J Mater Sci: Mater Med (2010) 21:1521–1530
DOI 10.1007/s10856-010-4029-1

Preparation and characterization of amphiphilic poly-N-vinylpyrrolidone nanoparticles containing indomethacin

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Received: 26 October 2009 / Accepted: 8 February 2010 / Published online: 23 February 2010

Abstract Amphiphilic poly-N-vinylpyrrolidone derivatives (Amph-PVP) with different molecular weight of hydrophilic PVP fragment and one terminal hydrophobic n-alkyl fragment of different length were synthesized for preparation of nano-scaled particles in aqueous media. To estimate novel polymer efficiency and perspective as basis for drug delivery systems, the polymeric micelle-like particles were prepared by dialysis and solvent evaporation methods. Indomethacin was incorporated into hydrophobic inner core of these nanoparticles as a typical model drug. From the dynamic light-scattering measurements, the size of particles formed was less than 200 nm with narrow monodisperse size distribution and nanoparticles size slightly increased with the amount of indomethacin encapsulated into inner core of Amph-PVP particles. The critical aggregation concentration values for prepared polymer samples determined by fluorescence spectroscopy were in micromole range which is lower than it is for common low molecular weight surfactants. As the hydrophobic fragment of amphiphilic polymer increased, the critical aggregation concentration values decreased. An increase of polymer hydrophilic fragment molecular weight produced larger nanoaggregates. In vitro release experiments using indomethacin-loaded Amph-PVP nanoparticles exhibited the sustained release behavior without any burst effect for most polymer samples.

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1 Introduction

For the last several decades the great attention is paid to the development of drug delivery systems with controlled release of biologically active substance [1–3]. There have been vast efforts to develop efficient systems for targeted delivery of drugs by using appropriate delivery systems [4, 5]. Targeting the drug to the desired site would not only improve the therapeutic efficiency but also enable a reduction of the amount of drug which must be administered to achieve a therapeutic response, thus minimizing unwanted toxic effects.

For all this period polymers played a major role in the development of drug delivery systems. Polymeric colloidal drug delivery systems offer a number of advantages over conventional dosage forms. Due to their small particle size, colloidal preparations are suitable to parenteral administration and may be useful as prolonged release injections for the delivery to a specific organ or target site [6, 7]. The advantages of polymeric colloidal delivery systems are easy control of particle size, good structural stability and stability during long-term storage, solubilization of hydrophobic drugs and ability to deliver drugs showing low interactions with biocomponents such as proteins and cells. The particles usually smaller than 1 μm, circulate in the blood stream without immobilization at capillaries, and permeate into the target cells through blood vessels [8]. This particular dosage form is expected to help protect the incorporated drug from enzymatic attack in plasma by covering the incorporated drug in a hydrophobic core of the particles [9].

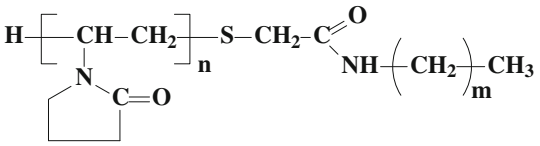
In most studies block copolymers composed of hydrophilic and hydrophobic segments are used for development of drug delivery systems which can form a micellar structure [2, 10] with a hydrophobic compact inner core

and a hydrophilic swollen outer shell in solvent, which is thermodynamically favorable for one block, but unfavorable for others [11, 12]. Such copolymers usually consist of suitable biodegradable polymers such as poly(lactic/glycolic acid) (PLGA) [13], poly-ε-caprolactone (PεCL) [14, 15], poly(L-lactide) (PLLA) [16] or polyethylene glycol (PEG) [17]. Micelles made of these copolymers have been investigated with a novel type of sustained release system for targeting drugs to specific sites of the organism in the past several years [18–20]. But in spite of mentioned above advantages all listed polymers have significant disadvantages limiting their application in medical practice, such as low ability for additional polymer functionalization and not proved safety of polymers and their derivatives after injection introduction to organism.

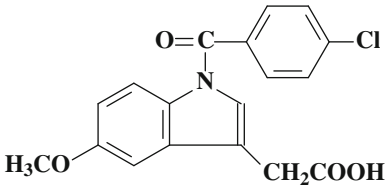
In this study we have suggested to use novel amphiphilic derivatives of poly-N-vinylpyrrolidone (PVP) as a basis for creation of highly-effective drug delivery systems. PVP has a long history of pharmaceutical applications and demonstrates a high degree of biocompatibility [21, 22]. The peculiarity of the prepared amphiphilic polymer structure is that these polymers consist of only one polymeric fragment (hydrophilic PVP) with one terminal long-chain aliphatic radical serving as hydrophobic fragment. Earlier we have described an easy original two-stage method of synthesis of such polymers and have shown that such a structure allows controlling easily hydrophilic-lipophilic balance of the polymer and size and properties of nano-scaled drug carriers made of amphiphilic PVPs [23].

