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Journal of Controlled Release 82 (2002) 105–114

journal of controlled release

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Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake

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Received 24 February 2002; accepted 7 May 2002

Abstract

Polyvinyl alcohol (PVA) is the most commonly used emulsifier in the formulation of poly lactide and poly (D,L-lactide-co-glycolide) (PLGA) polymeric nanoparticles. A fraction of PVA remains associated with the nanoparticles despite repeated washing because PVA forms an interconnected network with the polymer at the interface. The objective of this study was to determine the parameters that influence the amount of residual PVA associated with PLGA nanoparticles and its effect on the physical properties and cellular uptake of nanoparticles. Nanoparticles were formulated by a multiple emulsion-solvent evaporation technique using bovine serum albumin (BSA) as a model protein. The parameters that affected the amount of residual PVA include the concentration of PVA and the type of organic solvent used in the emulsion. The residual PVA, in turn, influenced different pharmaceutical properties of nanoparticles such as particle size, zeta potential, polydispersity index, surface hydrophobicity, protein loading and also slightly influenced the in vitro release of the encapsulated protein. Importantly, nanoparticles with higher amount of residual PVA had relatively lower cellular uptake despite their smaller particle size. It is proposed that the lower intracellular uptake of nanoparticles with higher amount of residual PVA could be related to the higher hydrophilicity of the nanoparticle surface. In conclusion, the residual PVA associated with nanoparticles is an important formulation parameter that can be used to modulate the pharmaceutical properties of PLGA nanoparticles.

Keywords: Polyvinyl alcohol; Particle size; Sustained release; Intracellular uptake; Hydrophobicity

1. Introduction

There is an increased interest in developing biodegradable nanoparticles since they offer a suitable means of delivering small molecular weight drugs, proteins or genes by either localized or targeted delivery to the tissue of interest [1]. Nanoparticles are colloidal systems that range in size typically from 10 to 1000 nm in diameter, and are formulated from a biodegradable polymer in which the therapeutic agent is entrapped in, adsorbed or chemically coupled onto the polymer matrix [2]. Although a number of different polymers have been investigated for formulating biodegradable nanoparticles, poly (D,L-

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0168-3659/02/$ – see front matter
PII: S0168-3659(02)00127-X

lactide-co-glycolide) (PLGA) and poly lactic acid (PLA), FDA approved biocompatible and biodegradable polymers, have been the most extensively studied [3,4]. Emulsion-solvent evaporation is the commonly used method to formulate PLA and PLGA nanoparticles and poly (vinyl alcohol) (PVA) is the emulsifier most commonly used to stabilize the emulsion since it forms particles of relatively small size and uniform size distribution [5,6]. A fraction of PVA remains associated with the nanoparticles despite repeated washing because PVA forms an interconnected network with the polymer at the interface [7]. Since the residual PVA associated with the surface of nanoparticles could be up to 13% w/w of nanoparticles [6], we hypothesized that it could influence the different physical properties of nanoparticles and their interactions with the surrounding environment including with that of the cell membrane. The overall goal of the study, therefore, was to determine the effect of residual PVA associated with nanoparticles on their physical and intracellular uptake properties.

2. Methods

2.1. Materials

PLGA (MW 143 900 Da, copolymer ratio 50:50) was purchased from Birmingham Polymers, Inc. (Birmingham, AL). Polyvinyl alcohol (PVA, average MW 30 000–70 000 Da), Bovine serum albumin (Fraction V) and Rose Bengal were purchased from Sigma (St. Louis, MO). 6-coumarin was obtained from Polysciences Inc. (Warrington, PA).

2.2. Nanoparticle formulation

Nanoparticles were formulated using a multiple emulsion-solvent evaporation technique as per the previously published protocol [8]. In brief, an aqueous solution of BSA (300 μl, 10% w/v) was emulsified in an organic phase consisting of 90 mg PLGA and 50 μg of 6-coumarin dissolved in 3 ml of chloroform to form a primary oil-in-water emulsion. The above emulsion was further emulsified in an aqueous PVA solution (12 ml, concentration of PVA was varied from 0.5 to 5% w/v depending upon the protocol) to form a multiple water-in-oil-in-water emulsion. The emulsification was carried out using a microtip probe sonicator set at 55 W of energy output (XL 2015 Sonicator® ultrasonic processor, Misonix Inc., Farmingdale, NY) for 2 min over an ice-bath. The emulsion was stirred overnight on a magnetic stir plate to allow the evaporation of chloroform. Nanoparticles were recovered by ultracentrifugation at 110 000×*g* for 20 min at 4 °C (Beckman Optima™ LE-80K, Beckman Instruments, Inc., Palo Alto, CA), washed twice with water to remove PVA and then lyophilized for 2 days (VirTis Company, Gardiner, NY). The fluorescent 6-coumarin dye incorporated in nanoparticles serves as a good marker for nanoparticles as the dye does not leach from the nanoparticles, and has been used in our previous studies to determine the cellular and tissue uptake of nanoparticles [8,9]. To study the effect of different organic solvents used to dissolve the polymer on the nanoparticle properties, an identical protocol was used except that the chloroform was replaced with the respective organic solvent (acetone or dichloromethane) and the PVA concentration was kept at 5% w/w in the aqueous phase for all the formulations.

