

Characterization of amphiphilic poly-*N*-vinylpyrrolidone derivatives by dynamic light scattering

Apostolos K. Rizos ^{a,*}, Ioannis Tsikalas ^a, Aristidis M. Tsatsakis ^b, Michail I. Shtilman ^c

^a *University of Crete, Department of Chemistry and Foundation for Research and Technology-Hellas (FORTH), P.O. Box 2208, Heraklion 71003, Crete, Greece*

^b *University of Crete, Department of Medicine, Crete 71110, Greece*

^c *Department of Polymers, D.I. Mendeleev University of Chemical Technology, 9 Miusskaya Square, Moscow 125047, Russia*

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Abstract

Liposomes have been widely considered as promising drug carriers for many years. Certain amphiphilic water-soluble polymers including amphiphilic derivatives of polyvinyl pyrrolidone (PVP) were found to be efficient steric protectors for liposomes *in vivo*. Amphiphilic PVP (SPVP) with molecular weight between 1800 and 6300 easily incorporates into the liposomal membrane and provides good steric protection for liposomes. The degree of this protection depends on both polymer concentration and molecular size of the PVP block. In order to investigate the influence of the hydrophobic and hydrophilic blocks on the properties of the resulting polymers, and their ability to provide steric protection, we have performed dynamic light scattering experiments (DLS) in which we measure the temporal autocorrelation function of fluctuations in the scattered intensity resulting from the diffusive motion of the particles. All SPVP derivatives form stable micelles in water solution.

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1. Introduction

Liposomes have been widely considered as promising drug carriers for many years [1–6]. Long-circulating drug carriers may be used to maintain a required level of a pharmaceutical agent in the blood for extended time intervals for better drug bioavailability. Surface modification of liposomes is intended to improve various physico-chemical properties and make them more stable in the biological environment. To impart *in vivo* longevity to liposomes, chemical modification with certain synthetic polymers is usually applied [7–13]. Polymers have been shown to protect individual molecules and solid particulates from interaction with different solutes. The important feature of protective

polymers is their flexibility (short segment length and free rotation of individual polymer units around inter-unit linkages). The molecular mechanism of polymer protective action is determined by the properties of a flexible polymer molecule in solution and includes the formation of a polymeric layer over the liposome surface which is impermeable to other solutes even at relatively low polymer concentrations. The more flexible the polymer, the larger the total number of its possible conformations and the higher the transition rate from one conformation to another.

Polymers that are candidates for steric protection of drugs and drug carriers should be soluble, hydrophilic and have highly flexible main chain. Polymer biocompatibility has to be added to the list if polymer liposomes are intended for medical use. Synthetic polymers of vinyl series, such as poly(vinyl pyrrolidone) (PVP) may serve as the most evident example of other potentially protective

* Corresponding author. Tel.: +30 2810393648; fax: +30 2810393601.
E-mail address: rizos@chemistry.uoc.gr (A.K. Rizos).

polymers [14–16]. PVP has a long history of pharmaceutical application and demonstrates a high degree of biocompatibility [17,18]. Amphiphilic derivatives of PVP have been described containing phospholipid residues and long-chain acyls as hydrophobic groups and serving as efficient steric protectors for liposomes. However, the choice of both the type of the hydrophobic group and MW of a polymer itself were usually quite arbitrary and based on the most general consideration.

In this study after developing various synthetic pathways for preparing amphiphilic PVP [19], we made an attempt to investigate the influence of hydrophobic and hydrophilic blocks on the properties of amphiphilic PVP polymers with static and dynamic light scattering.

2. Experimental

2.1. Synthesis of functional PVP derivatives

The PVP derivatives have been successfully prepared by free-radical polymerization of VP in the presence of various mercaptans that are able to perform the chain transfer due to the formation of free radical produced by elimination of a hydrogen atom from a SH-group. The hydrophobic stearyl end groups were introduced into these polymers by direct reaction of amino-PVP with stearyl chloride as described before [18]. All amphiphilic SPVP samples were water soluble.

2.2. Static and dynamic light scattering

The beam from a solid state diode laser (Coherent Model 2020), operating at 532 nm with vertically polarized light, was focused onto the sample cell through a temperature-controlled chamber (temperature controlled to within ± 0.01 °C) filled with refractive-index-matching toluene. The incident and scattered beams were polarized with Glan and Glan-Thompson polarizers with extinction coefficients higher than 10^{-6} and 10^{-7} , respectively. The sample solutions were filtered through 0.45 μm filters (Millipore) directly into pre-cleaned light scattering cells of highest quality. The light scattering process defines a wave vector $q = (4\pi n/\lambda)\sin(\theta/2)$, where λ is the wavelength of the incident light in a vacuum, θ is the scattering angle, and n is the refractive index of the medium. In this study the full homodyne intensity autocorrelation function $g(q, t)$ was measured at room temperature at different scattering angles ranging between 15° and 150° with an ALV-5000 multibit, multi- τ full digital correlator that covered a broad dynamic range of about 10 decades. The measured intensity autocorrelation function $G(q, t)$ is related to the desired normalized field correlation function $g(q, t)$ by