At concentrations above the so-called critical aggregation concentration (CAC), almost all amphiphilic polymer chains aggregate to form micelles, while below the CAC, only isolated chains are observed in the solution [10]. Since most drugs have a hydrophobic character, these drugs can be easily incorporated into the particle core by a covalent or a non-covalent bonding through hydrophobic interactions with the experimental methods such as direct dilution, dialysis [11], salting out procedure, or solvent evaporation method [24]. In our previous work [25] we have demonstrated the possibility of immobilization of model proteins (Bowman-Birk soybean proteinase inhibitor and its derivatives) on nano-scaled aggregates made of amphiphilic PVPs.

The objective of the present study was to prepare polymeric nano-scaled particles composed of hydrophobically α-end-capped poly-N-vinylpyrrolidone (Fig. 1a) (chosen as promising biocompatible polymer) in order to evaluate their potential and efficiency as drug carriers. Indomethacin (IMC) (1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid), as shown in Fig. 1b, is known as a typical model drug in no-steroidal anti-inflammatory drugs (NSAIDs) [26–28]. In this study, indomethacin was used as a model drug to incorporate into



(a) Amph-PVP



(b) Indomethacin

Fig. 1 Structural formula of N-vinylpyrrolidone amphiphilic polymer (**a**) and indomethacin (**b**)

nano-aggregates, due to its hydrophobic character. To evaluate capability of carriers for drugs, we prepared IMC-loaded polymeric particles by different methods. The characteristics of these particles were investigated through dynamic light-scattering (DLS), transmission electron microscopy (TEM) and fluorescence probe technique. In addition, the drug loading efficiency of incorporated IMC was investigated by ultraviolet (UV) spectrophotometer. The drug release properties of prepared nanoparticles have also been investigated.

2 Experimental

2.1 Materials

N-vinylpyrrolidone (VP) and indomethacin (IMC) were obtained from Sigma (USA). Substrate for electron microscopy—0.2 % polyvinylformal, was from Merk (Germany). All other chemicals used were reagent grade and used as purchased without further purification. All solvents and components of buffer solutions were analytical grade preparations. Distilled-deionized water was prepared with a Milli-Q Plus System (Millipore, USA).

2.2 Synthesis of N-vinylpyrrolidone amphiphilic polymer

Amphiphilic N-vinylpyrrolidone polymers (Amph-PVP) were prepared using originally developed two-stage method as described in our previous papers [23, 25]. On the first stage poly-N-vinylpyrrolidone (PVP) with one terminal carboxylic group was synthesized by free-radical polymerization of N-vinylpyrrolidone in the presence of initiator (azobisisobutyronitrile (AIBN)) and chain-transmitter (mercaptoacetic acid (MAA)). The reaction was

carried under dry argon atmosphere in dioxane solution for 2.5 h at 60°C. The yield of polymers was 75% ÷ 90%. MW values were determined by titration or steam osmometry using "Knauer" osmometer (Germany). Polydispersity of one end functionalized polymers was determined by high-performance liquid chromatography (GFHPLC; TSK Gel G4000PwxL; Toso Co., Ltd., Japan). For PVP 2500, PVP 3500, PVP 5000 and PVP 8500 samples Mw/Mn values were 1.09, 1.11, 1.12 and 1.14 respectively.

On the second stage hydrophobic n-alkyl groups (octadecyl, hexadecyl, dodecyl, hexyl) were attached to reactive terminus of the PVP molecule. For this purpose, the solution of carboxy-PVP in isopropanol was supplemented with an excess of N, N-dicyclohexylcarbodiimide (DCC) in an equal volume of the same solvent. The mixture was stirred at 5°C for 2 h, and then excess of appropriate aliphatic amine dissolved in isopropanol was added. The mixture was incubated for 3 h at 65°C until full connection of hydrophobic alkyl groups. Then, polymers were isolated by precipitation, dried to constant weight and their yield was determined.

2.3 Preparation of indomethacin-loaded nanoparticles from amphiphilic poly-N-vinylpyrrolidone

Amphiphilic N-vinylpyrrolidone polymeric nanoparticles were prepared using dialysis and solvent evaporation methods. Indomethacin (IMC) which has hydrophobic nature was used as a model drug.

In dialysis method amphiphilic PVP was dissolved in dimethylformamide followed by the addition of IMC with various weight ratios to polymer (1:0.1–1:1) and stirred at room temperature. To form IMC-loaded nanoparticles and remove free IMC, the solution was dialyzed for 24 h against 3 l of ultra pure water using regenerated cellulose dialysis membranes (molecular weight cut-off: 3.5×10^3 and 6×10^3–8×10^3, Membrane Filtration Products Inc., USA). The nano-aggregates solution was sonicated using ultrasonic dispergator Sonoplus HD 2070 (Bandelin, Germany), and then centrifuged (Heraeus, Martin Christ GmbH, Germany) to remove unloaded IMC and aggregated particles.

