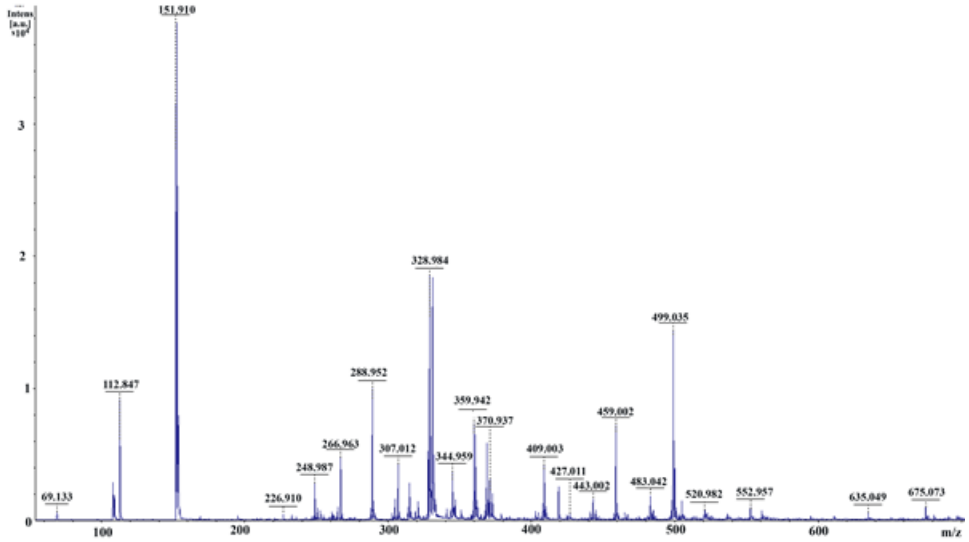


Figure 4. MALDI-TOF MS spectrum of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid in the negative ion mode.

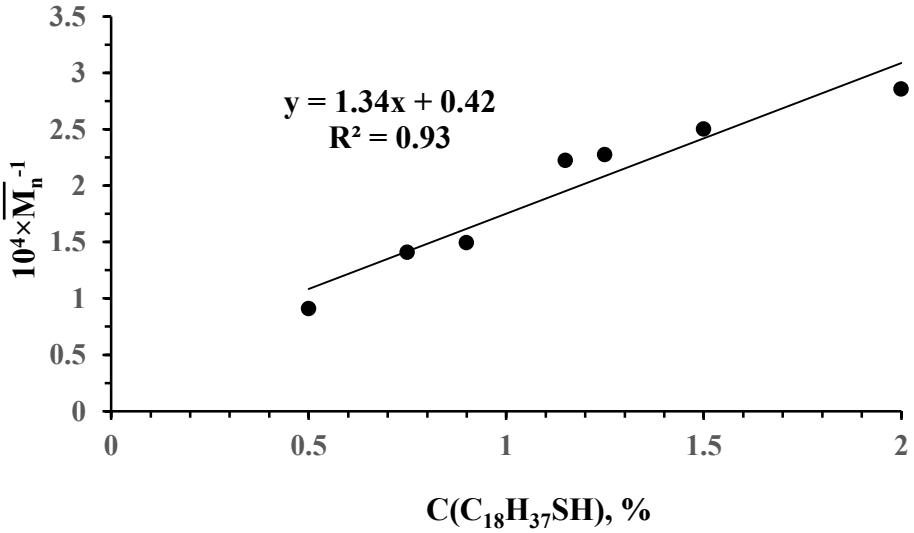


Figure 5. The dependence of the number average molecular weight $\bar{M}_n^{-1}$ on the concentration of *n*-octadecyl mercaptan for radical copolymerization of N-vinyl-2-pyrrolidone and acrylic acid.

