Temperature-Responsive Properties and Drug Solubilization Capacity of Amphiphilic Copolymers Based on N-Vinylpyrrolidone and Vinyl Propyl Ether

Daulet E. Zhunuspayev,1,2 Grigoriy A. Mun,3 and Vitaliy V. Khutoryanskiy4,5

1 School of Pharmacy, University of Reading, Whiteknights, P.O. Box 224, Reading RG6 6AD, United Kingdom, and 2 Kazakh National University, Department of Chemical Physics & Macromolecular Chemistry, 95 Karasai Bulay Street, 050012 Almaty, Kazakhstan

Received November 20, 2009. Revised Manuscript Received February 3, 2010

A series of amphiphilic copolymers were synthesized by free-radical copolymerization of N-vinylpyrrolidone (NVP) with vinyl propyl ether (VPE), and the structure of the copolymers was characterized by elemental analysis and gel permeation chromatography. The reactivity of VPE in copolymerization was found to be significantly lower than the reactivity of NVP, which resulted in a decrease of copolymers' yields and molecular weights with higher content of VPE in the feed mixture. An investigation of the behavior of the copolymers in aqueous solutions at different temperatures by dynamic light scattering revealed the presence of lower critical solution temperature, which depended on the content of VPE ranging within 23–38 °C. Aqueous solutions of these copolymers were studied by fluorescent spectroscopy with pyrene as a polarity probe to reveal the formation of hydrophobic domains. The copolymers were found to be useful for enhancing the solubility of riboflavin in water.

Introduction

Poly(N-vinylpyrrolidone) (PVP) is a nontoxic water-soluble polymer with a number of unique physicochemical and biological properties, which resulted in its numerous applications in drug delivery, development of biomaterials, contact lenses, etc. One of the interesting properties of PVP is its ability to bind various small and large molecules such as iodine,1 amphiphilic compounds,2 drugs,3 tannins,4 and polycarboxylic acids.6 Depending on the chemical nature of these molecules, the mechanism of binding may involve electrostatic interactions, hydrogen bonding, and hydrophobic effects. In aqueous solutions of PVP exhibits a weakly amphiphilic character due to the simultaneous presence of highly polar hydrophilic amide groups and apolar methylene (CH2) and methine (CH) groups in the backbone and the ring. However, in spite of this amphiphilicity, PVP does not show any signs of phase separation in aqueous solutions over the temperature range of 0–100 °C.7 In the presence of some additives, such as inorganic salts and organic molecules, PVP may exhibit lower critical solution temperature (LCST). For example, Gargallo and co-workers8 have reported the phase separation of PVP in 0.55 mol/L solutions of Na2SO4 at 28 °C. The temperature of phase separation (Tps) was also found to decrease with increasing concentrations of PVP and also by adding sodium dodecyl sulfate (SDS). A further addition of SDS results in the opposite effect: an increase in the Tps, which was related by Smolova et al.9 to repulsive electrostatic interactions between the charges brought by SDS molecules bound to PVP. Similar reduction in the Tps was also reported by Salamova et al.9 in solution mixtures of PVP with a number of inorganic salts such as K2PO4, KH2PO4, Na2CO3, etc. Kirci and Guner10 have reported the presence of temperature-induced phase separation in aqueous mixtures of PVP with phenolic derivatives (phenol, catechol, resorcinol, hydroquinone, and chlorogluconol) and also discussed existing literature on similar behavior in the presence of other additives. In summary, the aqueous solutions of PVP may exhibit temperature-induced phase separation in the presence of some cosolutes; however, this interesting property cannot be exploited for biomedical and pharmaceutical applications because of the toxicity and biological incompatibility of these small molecules.