In the solvent evaporation (emulsion) method different weight ratios of amphiphilic PVP and IMC were dissolved in small amount of chloroform. This mixture was then emulsified in an aqueous phase with ultrasonic dispergator (Sonoplus HD 2070, Bandelin, Germany). The organic solvent was then removed and the resulting suspension concentrated by evaporation under reduced pressure (rotary evaporator Laborota 4010, Heidolph, Germany).

IMC-loaded nanoparticle suspensions, obtained in both processes, were frozen and lyophilized by Alpha I-4LD freeze dryer system (Martin Christ GmbH, Germany) to obtain dried nanoparticle products. Thermogravimetric analysis of freeze-dried nanoparticles confirmed that there were no residues of organic solvents left in the drug-loaded particles.

The plain nanoparticles without IMC were prepared by the same methods just without addition of indomethacin.

2.4 Particle characterization

To estimate the critical aggregation concentration (CAC) values for amphiphilic PVP samples, fluorescence measurement was carried using pyrene as fluorescent probe. The method is based on solubilization of hydrophobic pyren by polymeric nanoparticles. For this purpose, aliquots of 10 μl of pyrene solution in acetone (10 mg/ml) per test tube were dried under vacuum. The tubes were supplemented with 2 ml of serial dilutions (10^{-4}–10^{-10} M) of various PVP samples, and shaken overnight at room temperature. The samples were filtered through 0.2 μm filter to remove the non-solubilized pyrene, and the fluorescence intensity of solubilized pyrene was measured using a Hitachi 650-10 S spectrofluorophotometer (Hitachi Instruments Inc, Japan). The emission wavelength was 390 nm for excitation spectra.

Average size and size distribution of nano-aggregates were determined by dynamic light scattering (DLS) using N5 Submicron Particle Size Analyzer (Beckman Coulter Inc., USA). Light scattering correlation function was obtained using helium–neon laser at a wavelength of 632.8 nm at 20°C and at scattering angle of 90°. A multibit, multi-τ full digital correlator (ALV-5000) was used that covered a dynamic range of about ten decades. For each sample, the mean diameter of six determinations was calculated by applying multimodal analysis.

For morphological examinations, nanoparticles were analyzed with transmission electron microscopy (TEM) using apparatus JEOL JEM-2100 (Germany) at a voltage of 120 kV. For sample preparation, a drop of particles suspension was placed on substrate from 0.2% solution of polyvinylformal applied on copper mesh.

2.5 Drug loading efficiency

The amount of IMC-loaded inner core of Amph-PVP particles was investigated using UV spectrophotometer (Hitachi 650-10 S). To remove unbound and immobilized on particle surface IMC, the solution was sonicated, centrifuged and then lyophilized. The nanoparticles which were obtained by freeze-drying then were disrupted by addition of ethanol and THF mixture (1:1). The amount of indomethacin entrapped was determined by measuring UV

absorbance at 319 nm. Drug loading efficiency (DLE) was measured using the following equation:

$$\mathrm{DLE}\,(\%) = \frac{A - B}{A} \times 100$$

where A is the total weight of IMC used and B is the weight of unloaded IMC in the precipitate after centrifugation.

2.6 Drug release measurements

Release behavior of IMC from Amp-PVP nanoparticles was investigated in vitro conditions. The appropriate amount of IMC-loaded particles with different composition was preliminarily weighed and suspended in 10 ml of a phosphate buffer solution (PBS, 0.1 M, pH 7.4). Then nanoparticle solution was introduced into dialysis membrane bag and the bag was placed in 500 ml of phosphate buffer solution release media, and the media was stirred at 37°C. At predetermined time intervals, 5 ml aliquots of the aqueous solution were taken from the release media. The concentration of released IMC was measured using a UV spectrophotometer at 319 nm. After determination the solution taken as a sample was replaced in release medium. Each result is an average of three parallel experiments.

3 Results and discussion

3.1 Synthesis of N-vinylpyrrolidone amphiphilic polymers

For preparation of poly-N-vinylpyrrolidone amphiphilic derivatives with one terminal hydrophobic fragment we used original two-stage process.

First we intended to prepare VP homopolymer α-end-capped with functional carboxylic group by free radical polymerization of monomer in the presence of AIBN as initiator and MAA acid as chain growth regulator and carboxyl group source. Such scheme of polymerization allowed us preparation of semitelechelic PVP with one end carboxyl group, controllable molecular weight and low polydispersity (Mw/Mn $\sim$1.1). The structure of obtained polymers was confirmed by FT-IR spectral analysis, ^{1}H-NMR spectroscopy and by agreement of molecular weight values determined by potentiometric titration of PVP carboxyl groups (on the basis of one carboxyl group per one polymer molecule) and by osmometry. The results of investigation of this process and its kinetics are of single interest and will be reported in a separate paper shortly.