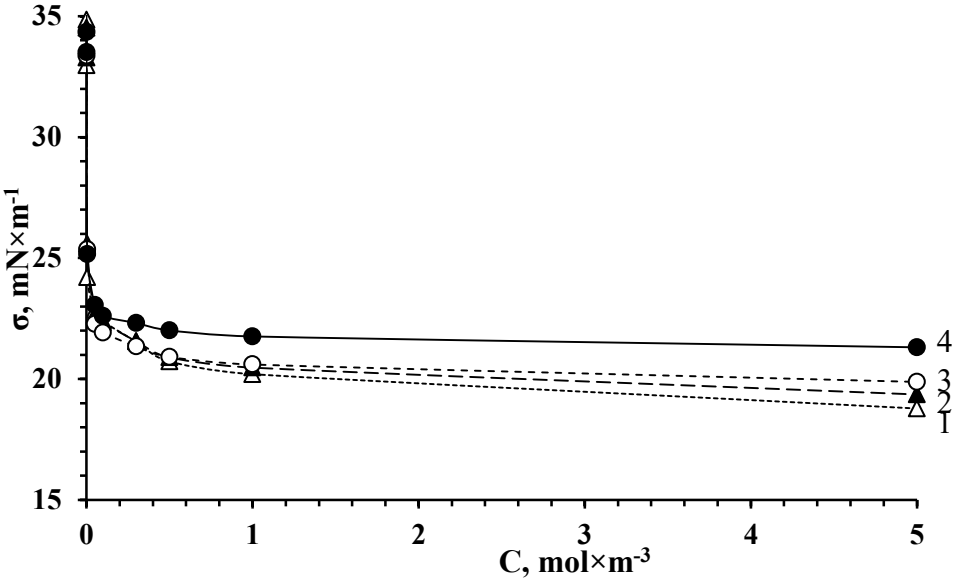


Figure 6. The isothermal dependences of the surface tension at water/toluene interface on the concentration of the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid of various molecular weights: (1) – 11000, (2) – 7100, (3) – 4400, (4) – 3500.

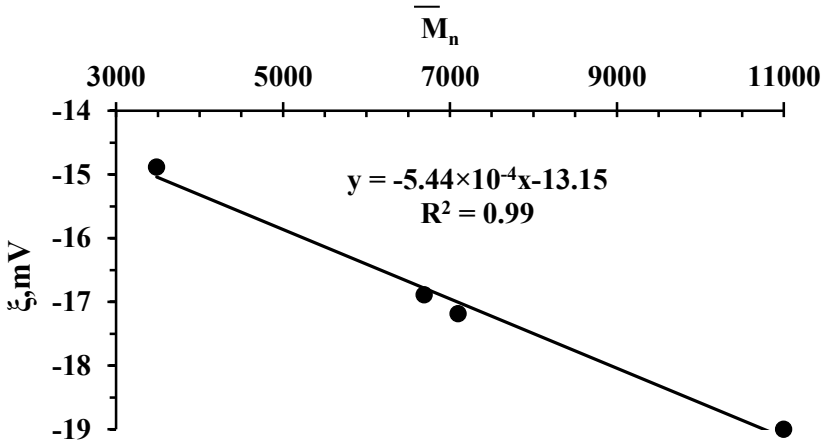


Figure 7. ζ-potential of nanoaggregates formed by the chains of the amphiphilic copolymer of N-vinyl-2-pyrrolidone and acrylic acid as a function of the number average molecular weight $\overline{M_n}$ (pH = 4).