During the past few years, we have published a series of studies reporting the design of temperature-responsive polymers: by copolymerizing hydrophilic 2-hydroxyethyl acrylate (HEA) with relatively hydrophobic monomers such as vinyl butyl ether,11 butyl acrylate,12 and 2-hydroxyethyl methacrylate.13 Homopolymer HEA is fully water-soluble and does not exhibit any temperature-induced phase separation, whereas the copolymerization of HEA with more hydrophobic monomers at certain ratios resulted in formation of water-soluble polymers exhibiting LCST behavior. Taking into consideration the possibility of...
inducing temperature-responsive phase separation in solutions of PVP in the presence of certain additives. It can be expected that copolymerization of N-vinylpyrrolidone (NVP) with relatively hydrophobic monomers should result in copolymeric systems which exhibit phase-separation behavior at temperatures of physiological range.

Recently, we also reported the successful copolymerization of NVP with relatively hydrophobic vinyl propyl ether (VPE) and studied the formation of complexes between these copolymers and poly(acrylic acid). However, we did not pay any attention to the temperature-responsive behavior of these copolymers in aqueous solutions nor studied their use for formulating poorly soluble drugs.

In the present work we have studied the copolymerization of NVP with VPE and evaluated the structure and physicochemical properties of the copolymers. We used free-radical copolymerization aiming at the development of a relatively cheap approach for the synthesis applicable to the possibility of larger scale industrial production of these copolymers. We detected the presence of temperature-responsive properties for aqueous solutions of the copolymers with relatively low content of VPE as well as probed the formation of hydrophobic domains/nanoparticles in the systems containing higher levels of the hydrophobic monomer. The ability of the copolymers, with various levels of VPE, to enhance the solubility of riboflavin in water was also studied, and the possible mechanisms of this enhancement are discussed in this paper.

Materials and Methods

Materials. N-Vinyl-2-pyrrolidone (NVP) and vinyl propyl ether (VPE) were purchased from Sigma-Aldrich (UK) and were purified from inhibitor by distillation. 1,4-Dioxane and 2,2-azoisobis(isobutyronitrile) (AIBN) were purchased from Acros. AIBN was recrystallized from ethanol before use. Pyrene, riboflavin, and urea were purchased from Sigma-Aldrich (UK) and used without purification. Ethanol, dimethylformamide, and sodium chloride were purchased from Fisher Scientific (UK) and used without purification. Dialysis membranes (molecular weight cutoff 12–14 kDa) were supplied by Medicell International Ltd. (UK).

Synthesis of PVP and NVP–VPE Copolymers. Copolymers NVP–VPE and PVP and PVP were synthesized by free radical copolymerization at 60 °C in ethanol solutions. The polymerization was conducted for 26 h with AIBN (0.01 mol) used as a radical initiator. Before copolymerization the monomer mixtures were saturated with nitrogen by bubbling for 10 min. Polymerization was terminated after 26 h by cooling the reaction vials with cold water. The copolymers were purified by dialysis against deionized water (volume 3 L; 20 changes during 4 days) and were recovered by freeze-drying. The purity of the copolymers was estimated by H NMR spectroscopy, and the composition of the copolymers was determined by elemental analysis for the content of nitrogen, which is present in NVP only.

Ge1 Permeation Chromatography (GPC). PVP and selected samples of the copolymers were analyzed by gel permeation chromatography using PL-GPC 50 Plus system (Varian, Inc., UK) equipped with 2 × PLgel 5 μm MIXED-D (300 × 7.5 mm) column set. The samples were prepared at nominally 2.0 mg/mL in dimethylformamide containing 0.1% LiBr. Full dilution was allowed to occur overnight prior to the injection of the samples. The analysis was performed in dimethylformamide containing 0.1% LiBr at 50 °C with a flow rate of 1 mL/min. Narrowly disperse poly(methyl methacrylate) (EasiVials) were used as standards for calibration.

Dynamic Light Scattering (DLS). The temperature-responsive behavior of water-soluble copolymers in aqueous solutions was studied by dynamic light scattering at 10–60 °C using a Malvern Zetasizer Nano-S (Malvern Instruments, UK). Each DLS experiment was repeated in triplicate by preparing and analyzing solutions of each polymer sample separately. The results were statistically treated and presented as an average ± standard deviation.