On the second stage prepared semitelechelic PVP was treated by appropriate amount of long-chain aliphatic amine (octadecyl, hexadecyl, dodecyl or hexyl amine) in the presence of DCC in order to attach single hydrophobic fragment to terminal carboxylic group of each PVP molecule. The total substitution of carboxyl groups was confirmed by FT-IR and NMR analysis and by titration under the same conditions as for semitelechelic polymer molecular weight estimation.

Applied two-staged method allows preparation of amphiphilic PVP derivatives with different size of hydrophilic and hydrophobic fragments (Table 1) which can be easily controlled for achieving optimal polymer properties.

The total yield of amphiphilic polymers was 85–95% except amphiphilies with the lowest PVP fragment molecular weight Mn = 2500 (yield 65–75%). Such low polymer yield for these samples can be explained by large fraction of low-molecular products in reaction mixture which cannot be isolated by methods used for amphiphilic

Table 1 Synthesized amphiphilic N-vinylpyrrolidone polymers and their properties

Sample	Hydrophilic fragment number average molecular weight		Hydrophobic fragment type	Poly-dispersity Mw/Mn	CAC (μM)	Particle size mean ± S.D. (nm)	
	Titration[a]	Osmometry[b]				Dialysis[c]	Emulsion[d]
PVP-OD8500	8530	8515	Octadecyl	1.14	9.9	184 ± 0.102	172 ± 0.078
PVP-OD5000	5050	5021	Octadecyl	1.12	8.9	131 ± 0.084	104 ± 0.072
PVP-OD3500	3510	3518	Octadecyl	1.11	6.7	77 ± 0.033	58 ± 0.012
PVP-OD2500	2530	2511	Octadecyl	1.09	5.5	55 ± 0.051	46 ± 0.061
PVP-HD3500	3510	3518	Hexadecyl	1.11	11.6	122 ± 0.031	102 ± 0.048
PVP-DD3500	3510	3518	Dodecyl	1.11	18.1	225 ± 0.095	212 ± 0.106
PVP-Hex3500	3510	3518	Hexyl	1.11	N.D.	N.D.	N.D.

[a] Determined by potentiometric titration of end carboxyl groups

[b] Determined by steam osmometry method

[c] Estimated for Amph-PVP nanoparticles prepared by dialysis method

[d] Estimated for Amph-PVP nanoparticles prepared by solvent evaporation technique

polymers preparation. That is why amphiphilic polymers with low molecular weight of hydrophilic PVP fragment cannot be observed as promising basis for indomethacin delivery systems from the technological and practical point of view.

3.2 Amph-PVP nanoparticles formation

As prepared PVP amphiphilic derivatives contain hydrophilic and hydrophobic fragments, at certain concentrations in aqueous media greater than some critical concentration (so-called critical aggregation concentration) they can aggregate with formation of core-shell type polymeric nanoparticle structures. Due to hydrophobic characters of terminal long-chain fragments, n-alkyl domain 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.

To obtain information on critical aggregation concentration (CAC) for different Amph-PVP samples and to study the influence of polymer hydrophilic/hydrophobic fragment size on the process of nanoaggregates formation, we estimated the fluorescence excitation spectra of pyrene at various concentrations of amphiphilic polymers. Excitation wavelength was 390 nm and pyrene concentration was kept constant at 6.0×10^{-6} M. Pyrene was chosen as fluorescent probe because of its photochemical properties and remarkably long life-time suitable for an effective probe [29]. Hydrophobic and very low-soluble in water pyrene preferentially solubilizes into the interior of the hydrophobic fragments of Amph-PVPs, so that it moves from water environments to hydrophobic particle cores. Therefore, the fluorescence intensity is affected by the change in the polymer concentration.

Figure 2 plots the intensity ratio of $I_{337.5}/I_{334}$ from pyrene excitation spectra vs. log C for Amph-PVP polymer samples. For example, CAC value of PVP-OD3500 was 6.7×10^{-6} mol/l, which was much lower than that of common low-molecular weight surfactants. This result indicates that Amph-PVP system can retain a micelle-like structure even in a much diluted solutions, featuring stable polymeric particles which may be useful as drug vehicles.