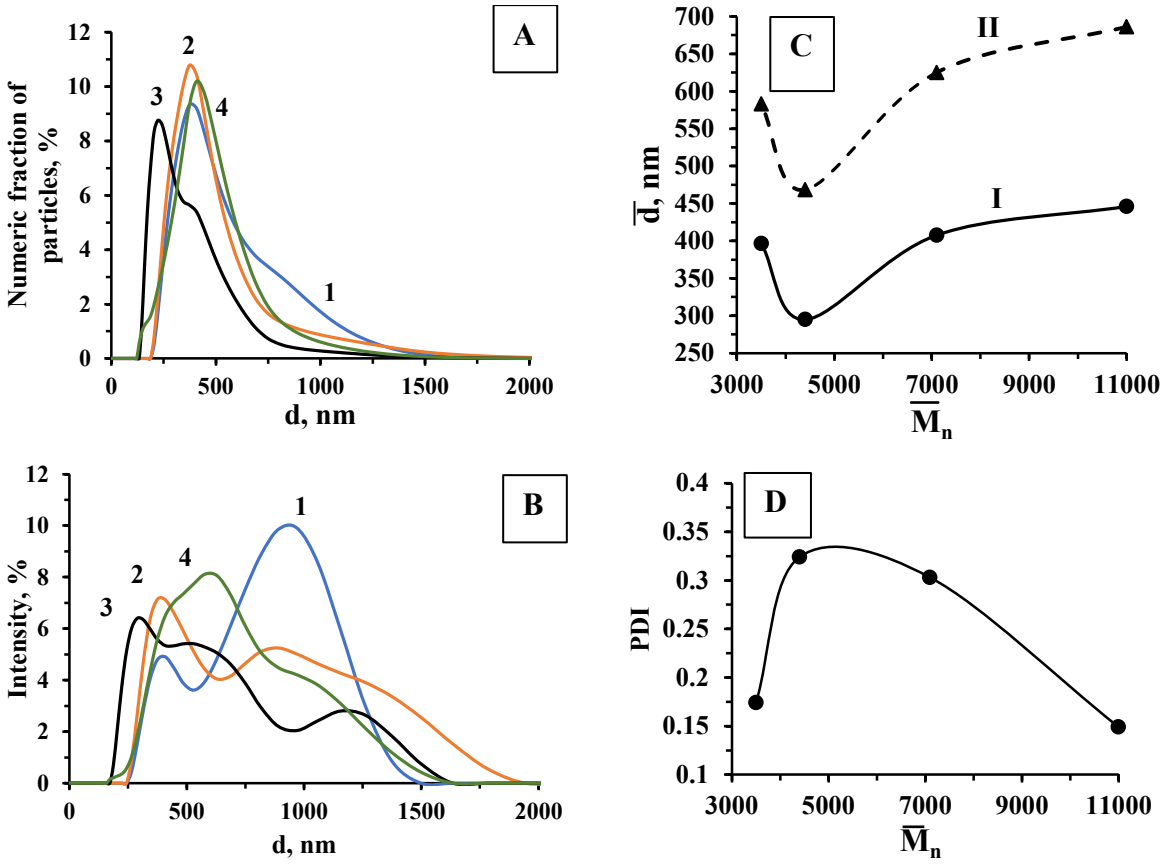


Figure 8. The sizes of the aggregates of the amphiphilic copolymer chains: A) – the number size distribution of nanoaggregates formed by the copolymer of N-vinyl-2-pyrrolidone and acrylic acid at various number average molecular weights $\overline{M}_n$; B) – the intensity size distribution of nanoaggregates formed by the copolymer of N-vinyl-2-pyrrolidone and acrylic acid at various number average molecular weights $\overline{M}_n$: (1) – 11000, (2) – 7100, (3) – 4400, (4) – 3500; C) – the (number – I, intensity – II) average size of aggregates as a function of $\overline{M}_n$; D) – the PDI of aggregates as a function of $\overline{M}_n$.