Fluorescence Spectroscopy with Pyrene Probe. A dilute aqueous solution of pyrene (2 μM) was prepared as described in ref. 14. Pyrene was initially dissolved in ethanol (0.4 mg/mL), then 100 μL of this solution was transferred into a volumetric flask (100 mL), and ethanol was dried under a stream of nitrogen gas for several minutes. The solution was then made up in deionized water and used after overnight standing. The solutions of polymers were prepared using the aqueous solution of pyrene as a solvent. The fluorescence emission spectra were recorded using a spectrofluorimeter FP-6200 (Jasco, UK) at an excitation wavelength of 355 nm at room temperature (20 ± 3 °C). The I/I0 ratio was calculated from the intensity of the first (373 nm) to the third (383 nm) vibronic peaks in the pyrene emission spectra.

Riboflavin Solubilization Experiments. In drug solubilization experiments, 4 mg of riboflavin was mixed with 4 mL of aqueous solution of polymers of different concentrations (5–20 mg/mL), and these mixtures were left stirring overnight at room temperature (20 ± 3 °C). Then, these mixtures were centrifugated for 2 mm at 2000 rpm, and 1 mL of supernatant was taken out, placed into volumetric flasks, and diluted with dimethylformamide up to 10 mL. The optical density of solutions was measured using UV–vis spectrophotometer (Jasco, UK) at 373 nm. The content of riboflavin in solutions was calculated using a calibration curve, and dimethylformamide–water (9:1) mixtures were used as a solvent.

Transmission Electron Microscopy (TEM). TEM images of copolymer nanoparticles and riboflavin formulations were acquired using a Philips CM20 analytical TEM at 200 kV. For sample preparation, the copper grids were brought into contact with dispersions of nanoparticles for 30 s and then dried off with a filter paper. All sample preparation experiments were performed at room temperature (20 ± 3 °C).

Results and Discussion

Synthesis and Characterization of Copolymers. The polymerization of NVP initiated by thermal decomposition of AIBN leads to the formation of a homopolymer with excellent yields (over 99%), whereas VPE-based homopolymer could not be formed under these conditions. It is well-known that vinyl ethers cannot be synthesized by free radical polymerization, and their polymers are usually manufactured using cationic processes. Indeed, our attempts to prepare homopolymers by free-radical polymerization of VPE have not been successful. However, the polymerization of the mixtures of NVP with VPE leads to the formation of the copolymers with reasonably good yields. Table 1 summarizes the data on compositions, molecular weights, and polydispersities of the polymers synthesized from different NVP–VPE feed mixtures.

The effect of the feed mixture composition on the content of VPE in copolymers as well as their yields is also shown in Figure 1.

The copolymers synthesized in this work have relatively high polydispersities (2.68–2.45), which is typical for materials formed by free-radical copolymerization. An increase in VPE content in the feed mixture results in reduction of the copolymer yields as well as their molecular weights. Moreover, the composition of the copolymers is predominantly enriched with NVP. For example,
Table 1. Composition and Molecular Weights of the Polymers