CAC values for amphiphilic polymer samples with different size of hydrophilic and hydrophobic parts are presented in Table 1. As one can see CAC values slightly decreases with decreasing hydrophilic PVP fragment molecular weight and dramatically decreases with increasing hydrophobic anchor chain length. And the PVP-Hex2500 sample with the shortest hydrophobic radical is not able to form aggregates at any concentration in water. Obtained data fully corresponds with results of other investigators [30–32], which reported that the micellization process is determined mainly by the nature and the length

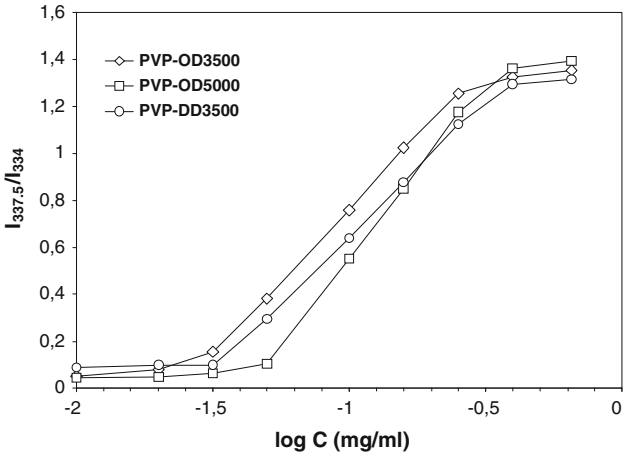


Fig. 2 Plots of the intensity ratio $I_{337.5}/I_{334}$ from pyrene excitation spectra vs. log C for different Amph-PVP samples ([Pyrene] = 6×10^{-6} M)

of the hydrophobic block, where the nature of soluble hydrophilic block has only a slight effect on the onset of micelle formation.

The best results were obtained for PVP-OD2500, so it possesses highest inclination for aggregate formation and hydrophilic drug solubilization. But the application of this particular polymer is limited by its rather low yield on synthesis stage. That is why, we have chosen PVP-OD3500 polymer as main object for further investigations as it can be prepared with high yield and its ability to form stable and compact micelle-like nano-scaled particles is commensurable with those of PVP-OD2500 which has the best results among all the prepared amphiphilic polymer samples.

3.3 Amph-PVP nanoparticles characterization

It is well known that morphology of amphiphilic polymer aggregates is quite diverse. Generally, it is supposed that amphiphilic polymers produce particles close to spherical form. In our study, Amph-PVP polymeric nanoparticles prepared both by dialysis and emulsion methods were observed with transmission electron microscopy measurements. As shown in Fig. 3a, it was confirmed that nano-sized particles of spherical shape have been prepared.

The size and size distribution of nanoparticles prepared by dialysis or solvent evaporation were measured by means of a dynamic light scattering method (DLS). Figure 4 shows the typical size distribution of Amph-PVP nanoparticles. From the DLS measurements, the average size was smaller than 200 nm and size distribution showed a narrow and monodisperse unimodal pattern as shown in Fig. 4 and Tables 1, 2 and 3. The size of Amph-PVP nanoparticles increased with the molecular weight of hydrophilic polymeric fragment and with decreasing of

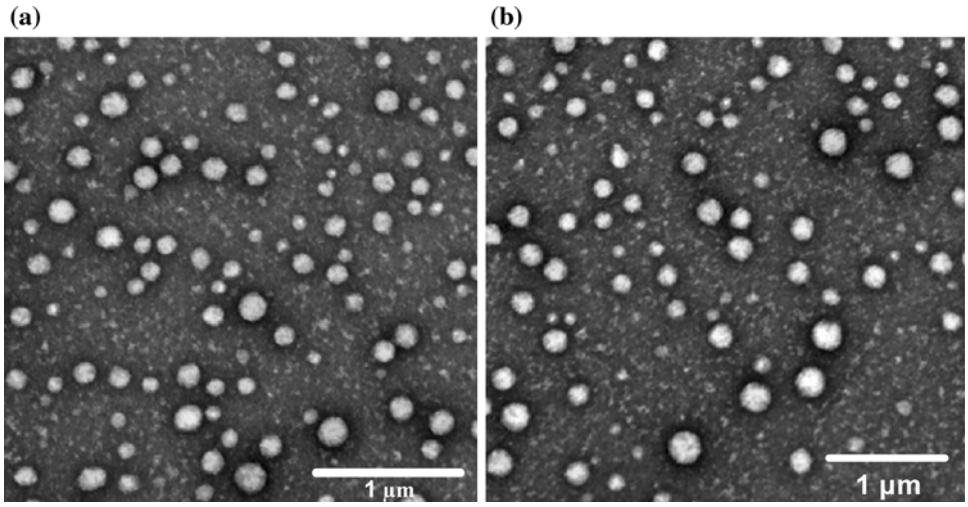


Fig. 3 Transmission electron micrographs: **a** nanoparticles prepared from PVP-OD3500; **b** PVP-OD3500 nanoparticles loaded with IMC (weight ratio 1:1)

hydrophobic terminal group length. These results fully correspond with data obtained for CAC studies.