2.3. Particle size analysis and zeta potential

Particle size and size distribution was determined by photon correlation spectroscopy (PCS) using quasi-elastic light scattering equipment. A dilute suspension of nanoparticles (100 μg/ml) was prepared in double distilled water and sonicated on an ice bath for 30 s. The sample was subjected to particle size analysis in the ZetaPlus™ particle size analyzer (Brookhaven Instrument Corp, Holtsville, NY). To measure zeta potential of nanoparticles as a function of pH, a suspension of nanoparticles was prepared as above in 0.001 M HEPES buffer of different pH (pH adjusted either with 0.1 M HCl or 0.1 M NaOH). The zeta potential was measured immediately using the ZetaPlus™ zeta potential analyzer.

2.4. Determination of residual PVA

The amount of PVA associated with nanoparticles was determined by a colorimetric method based on

the formation of a colored complex between two adjacent hydroxyl groups of PVA and an iodine molecule [10]. Briefly, 2 mg of lyophilized nanoparticle sample was treated with 2 ml of 0.5 M NaOH for 15 min at 60 °C. Each sample was neutralized with 900 μl of 1 N HCl and the volume was adjusted to 5 ml with distilled water. To each sample, 3 ml of a 0.65 M solution of boric acid, 0.5 ml of a solution of I_2/KI (0.05 M/0.15 M), and 1.5 ml of distilled water were added. Finally, the absorbance of the samples was measured at 690 nm after 15 min incubation. A standard plot of PVA was prepared under identical conditions.

2.5. Hydrophobicity of nanoparticles

The binding constant of Rose Bengal to the surface of the nanoparticles was used as the measure of surface hydrophobicity [11]. A sample of various formulations of nanoparticles (1 mg) was incubated with different concentrations (5–40 μg/ml) of Rose Bengal dye for 3 h at room temperature. The samples were centrifuged at 14 000 rev./min for 30 min in a microcentrifuge (Eppendorf 5417R, Brinkmann Instruments, Westbury, NY) to spin down the particles. The supernatant from each sample was analyzed spectrophotometrically at 542.7 nm to determine the unbound dye. The dye solution without nanoparticles as a control was run each time under similar conditions to account for the dye that might bind to the centrifuge tubes. The binding constant was calculated using a Scatchard plot according to the equation:

$$r/a = KN - Kr$$

where r is the amount of Rose Bengal adsorbed per mg nanoparticles (μg/mg); a is equilibrium concentration of Rose Bengal (μg/ml); K is the binding constant (ml/μg); and N is the maximum amount bound (μg/mg).

2.6. Determination of protein loading

Protein loading in the nanoparticles was determined by analyzing the washings from the nanoparticle formulation for the protein that is not encapsulated. Protein content was measured by BCA protein assay kit (Pierce, Rockford, IL). The amount of protein encapsulated in the nanoparticles was determined from the total amount of protein added and the amount of protein that was not encapsulated.

2.7. In vitro BSA release from nanoparticles

Release of BSA from the nanoparticles formulated using either 0.5 or 5% PVA as emulsifier was determined in phosphate buffer saline (PBS, 0.15 M, pH 7.4) at 37 °C utilizing double chamber diffusion cells placed on a shaker at 100 rev./min (Environ® orbital shaker, Lab Line, Melrose Park, IL). The donor chamber was filled with 2.5 ml of nanoparticle suspension (2.5 mg/ml) and the receiver end was filled with buffer. A Millipore® hydrophilic low protein binding membrane (Millipore Co., Bedford, MA) with 0.1 μm pore size was placed between the two chambers. The protein is freely permeable across the membrane. At predetermined intervals, the receiver chamber fluid was replaced with fresh buffer and the BSA content was analyzed using BCA protein assay.