<table>
<thead>
<tr>
<th>Feed mixture</th>
<th>NVP-VPE, mol %</th>
<th>(f_{\text{NVP, mL}})</th>
<th>(f_{\text{VPE, mL}})</th>
<th>(f_{\text{chloro, mL}})</th>
<th>Composition of copolymers, mol %</th>
<th>Apparent (M_n), kDa</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.0:0.0</td>
<td></td>
<td>6.00</td>
<td>0.00</td>
<td>14.0</td>
<td>100.0:0.0</td>
<td>182.0</td>
<td>2.42</td>
</tr>
<tr>
<td>90.0:10.0</td>
<td></td>
<td>5.31</td>
<td>0.62</td>
<td>14.0</td>
<td>86.6:13.4</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>80.0:20.0</td>
<td></td>
<td>4.99</td>
<td>1.26</td>
<td>14.0</td>
<td>83.7:16.3</td>
<td>98.4</td>
<td>2.45</td>
</tr>
<tr>
<td>70.0:30.0</td>
<td></td>
<td>4.33</td>
<td>1.95</td>
<td>14.0</td>
<td>78.6:21.4</td>
<td>&quot;</td>
<td>2.24</td>
</tr>
<tr>
<td>60.0:40.0</td>
<td></td>
<td>3.80</td>
<td>2.66</td>
<td>14.0</td>
<td>77.5:22.5</td>
<td>70.5</td>
<td>2.20</td>
</tr>
<tr>
<td>50.0:50.0</td>
<td></td>
<td>3.25</td>
<td>3.41</td>
<td>14.0</td>
<td>74.5:25.5</td>
<td>61.3</td>
<td>2.08</td>
</tr>
<tr>
<td>40.0:60.0</td>
<td></td>
<td>2.67</td>
<td>4.20</td>
<td>14.0</td>
<td>70.8:29.2</td>
<td>48.6</td>
<td>2.08</td>
</tr>
<tr>
<td>30.0:70.0</td>
<td></td>
<td>2.05</td>
<td>5.03</td>
<td>14.0</td>
<td>70.8:29.2</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>20.0:80.0</td>
<td></td>
<td>1.41</td>
<td>5.91</td>
<td>14.0</td>
<td>62.4:37.6</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>10.0:90.0</td>
<td></td>
<td>0.72</td>
<td>6.53</td>
<td>14.0</td>
<td>50.6:49.4</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.0:100.0</td>
<td></td>
<td>6.00</td>
<td>0.00</td>
<td>14.0</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*The molecular weights of these samples were not analyzed.*

Figure 1. Effect of VPE content in the feed mixture on VPE content in the copolymers and their yields. Each synthesis was conducted at least three times, and the data are presented as a mean ± standard deviation.

the feed mixtures containing 20.0 and 40.0 mol % of VPE lead to the formation of the copolymers having 16.3 and 22.5 mol % of this monomer incorporated into their structure, respectively. This clearly indicates lower reactivity of VPE in copolymerization and is in good agreement with existing literature.**Vinyl ethers typically exhibit lower reactivity in copolymerization with the majority of monomers because of inefficiency of ether substituents to stabilize the growing radicals.** Indeed, the reactivity ratio values calculated for vinyl ethers in copolymerization are often close to \(r_1 = 0\), whereas their comonomers exhibit higher \(r_2\) values. This means that the growing macroradicals with vinyl ether are not able to add further vinyl ether monomers but more likely react with comonomer molecules. The microstructure of these copolymers usually consists of more active comonomer sequences (microblocks) interrupted with single units of vinyl ethers. It can be anticipated that similar microstructure is formed in the case of NVP–VPE copolymers, synthesized in the present study.

Temperature-Responsive Behavior in Aqueous Solutions

Copolymers containing less than 29.2 mol % of VPE were found to be soluble/dispersible in water, giving fully transparent or slightly cloudy solutions at room temperature. The behavior of PVP as well as NVP–VPE copolymers in aqueous solutions was studied at different temperatures using dynamic light scattering (Figure 2). As expected, no signs of phase separation or even any increase in laser light scattering intensity were observed in solutions of PVP as well as the copolymers containing 13.4 and 16.3 mol % of VPE. However, the copolymers containing 21.4, 22.5, 25.5, and 29.2 mol % of VPE exhibited phase separation upon increase in temperature, which can even be detected visually because of clouding of their solutions. Although the phase separation in solutions of NVP–VPE copolymers is as sharp as in the case of a classical temperature-responsive polymer such as poly(N-isopropylacrylamide), it is observed in the temperature range suitable for biomedical applications (23–38 °C). The temperatures of phase separation are consistent with the composition of the copolymers.

The copolymer containing 21.4 mol % of VPE begins to form cloudy solutions above 38 °C. More hydrophobic samples containing 22.5, 25.5, and 29.2 mol % of VPE undergo a phase separation above 30, 25, and 23 °C, respectively. This reduction in \(T_{\text{rs}}\) values is related to a gradual increase in the role of hydrophobic effects in the copolymers, which intensify at higher temperatures and lead to the phase separation in solutions.