The influence of polymer concentration on the size of prepared particles was also studied. At low concentrations near CAC values Amph-PVP formed micelle-like spherical particles in water solutions with the average size between less 200 nm and narrow size distribution. At higher polymer concentrations (dozen times higher than CAC), polymeric nanoparticles associated, with the formation of larger aggregates (up to 1 μm) with complex structure. The size of polymeric particles at high polymer concentration can be easily controlled and lowered by sonication or changing particles preparation conditions.

For all Amph-PVP samples the size of the particles prepared by emulsion method was smaller than of those prepared by dialysis (Table 1). Thus the solvent evaporation method is more suitable and preferred for preparation of polymeric nanoparticles.

To examine the existence of any residual organic solvents, nanoparticle solution obtained through dialysis or solution evaporation procedure was investigated by gas chromatography mass spectroscopy (GCMS QP-2010, Shimadzu, Japan). In the spectrum, the peak of DMF was not detected. Thus, it indicated that organic solvent used for preparation of nanoparticles was completely removed through the dialysis or rotary evaporation process.

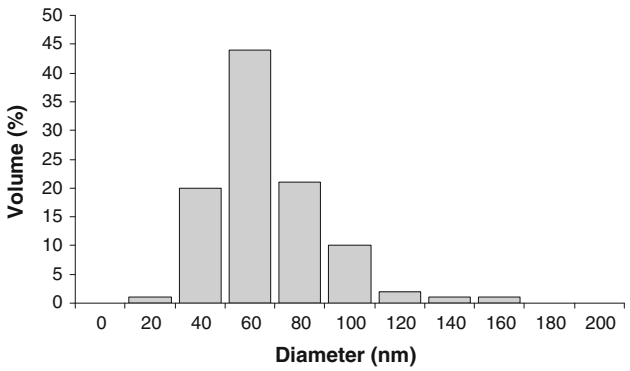


Fig. 4 Typical size distribution profile of Amph-PVP nanoparticles (PVP-OD3500) by dynamic light scattering measurement

3.4 Drug loading efficiency study

IMC-loaded nanoparticles were prepared using the same techniques as for hollow nanoparticles. The IMC molecules were gradually entrapped into the hydrophobic core of Amph-PVP nanoparticles via self-assembly.

The amount of IMC incorporated into Amph-PVP nanoparticles was calculated by difference between total weight of IMC used for encapsulation in nanoparticles and unloaded IMC in the precipitate, determined by UV spectrophotometry after removing free IMC and IMC bounded on the surface of Amph-PVP particles by centrifugation-sonication technique.

Table 2 shows the effect of solvents on the size of nanoparticles and IMC loading efficiency when nanoparticles were prepared by dialysis or emulsion method. For solvent evaporation method, when we use chloroform (CF) as a solvent, the size of obtained nanoparticles is smaller and the drug loading efficiency is higher than those obtained in dimethylformamide (DMFA) or in tetrahydrofuran (THF). This result shoes that CF is a more favorable solvent than DMFA and THF for emulsion preparation of Amph-PVP nanoparticles. As for dialysis method, DMFA contributes considerably to the formation of smaller and more stable particles. In this case, in contrast to emulsion method, an organic solvent used to dissolve amphiphilic polymer should be miscible with water. Therefore, it could be expected that miscibility of polymer and solvent or water and solvent affect the formation of nanoparticles.

Table 2 Influence of solvent type on size and drug loading efficiency of IMC-loaded Amph-PVP nanoparticles

Sample	Polymer:IMC weight ratio	Solvent	Particle size mean ± S.D. (nm)		DLE (%)	
			Dialysis	Emulsion	Dialysis	Emulsion
PVP-OD3500	1:1	CF	N.D.	105 ± 0.034	N.D.	42.4
PVP-OD3500	1:1	DMFA	124 ± 0.030	118 ± 0.054	37.3	39.6
PVP-OD3500	1:1	THF	141 ± 0.044	134 ± 0.062	27.4	31.2
PVP-HD3500	1:0.5	CF	N.D.	97 ± 0.049	N.D.	99.1
PVP-HD3500	1:0.5	DMFA	128 ± 0.092	105 ± 0.043	98.6	90.6
PVP-HD3500	1:0.5	THF	146 ± 0.089	136 ± 0.088	69.5	76.1

Table 3 Influence of polymer concentration and structure, polymer/drug weight ratio and solubilization technique on particle size of IMC-loaded Amph-PVP nanoparticles (C_p, amphiphilic polymer concentration)