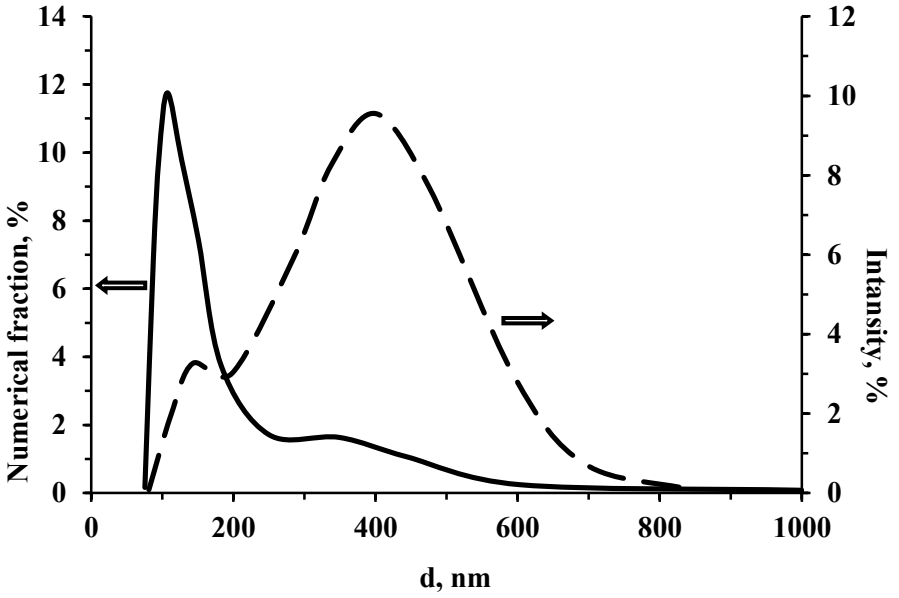


Figure 9. The number and intensity size distributions for the nanoaggregates formed by the N-vinyl-2-pyrrolidone and acrylic acid copolymer ($\bar{M}_n$ = 4400).

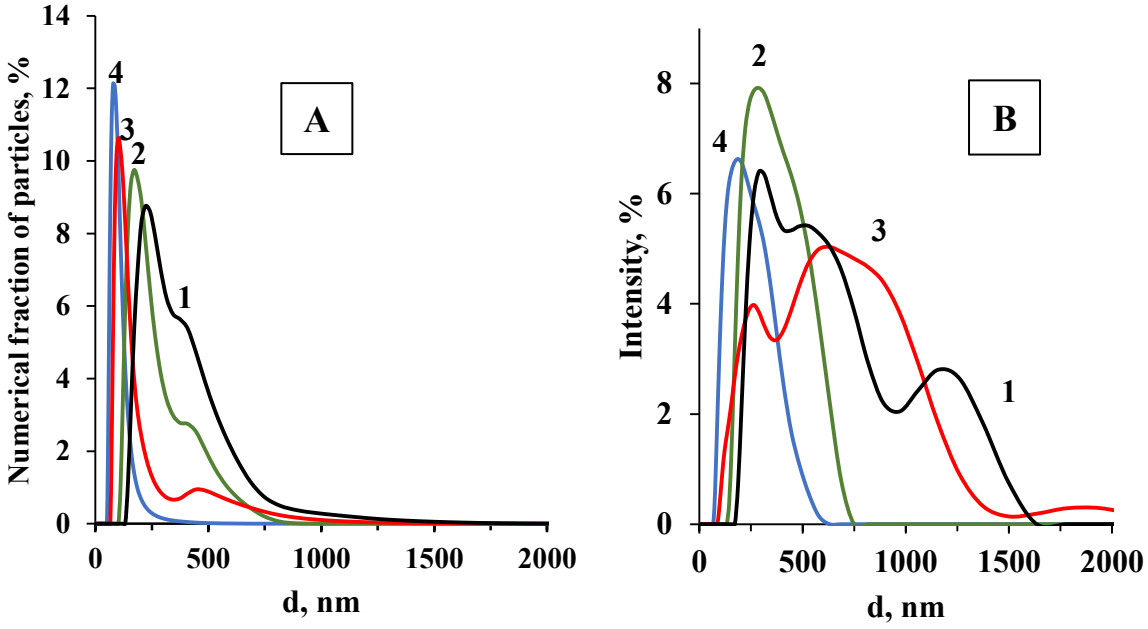


Figure 10. The number average (A) and intensity average (B) size distributions of the nanoaggregates for paclitaxel immobilization: 1 – initial aggregates of the amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymer ($\bar{M}_n = 4400$); 2 – after loading with the paclitaxel solution in chloroform; 3 – after removal of chloroform by distillation; 4 – after filtration (filter pore size 450 nm).