The phase separation behavior of the copolymers was also studied at various concentrations to elucidate the role of intermolecular versus intramolecular aggregation. Figure 3 shows the results of dynamic light scattering experiments recorded for solutions of NVP–VPE (74.5:25.5 mol %) of different concentrations ranging from 1 to 10 mg/mL. These data show a clear concentration dependence of the light scattering intensity and the temperatures of phase separation. The most concentrated sample (10 mg/mL) begins to separate into two phases at 23–25 °C. The phase separation of the samples of lower concentration is less intensive and observed at higher temperatures. For example, the increase in the scattering intensity for 1 mg/mL sample is observed only above 35 °C. The concentration dependence of the phase separation phenomenon indicates its intermolecular nature, where several macromolecules self-assemble and form aggregates of micellar nature.

As it was discussed in the Introduction, the presence of various low molecular weight additives in solutions can decrease or increase the temperature of phase separation depending on the way their molecules interact with a polymer. Here, we have studied the effect of three types of additives such as NaCl, urea, and ethanol on the phase behavior of NVP–VPE (74.5:25.5 mol %) in aqueous solutions (Figure 4). The presence of inorganic salt in solution can reduce the \(T_{\text{rs}}\) values quite significantly; in 3% solutions of NaCl this copolymer undergoes phase separation at 15–20 °C, in 10 and 15% the solutions remain cloudy even at less than 10 °C (Figure 4a). This effect of inorganic salt can be explained by deterioration of the thermodynamic quality of water as a solvent with respect to a polymer. Indeed, water molecules prefer to solvate Na⁺ and Cl⁻ ions rather than noncharged macromolecules of the copolymer, resulting in their partial

Figure 2. Intensity of light scattering by 10 mg/mL aqueous solutions of PVP (1), NVP–VPE 86.6:13.4 (2), 83.7:16.3 (3), 78.6:21.4 (4), 77.5:22.5 (5), 74.5:25.5 (6), and 70.8:29.2 mol % (7) as a function of temperature. Inset: images of 10 mg/mL NVP–VPE (74.5:25.5 mol %) solutions at different temperatures. Each experiment was conducted at least three times, and the data are presented as a mean ± standard deviation.

Figure 3. Intensity of light scattering by aqueous solutions of NVP–VPE (74.5:25.5 mol %) as a function of temperature at different copolymer concentrations: 1 (1), 2 (2), 4 (3), 6 (4), 8 (5), and 10 mg/mL (6). Each experiment was conducted at least three times, and the data are presented as a mean ± standard deviation.

desorption and better sensitivity to the hydrophobic effects, whose role increases with temperature. Urea is a small molecular weight-water-soluble compound with a well-documented ability to denature proteins, to enhance aqueous solubility of certain polymers, and to affect micellization of low molecular weight surfactants and Pluronic. All these unique characteristics of urea are believed to result from its strong ability to bind to various macromolecules via hydrogen bonding. Urea addition was found to have an interesting effect on the temperatures of phase separation of our copolymers (Figure 4b). The $T_p$ values of the copolymer are practically not affected in solutions of 5 and 10% of urea; however, when its concentration is increased to 15, 20, 25, 30, and 40%, the phase separation is shifted to a higher temperature region and observed at $>40^\circ$C. Interestingly, even the presence of 40% of urea in solutions cannot prevent the phase separation completely. The effect of urea on the phase separation temperatures can be explained by formation of hydrogen bonds with carbonyl groups of NVP in the copolymer and additional solvation of its macromolecules. However, urea is possibly unable to penetrate into the hydrophobic domains formed by the propyl groups of VPE and therefore cannot prevent the phase separation of the copolymers at higher temperatures. In the presence of ethanol (Figure 4c) only a small shift in the onset of phase separation toward the higher temperatures is observed initially, but as the concentration of the organic solvent in the mixture increases, the growth in the scattering intensity with temperature becomes less intensive. In solutions containing 25 and 30% of ethanol the phase separation is not observed at all in the studied temperature range. Unlike urea, ethanol is able to penetrate into the hydrophobic domains and solvate both the polar and non-polar functional groups of the copolymer macromolecules. When the efficient solution is achieved, the hydrophobic effects become negligible and cannot cause a phase separation of the copolymer even at relatively high temperatures.