Sample	Polymer//IMC weight ratio	Particle size mean ± S.D. (nm)			
		Dialysis		Emulsion	
		$C_p \sim CAC$	$C_p = 10$ mg/ml	$C_p \sim CAC$	$C_p = 10$ mg/ml
PVP-OD3500	1.0:0.0	77 ± 0.033	287 ± 0.052	58 ± 0.012	245 ± 0.092
PVP-OD3500	1.0:0.5	92 ± 0.024	168 ± 0.044	71 ± 0.025	139 ± 0.071
PVP-OD3500	1.0:1.0	116 ± 0.075	124 ± 0.030	98 ± 0.062	105 ± 0.034
PVP-OD8500	1.0:0.0	184 ± 0.102	470 ± 0.121	172 ± 0.078	416 ± 0.034
PVP-OD8500	1.0:0.5	198 ± 0.082	325 ± 0.065	188 ± 0.091	262 ± 0.041
PVP-OD8500	1.0:1.0	212 ± 0.047	206 ± 0.055	196 ± 0.036	204 ± 0.059
PVP-DD3500	1.0:0.0	230 ± 0.109	540 ± 0.092	198 ± 0.058	506 ± 0.112
PVP-DD3500	1.0:0.5	265 ± 0.068	412 ± 0.077	238 ± 0.062	350 ± 0.075
PVP-DD3500	1.0:1.0	292 ± 0.043	276 ± 0.054	262 ± 0.038	195 ± 0.049

At the same time, drug loading efficiency values determined for Amph-PVP particles prepared by emulsion method (using chloroform as solvent) are in most cases higher than those for particles prepared by dialysis (using dimethylformamide as a solvent), which confirms that emulsification technique is more effective and preferable for IMC encapsulation.

Table 3 shows the effect of weight ratio of IMC to Amph-PVP on the size of prepared nanoparticles. Increasing amount of IMC in nanoparticles relatively to amphiphilic polymer increases the size of drug-loaded aggregates. However, the further increasing of IMC ratio exceeding 1:1 leads to abrupt increase of polymeric particles size caused by aggregation between particles due to the IMC hydrophobic nature. That is why, in the drug loading experiments, the weight ratio of polymer to IMC was fixed to less than 1:1 for all samples.

Figure 5 illustrates the effect of total amount of used IMC on the drug loading efficiency of Amph-PVPs. It shows that loading efficiency reached approximately 100% when IMC:Amph-PVP weight ratio was 0.5:1 or less, indicating that IMC was totally solubilized by polymeric nanoparticles. This agreed with experimental observation that prepared colloid solutions were stable without any precipitate. With increasing of added IMC the precipitate in the system gradually appeared. As a result IMC loading efficiency significantly decreased at drug:polymer weight ratio increased from 0.5:1 up to 1:1.

The influence of amphiphilic polymer structure on loading efficiency of IMC was also studied (Fig. 5). The best results were obtained for Amph-PVP with low molecular weight of hydrophilic polymeric fragment and the largest octadecyl hydrophobic group and fully corresponded with results of fluorescence analysis. Such polymers have lowest CAC values and can form nanoparticles and solubilize IMC more easily due to their optimal hydrophobic/hydrophilic balance. Especially, for polymer PVP-OD3500, when weight ratio of IMC to polymer is 1:1 we can prepare nanoparticles with relatively high DLE of about 42.4%.

The introduction of IMC into Amph-PVP nanoparticles also influences particles size (Table 3). The results

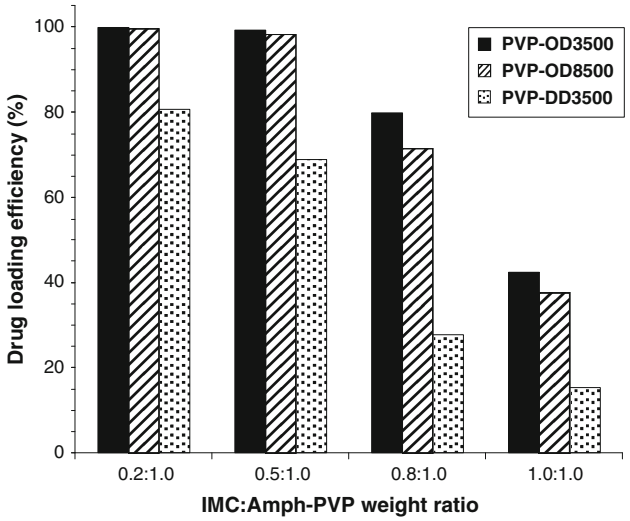


Fig. 5 Effect of indomethacin to amphiphilic poly-N-vinylpyrrolidone weight ratio on drug loading efficiency of polymeric nanoparticles prepared by solvent evaporation technique