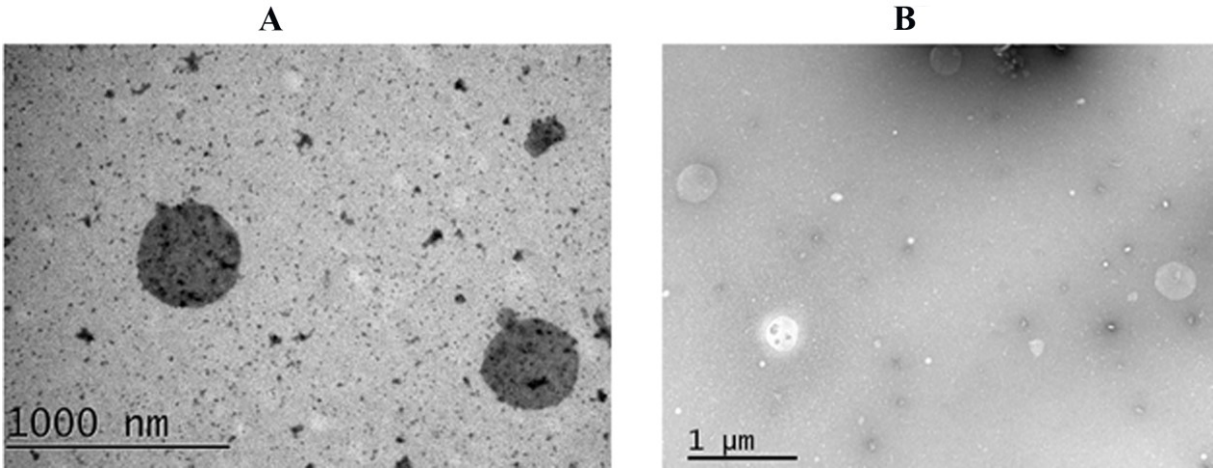


Figure 11. TEM micrographs: A – initial aggregates of the amphiphilic N-vinyl-2-pyrrolidone and acrylic acid copolymer ($\bar{M}_n = 4400$); B – aggregates after loading with paclitaxel and filtration (filter pore size 450 nm).

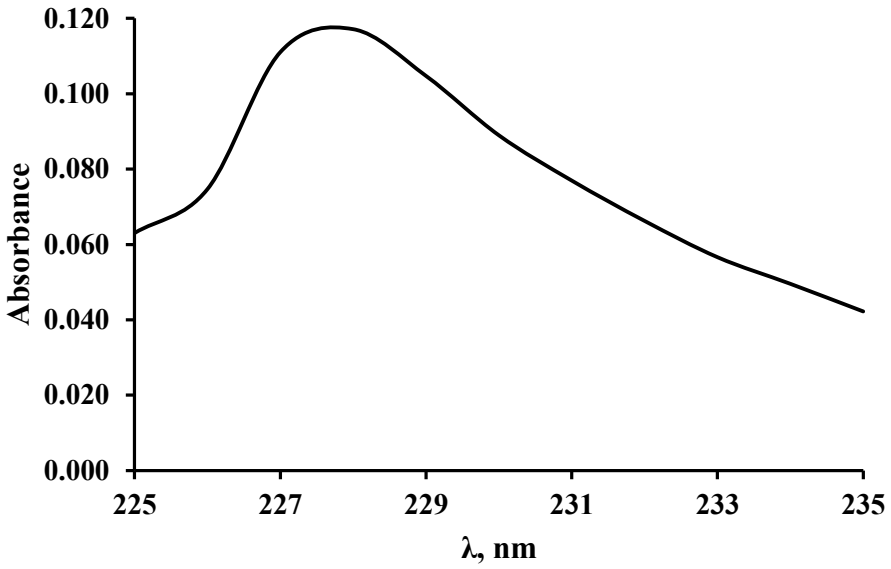


Figure 12. The UV spectrum obtained by subtraction of the UV spectrum of the paclitaxel-free aggregates from the UV spectrum of the aggregates loaded with paclitaxel at 225 nm – 235 nm after dilution of the water dispersion by 100 times.