Hydrophobic Domains in Solutions of the Copolymers. To study the formation of hydrophobic domains in aqueous solutions of NVP–VPE further, we used fluorescence spectroscopy with pyrene as a polarity probe. This technique has often been used to investigate the aggregation in hydrophilically modified polymers, formation of polymer–polymer complexes, and micellar behavior of surfactants. Pyrene is a highly hydrophobic molecule with unique ability to migrate into the areas with less polar environment and change its fluorescence. Although the solubility of pyrene in water is very low ($8 \times 10^{-7}$ mol/L), its

DOI: 10.1021/la0904403k 7593
PVP and NVP–VPE (86.6:13.4, 77.5:22.5, and 70.8:29.2 mol %) copolymers with increasing polymer concentration. An insignificant reduction in $I_1/I_2$ ratio is observed for PVP and NVP–VPE (86.6:13.4 mol %), indicating the absence of any hydrophobic domains and aggregation in the studied concentration range. This result is consistent with the dynamic light scattering data described in the previous section. Indeed, neither PVP nor NVP–VPE (86.6:13.4 mol %) shows any signs of phase separation upon increase in temperature because these polymers are not amphiphilic enough to undergo a hydrophobic aggregation. The copolymers containing 22.5 and 29.2 mol % of VPE show a more pronounced reduction in $I_1/I_2$ ratio upon increase in their concentration. The characteristic inflection point is observed at about 0.1 mg/mL for the copolymer with 29.2 mol % of VPE, which is likely to correspond to the critical aggregation concentration (cac), above which the formation of micellar aggregates occurs. However, these inflection points are not clearly visible for less hydrophobic copolymers.

To evaluate the influence of the amphiphilicity of the macromolecules on the formation of hydrophobic domains, we studied the effect of copolymer composition on the pyrene emission characteristics in 1 mg/mL aqueous solutions (Figure 6). When the content of VPE in the copolymers is below 16.3 mol %, no significant changes in the $I_1/I_2$ are observed, which indicates that the macromolecules do not form hydrophobic domains and do not aggregate in solutions at this concentration. However, when the content of VPE in the copolymers is increased, a notable reduction in $I_1/I_2$ is observed, indicating enhancement in pyrene solubility in the hydrophobic domains formed by copolymer aggregates. Thus, a hydrophobic association in aqueous solutions of NVP–VPE is observed only when the content of VPE has reached certain levels favoring the formation of hydrophobic domains.

To confirm the presence of hydrophobic aggregates in solutions formed at relatively high concentrations of the copolymers, we studied 10 mg/mL samples of NVP–VPE (70.8–29.2 mol %) by transmission electron microscopy (Figure 7). This experiment revealed the formation of spherical nanoparticles, with size between 45 and 63 nm. It should be noted that the sample preparation for TEM did not involve any staining with heavy metal salts, and a relatively good contrast of the particles confirms their dense structure able to absorb accelerated electrons. The further TEM attempts to visualize the formation of aggregates or nanoparticles in solutions of the copolymers containing lower levels of VPE were not successful, which is likely related to their looser structure and lower density.

The formation of aggregates in solutions of this copolymer is also confirmed by dynamic light scattering measurements, which reveals the presence of nanoparticles of two size populations: 68–220 and 255–955 nm (see Supporting Information). The discrepancy in the sizes resulting by TEM and dynamic light scattering is likely related to the difference in the sample preparation and different degree of nanoparticles' hydration; sample preparation techniques used in TEM experiments involves partial drying, whereas the dynamic light scattering experiments were performed for fully hydrated particles. Partial drying of the samples used for TEM experiments may have resulted in further aggregation of larger particles, which concentrated at the edges of a copper grid forming objects of irregular shape and dimensions and did not attract our attention during TEM experiments. The nanoparticles formed by the copolymers with higher VPE content (49.4 mol %) were found to have a monomodal size distribution and dimensions of 396–712 nm.