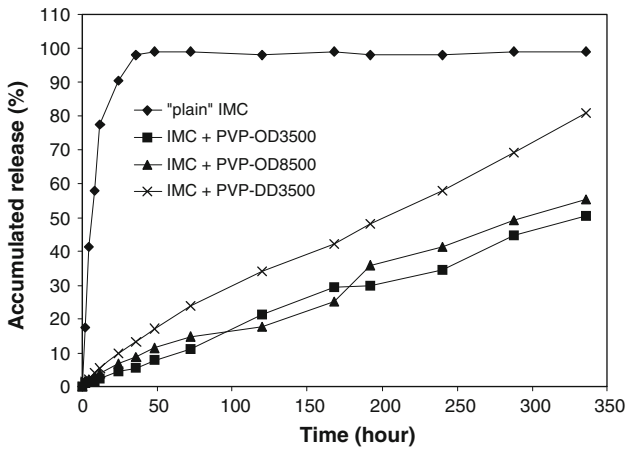


Fig. 6 In vitro release profiles of IMC from Amph-PVP nanoparticles with different drug loading efficiency in pH 7.4 PBS at 37°C

obtained by DLS measurements showed that at low polymer concentration (near CAC value) the size of nanoparticles increases depending on amount of introduced IMC. At the same time, the size of IMC-loaded nanoparticles does not exceed 200 nm (Fig. 3b) and the size distribution of drug-loaded nanoparticles is relatively identical to that before IMC loading. In the case of higher polymer concentration (dozen times higher CAC value) introduction of IMC leads to the large decrease of loaded nanoparticles size close to the size values obtained for low Amph-PVP concentration. We can assume that in this case the introduction of hydrophobic IMC molecules in the system compacts and regulates the structure of colloid aggregates. At low ratio of IMC to Amph-PVP in the mixture the strengths of interaction between polymer chains prevail, but with increasing of hydrophobic drug concentration in the mixture the main contribution is made by hydrophobic interactions between IMC and amphiphilic polymer. As a result the small micelle-like IMC-loaded nanoparticles are formed instead of large complex polymeric aggregates.

3.5 Drug release study

The indomethacin release properties from Amph-PVP nanoparticles were investigated using a dialysis membrane bags in phosphate buffer solution (pH 7.4, 37°C). Figure 6 shows a release profile of IMC from Amph-PVP nanoparticles with different structure and DLE as a function of time. It is a plot of accumulated release as a % of the actual IMC load, determined from the loading efficiency.

Since the initial burst effect was very small or was not observed at all for some polymer samples, we can conclude that Amph-PVP nanoparticles can be prepared without any residual drug on their surface. While plain IMC exhibited rapid release of 98% within 36 h, the IMC which was loaded into the inner core of polymeric nanoparticles showed controlled release of 45–50% for 12 days. Release rate of drug was much decreased in proportion to the loading amount of drug within nanoparticles. Because the increase of IMC with hydrophobic property within a nanoparticle can enhance an interaction between IMC and long-chain alkyl group as a hydrophobic part, it can induce a decrease in a drug release rate and amounts of drug released.

For revealing the influence of polymer hydrophilic and hydrophobic fragments size on drug release profile we compared the release behavior of PVP-OD3500 and PVP-OD8500 samples which have same hydrophobic group and similar DLE but different molecular weight of hydrophilic polymeric part and of PVP-OD3500 and PVP-DD3500 as they have similar PVP fragment molecular weight but different length of hydrophobic anchor (Fig. 6). The release profiles obtained for such IMC-loaded nanoparticles show that the major factor affecting the drug release rate is binding affinity between the hydrophobic core of polymer formed by alkyl chains and drug. That is why nanoparticles prepared from polymers with longer hydrophobic fragment (octadecyl group) in all cases shows slower release profile. The molecular weight of polymeric hydrophobic fragment does not provide any significant influence on IMC release from nanoparticles.

4 Conclusions

Amphiphilic PVP derivatives with different molecular weight of hydrophilic polymeric fragment and one end n-alkyl group with different length of aliphatic chain as hydrophobic fragment were prepared using original two-

step method. Amph-PVP nanoparticles loaded with indomethacin hydrophobic drug in their inner core were prepared by dialysis and solvent evaporation method using the solution behavior of amphiphilic polymers in selective solvents. The size of Amph-PVP particles increases with molecular weight of PVP hydrophilic fragment, with decrease of hydrophobic n-alkyl fragment length and with increasing of loading amount of drug. However, the size of IMC-loaded Amph-PVP nanoparticles for tested polymer samples was less than 200 nm and the size distribution was relatively identical to that before IMC loading. The critical aggregation concentration values for Amph-PVP samples are much lower than that of common low-molecular weight surfactants. The drug loading efficiency for Amph-PVP nanoparticles was up to 42.4% when the weight ratio of IMC to polymer was 1:1. In vitro release of IMC from the IMC-loaded Amph-PVP nanoparticles in PBS medium showed the significant sustained release behavior for most polymer samples without any burst effects. Thus such Amph-PVP nanoparticles are being considered as promising biocompatible drug vehicles for controlled release and site-specific drug delivery systems.

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