$$G(q, t) = A \left[1 + f |ag(q, t)|^2 \right], \quad (1)$$

where f is the instrumental factor, calculated by means of a standard, a is the fraction of the total scattered intensity

associated with density fluctuations with correlation times longer than 10^{-6} s and A is the baseline. The correlation functions were analyzed using the inverse Laplace transformation (ILT) of the time correlation functions with the RE-PES algorithm [20], which minimizes the sum of the squared differences between the experimental and calculated intensity–intensity autocorrelation functions using nonlinear programming.

$$\begin{aligned} ag(q, t) &= \int_0^\infty A(\tau) \exp(-t/\tau) d\tau \\ &= \int_0^\infty \tau A(\tau) \exp(-t/\tau) d \ln \tau. \end{aligned} \quad (2)$$

Thus, relaxation time distributions are given in the form of $\tau A(\tau)$ versus $\log \tau$ plots. Relaxation rates are obtained from the moments of the peaks in the relaxation time distribution.

Static light scattering (SLS) experiments were performed at different scattering angles within the range $15^\circ \leq \theta \leq 150^\circ$ and the time averaged scattered intensities were corrected for variations in the scattering volume. The static light scattering data were treated according to the simplified Zimm procedure, where the intensity of the scattered light is related to the weight average molecular weight, the second virial coefficient A_2 and the radius of gyration R_G . We find that the common Zimm plot

$$Kc/R(\theta, c) = \frac{1}{M_w} \left[1 + \frac{(R_G q)^2}{3} \right] + 2A_2 c \quad (3)$$

can describe the experimental data and thus produce an estimated value for the radius R_G of the sphere occupied by the polymer chain. In Eq. (3) $K = 4\pi^2 n_0^2 (dn/dc)^2 / (N_A \lambda_0^4)$ is the optical constant with n_0 the solvent refractive index, dn/dc the refractive index increment and $R(\theta, c)$ the Rayleigh ratio as obtained by calibration measurements.

3. Results

Since amphiphilic PVP derivatives, especially those with higher MW values, contain significantly larger hydrophilic fragments than the hydrophobic ones, one can assume that hydrophobic fragments, minimizing their contacts with water, may ‘fold’ themselves into the globule formed by the hydrophilic block. All tested SPVP derivatives, form micelles in water solutions with the average size between 5 and 8 nm and narrow size distribution as follows from the particle size measurements. However, the critical micelle concentration (CMC) values for various micelles depend on both the type of the hydrophobic residue and the molecular size of the hydrophilic PVP block. In general, the increase in the length of the PVP block increases the CMC value. The CMC values for amphiphilic PVP with PVP block size above 15000 were around 10^{-4} M. For very short PVP blocks (below 1500), CMC values for amphiphilic PVP samples were around 10^{-6} M. One may interpret these results in terms of the relative intensity of hydropho-

bic interactions between fatty acyls forming the micelle core and keeping the micelle together and the energy of the free motion of hydrophilic PVP chains forming the micelle shell in water and destabilizing the micelle. A water-soluble flexible polymer like PVP statistically exists in solution as a distribution of possible conformations. When PVP chains are short, C18 stearyl provides effective micellization with low CMC values. When PVP chains are long, the energy of free motion of the PVP block in water is higher than for short blocks and the CMC value increases [16].

Typical sizes and size distributions for SPVP (MW 1500) and SPVP (MW 8000) micelles were presented previously [19]. We found that the micelles have rather narrow size distribution with the mean size being smaller for SPVP (MW 1500) micelles (ca. 5 nm) than for SPVP (MW 8000) micelles (ca. 10 nm). Based on our results with micellization studies [19], we have chosen SPVP derivatives with intermediate size of PVP block (from 1800 to 4700) for further experiments with light scattering. The dynamic light scattering experimental correlation functions were treated in the homodyne limit. Fig. 1 shows experimental correlation functions for SPVP1 and SPVP2 samples at different scattering angles accompanied by the ILT distributions of the experimental correlation functions. This analysis revealed two relaxation modes. The faster one corresponds to the same dynamic process and is related to the diffusion dynamics of the SPVP micelles in solution. This mode is diffusive since the characteristic relaxation rates Γ ($\Gamma = 1/\tau$) depends linearly on q^2 . The slow mode is generated by much larger micelles. From the characteristic relaxation rates one can obtain the translational diffusion coefficient D ($D = \Gamma/q^2$) and hence the equivalent hydrodynamic radius R_h using the Stokes–Einstein equation for a sphere:

$$R_h = \frac{kT}{6\pi\eta_0 D}, \quad (4)$$

where η_0 is the solvent shear viscosity and k_B is the Boltzmann constant at the absolute temperature T . Fig. 2 displays the change in R_h for both PVP and SPVP as a function of molecular weight. There is a strong increase in size of the PVP micelles as compared to the weak change in size of the SPVP derivatives. There is a clear difference in size of the PVP micelles as compared to the SPVP micelles for the lower molecular weights, however this difference diminishes sharply with the increase in size of the PVP block. Fig. 3 displays normalized distributions of relaxa-