---

Figure 4. Effect of sodium chloride (a), urea (b), and ethanol (c) on the temperature of phase separation of the copolymers NVP–VPE (77.5:22.5 mol %) in aqueous solutions. Concentration of the copolymer is 10 mg/mL.

Aqueous solutions give well-resolved fluorescence spectra (data are not shown). The change in the ratio of the intensities of the first (373 nm) to the third peaks (383 nm) in its fluorescence emission spectra ($I_1/I_2$) allows the evaluation of the polarity of a particular environment. The values of $I_1/I_2$ observed for pyrene dissolved in deionized water are typically around 1.45–1.9,25,29,30 and the lowering of the ratio indicates the less polar environment. Figure 5 shows the changes in $I_1/I_2$ ratio for aqueous solutions of

---

Solubilization of Riboflavin. Water-soluble amphiphilic polymers able to solubilize nonpolar molecules in aqueous solutions are highly promising for formulating poorly soluble drugs. A number of studies have been published on the application of amphiphilic block copolymers such as Pluronics\textsuperscript{31,32} as well as hydrophobically modified polysaccharides\textsuperscript{33,34} for formulating poorly soluble drugs.

\textsuperscript{33} Francis, M. F.; Piredda, M.; Winnik, F. M. J. Control. Release 2003, 93, 59-68.

\textit{Langmuir} 2010, 26(10), 7590-7597

DOI: 10.1021/la904403k 7595
Riboflavin or vitamin B₁₂ is a relatively nonpolar molecule, with a solubility in water of around 0.1 mg/mL. It has often been used as a model drug in formulation studies or as a fluorescent probe. Riboflavin has also been used as a photosensitive cross-linker in the treatment of keratoconus, where the drug is administered via the ocular route.

In the present study, we evaluated the ability of PVP and NVP–VPE copolymers to enhance the solubility of riboflavin in water. For this purpose, riboflavin crystals were dispersed in aqueous solutions of PVP and NVP–VPE of various compositions and left stirring overnight at room temperature. The content of riboflavin remaining in solution was evaluated spectrophotometrically after removing undissolved drug crystals using centrifugation. Figure 8 shows the solubility of riboflavin in deionized water and solutions of PVP and NVP–VPE of various concentrations (5, 10, and 20 mg/mL). The solubility of riboflavin in deionized water was found to be 0.12 ± 0.02 mg/mL, which broadly agrees with the literature data. The solubility of riboflavin in 5 mg/mL solutions of PVP remains at the same level as in deionized water, but a further increase in the polymer concentration up to 20 mg/mL improves the solubility of the drug up to 0.36 mg/mL. The ability of PVP to stabilize riboflavin solutions at high polymer concentrations (20 mg/mL) cannot be simply related to the solubilization effects as this polymer does not form hydrophobic aggregates at the studied concentration range. It is likely that this enhancement is caused by partial adsorption of PVP macromolecules on the surface of riboflavin microcrystals driven both by hydrogen bonding and hydrophobic effects and providing an improvement in the formulation colloidal stability. Indeed, the ability of PVP to interact with various drug molecules and stabilize solid drug dispersions has often been related to polymer–drug hydrogen bonding. However, it should be noted that in our case riboflavin formulations stabilized by this mechanism slowly undergo aggregation and gradual formation of crystalline precipitates.