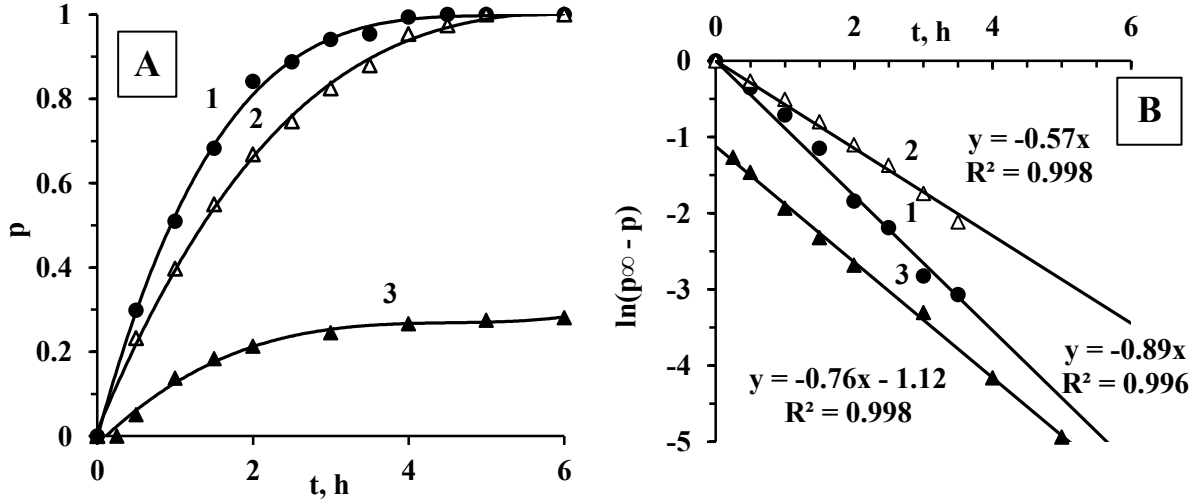


Figure 13. The kinetic release curves (A – in the p vs. t coordinates; B – in the $\ln(p_\infty - p)$ vs. t) coordinates: 1 – pure doxorubicin; 2 – doxorubicin in the presence of poly(N-vinyl-2-pyrrolidone); 3 –

doxorubicin in the presence of an N-vinyl-2-pyrrolidone and acrylic acid amphiphilic copolymer (curves 1 and 2 show the previously obtained data[28]).

Table 1. The CAC, the effective surface area occupied by the macromolecule fragments in the adsorbed layer (S_0) and the thickness of the adsorbed layer (δ) for the amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid of different number average molecule weight.

$\overline{M_n}$	$S_0 \times 10^{-18}$, [m^2]	$\delta \times 10^{-9}$, [m]	CAC, [$mol \times m^{-3}$]
11000	5.14	2.96	0.0023
7100	2.34	4.38	0.0028
4400	2.23	2.73	0.0027
3500	3.28	1.48	0.03

Amphiphilic copolymers of N-vinyl-2-pyrrolidone and acrylic acid with a hydrophobic *n*-octadecyl thio end-group capable of self-assembly in water and noncovalent immobilization of paclitaxel and doxorubicin were synthesized. Diameters of aggregates were defined by interplay of steric stabilization and interchain coupling through hydrogen bonding. The aggregates are promising carriers for combination therapies of late-stage cancer and decreasing cardiac toxicity of doxorubicin.