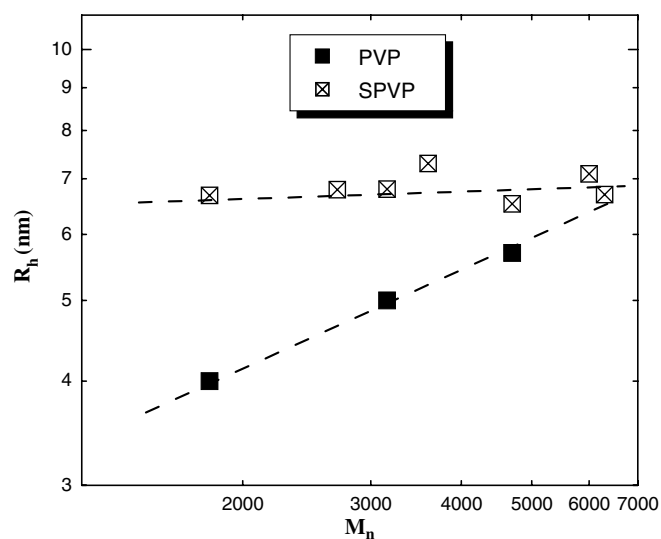


Fig. 2. The hydrodynamic radius as a function of molecular weight of PVP and SPVP polymeric derivatives.

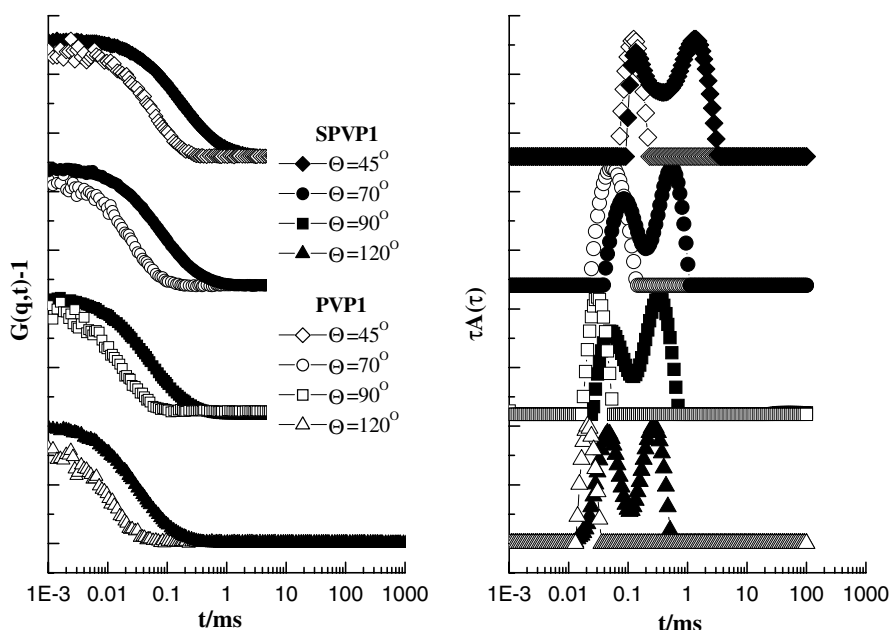


Fig. 1. Experimental correlation functions of PVP and SPVP polymeric derivatives at a fixed concentration c of 0.3% by weight, along with their normalized distributions of relaxation times, at several values of the scattering wave vector q .

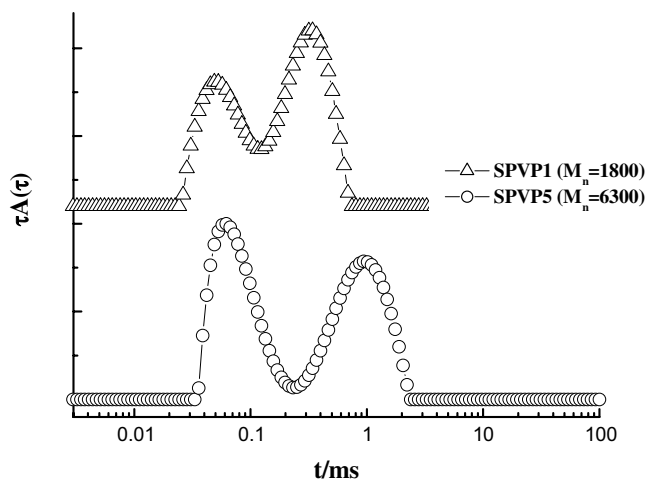


Fig. 3. Normalized distributions of relaxation times of the experimental correlation functions of the SPVP1 and SPVP5 samples at a scattering angle of 90° .

tion times of the experimental correlation functions of the SPVP1 and SPVP5 samples at a scattering angle of 90° .