The NVP–VPE copolymer containing 15 mol % of VPE does not show any difference in the ability to stabilize and/or solubilize riboflavin compared to PVP, and this is consistent with the fluorescence results discussed in the previous section. Indeed, the copolymers containing less than 20 mol % of VPE are not able to form hydrophobic domains that can efficiently enhance the solubility of nonpolar molecules. A notable 6-fold enhancement in the levels of riboflavin remaining in the formulations is observed when 20 mg/mL solutions of the copolymers containing 16.3, 21.4, 22.5, and 25.5 mol % VPE were used. This significant enhancement in riboflavin loading capacity is possibly achieved through the complementary effects of the two stabilization mechanisms: (1) drug solubilization in the hydrophobic domains formed by the copolymers and (2) colloidal stabilization of drug microcrystals through the adsorption of macromolecules on their surface. When the content of VPE in copolymers exceeds 30 mol %, their ability to solubilize and stabilize riboflavin goes down again. This is likely associated with the changes in the structure of the polymeric aggregates. As discussed above, copolymers containing high levels of VPE are able to form nanoparticles that are possibly too tight and dense to incorporate riboflavin at the molecular level and also are less efficient in stabilization of the drug microcrystals against further aggregation and precipitation.

Further evidence supporting the presence of the two mechanisms of the stabilization of riboflavin in solutions was obtained by TEM analysis of the formulations formed by NVP–VPE copolymer containing 22.5 mol % of VPE (Figure 9). The TEM image clearly shows the drug microcrystals incorporated within the polymeric aggregates.

Thus, two major mechanisms may be responsible for stabilization of riboflavin in aqueous solutions of the studied polymers; however, a relative contribution of each mechanism may differ depending on the amphiphilicity of their macromolecules. The copolymers having higher levels of VPE stabilize riboflavin formulations predominantly through the solubilization of drug molecules in hydrophobic domains formed by VPE segments in aqueous solutions. PVP and the copolymers with lower levels of VPE do not form hydrophobic domains, but their macromolecules may adsorb at drug microcrystal surfaces providing their stabilization against aggregation.
Conclusions

Amphiphilic copolymers can be synthesized by free-radical copolymerization of N-vinylpyrrolidone and vinyl propyl ether. Vinyl propyl ether was found to be less reactive in the copolymerization, resulting in its lower levels of incorporation in the copolymers compared to the feed mixtures as well as lower reaction yields and reduced molecular weights.

The copolymers exhibit temperature-responsive properties in aqueous solutions; i.e., they undergo a phase separation upon increase in temperature. These temperature-induced transitions are observed in the range of 23–38 °C depending on the copolymer composition and their concentration in solutions. The proximity of this range to a physiological temperature opens some possibilities for application of these copolymers in drug delivery. A further development of these copolymers in the form of aqueous compositions existing as free-flowing solutions at low temperatures and forming gels at human body temperature is of particular interest for formulating in situ gelling drug delivery systems.

A fluorescent spectroscopy study of these polymers in aqueous solutions using pyrene as a polarity probe revealed the formation of hydrophobic domains for the copolymers containing more than 20 mol % of vinyl propyl ether. The copolymers containing higher levels of vinyl propyl ether were also found to form dense nanoparticles.

The ability of the amphiphilic copolymers to enhance the solubility and stability of riboflavin in aqueous solutions has been demonstrated. It is believed that this enhancement is due to the two complementary mechanisms such as the solubilization of the drug molecules in the hydrophobic domains formed by the copolymers in solutions as well as the stabilization of riboflavin microcrystals against aggregation and precipitation. The 20 mg/mL solutions of the copolymers containing intermediate levels of vinyl propyl ether provide a 6-fold enhancement in the solubility of riboflavin.

Acknowledgment. D.E.Z. acknowledges the International Association for the promotion of cooperation with scientists from the New Independent States of the former Soviet Union (INTAS) for the Young Scientist Fellowship grant (ref. 06-1000020-6265). The authors are grateful to Dr. B. MacCreath (Varian, Inc, UK) for gel permeation chromatography analysis of the polymers. The help of Dr. C. Stan and Dr. P. Harris at the Centre for Advanced Microscopy (University of Reading) in transmission electron microscopy experiments is greatly appreciated. Dr. C. Conlon is also acknowledged for useful discussions.

Supporting Information Available: Size distributions of selected NVP–VPE samples in aqueous solutions (obtained by dynamic light scattering). This material is available free of charge via the Internet at http://pubs.acs.org.

Langmuir 2010, 26(10), 7590–7597

DOI: 10.1021/la904403k 7597