4. Discussion

There is a clear shift of the distribution peaks to longer times with increasing molecular weight. The hydrophobic character of SPVP1 is manifested with the appearance of the slower relaxation peak of the inverse Laplace transforms shown in Fig. 1. The existence of the micelles becomes obvious through the simultaneous appearance of small angle excess scattering in the static light scattering plot (see Fig. 4) from which we can extract their size which is well defined. A combination of the hydrodynamic radius

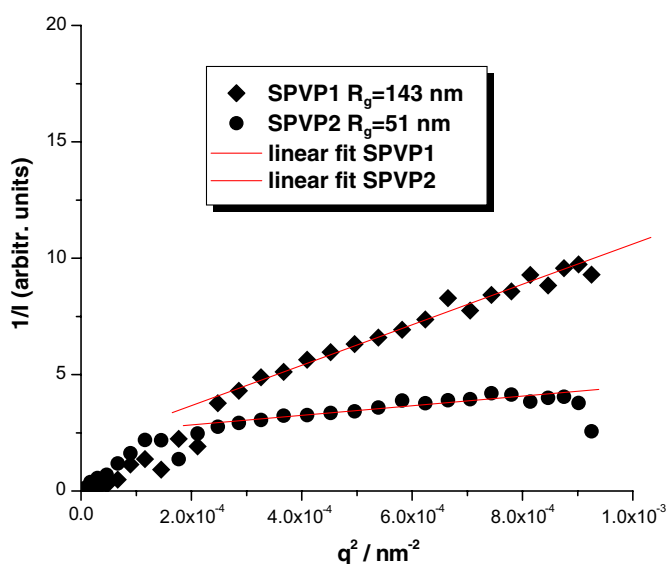
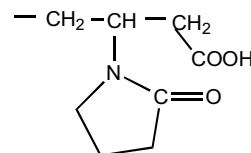


Fig. 4. Static light scattering Ornstein–Zernicke plot of the inverse of the scattering intensity versus the square of the scattering wave vector for the SPVP1 and SPVP2 PVP derivatives. Here the intensity of the scattered light is used for the estimation of the radius of gyration R_G .

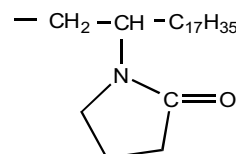
Table 1

Polyvinyl pyrrolidone (PVP) and amphiphilic derivatives of polyvinyl pyrrolidone (SPVP)

Sample	Description	M_n
PVP1	PVP with one end carboxylic group	1800
SPVP1	Same as above with one stearyl group ($C_{17}H_{35}$)	1800
SPVP2	PVP with one end stearyl group ($C_{17}H_{35}$)	2700
PVP3	PVP with one end carboxylic group	3600
SPVP3	Same as above with one stearyl group ($C_{17}H_{35}$)	3600
PVP4	PVP with one end carboxylic group	4600
SPVP4	Same as above with one stearyl group ($C_{17}H_{35}$)	4600
SPVP5	PVP with one end stearyl group ($C_{17}H_{35}$)	6300



PVP with one end carboxylic group



PVP with one end stearyl group ($C_{17}H_{35}$)

(R_h) and the root mean square radius (R_G) enables a detailed insight into the molecular architecture. For this purpose the shape factor or asymmetry factor $\rho = R_G/R_h$ is defined. Values of ρ vary from 0.78 for ideal homogeneous spheres to >2 for extended coils and prolate ellipsoids. Thus, the observed values of ρ between 0.7 and 0.85 can be correlated with the effective shape of a sphere for both PVP and SPVP. It should be emphasized that, although the larger micellar species dominate the scattered intensity due to their heavy weighting they constitute a very low number fraction (see Table 1).

5. Conclusions

The spontaneous micellization of SPVP has been investigated with static and dynamic light scattering. SPVP forms stable micelles. In comparison to PVP with one end carboxylic group we observe a weak increase in size of the micelles as a function of the molecular weight. In addition, the larger size amphiphilic SPVP micelles that appear in solution and the size of the second slow relaxation peak appear to correlate with the increasing hydrophobic character of the SPVP derivatives with decreasing molecular weight.

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