2.8. Intracellular nanoparticle uptake

Human arterial smooth muscle cells (HASMC, Cascade Biologics, Portland, OR) were used for investigating the cellular uptake of nanoparticles. HASMCs were maintained on Medium 231 supplemented with smooth muscle growth supplement (Cascade Biologics). For nanoparticle uptake study, 24-well plates were seeded with cells at 50 000 per well density and the cells were allowed to attach for 24 h. The medium in each well was replaced with 1 ml of freshly prepared nanoparticle suspension in the medium (100 or 200 μg/well) and the plates were incubated for 1 h. The cells were then washed three times with PBS to remove the nanoparticles which were not internalized. The cells were then lysed by incubating them with 0.1 ml of 1× cell culture lysis reagent (Promega, Madison, WI) for 30 min at 37 °C. A 5 μl of each cell lysate aliquot was used for the cell protein determination and the remaining portion was lyophilized. The dye from the nanoparticles in the lyophilized samples was extracted by shaking each sample with 1 ml methanol at 37 °C for 48 h at 150 rev./min using an Environ® orbital shaker. The samples were centrifuged at 14 000 rev./min for 10

min in a microcentrifuge to remove cell debris. The supernatant was analyzed for 6-coumarin by a high-performance liquid chromatography (HPLC) method described previously [8]. A standard plot with different concentration of nanoparticles was constructed simultaneously under similar conditions to determine the amount of nanoparticles in cell lysates. The data was normalized to per milligram cell protein.

3. Results and discussion

3.1. Factors influencing the amount of residual PVA

In the formulation of PLGA nanoparticles, a fraction of PVA that is used as the emulsifier forms a stable network on the polymer surface and can not be removed during the washing procedure [12]. Initially, we investigated the effect of various formulation factors that could influence the amount of residual PVA that stays associated with PLGA nanoparticles. These include the concentration of PVA in the external aqueous phase and the organic solvent used to make the polymer solution.

3.1.1. Influence of the organic solvent used

The polarity of organic solvent used in the emulsion formation during the nanoparticle formulation might affect the amount of PVA adsorbing at the polymer-organic solvent–water interface. Therefore, nanoparticles with three different organic solvents (acetone, dichloromethane and chloroform) were formulated under identical conditions. It was found that the residual PVA associated with the nanoparticles increased with increasing solvent miscibility with water (Table 1). The results can be explained on the basis that more PVA might have partitioned into the polymeric phase containing an organic solvent that is more miscible with the aqueous phase, resulting in the higher deposition of PVA on the surface of nanoparticles. The results also suggest that the composition of the co-solvent system such as the combination of chloroform and acetone that is sometimes used in the formulation could also affect the residual PVA associated with nanoparticles, and hence their pharmaceutical properties.

Table 1
Effect of organic solvent on %residual PVA associated with nanoparticles

Solvent	Miscibility in water (Parts)[a]	Residual PVA (%±S.E.M) (w/w) ($n=3$)
Acetone	Freely miscible	7.45±0.62
Dichloromethane	1 in 50	6.15±0.35
Chloroform	1 in 200	4.87±0.49

[a] Merck index.

3.1.2. PVA concentration in the external aqueous phase

To study the influence of different PVA concentrations in the external aqueous phase on the residual PVA associated with nanoparticles, we prepared nanoparticles by using chloroform as a organic solvent and with an external aqueous phase consisting of different concentrations of PVA. As shown in Fig. 1, an increase in PVA concentration in the external aqueous phase from 0.5 to 5% w/v results in the increase in the residual PVA amount associated with nanoparticles. The mechanism of PVA binding has been proposed to be due to the interpenetration of PVA and PLGA molecules during nanoparticle formulation. The hydrophobic segments of PVA penetrate into the organic phase and remain entrapped into the polymeric matrix of the nanoparti-

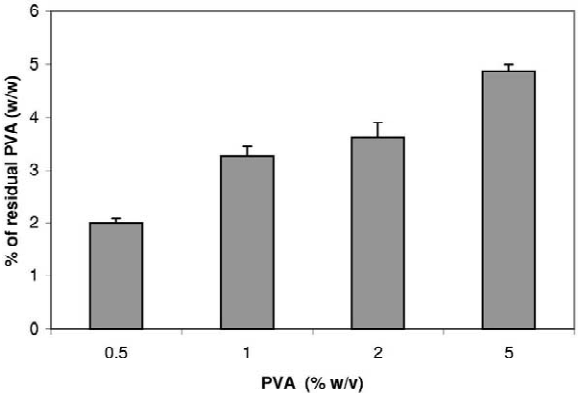

Fig. 1. Effect of PVA concentration in the external aqueous phase of the multiple emulsion used for nanoparticle formulation on the percent of residual PVA associated with nanoparticles, data as mean±S.E.M. ($n=3$).

cles [12]. The binding of PVA on the particle surface is likely to happen when the organic solvent is removed from the interface in which interpenetration of PVA and PLGA molecules takes place. Partially hydrolyzed PVA is a copolymer of poly(vinyl acetate) and poly (vinyl alcohol) with considerable block copolymer character. The hydrophobic vinyl acetate part serves as an anchor polymer at the oil interface for binding to the surface of PLGA polymer during the formulation. Thus, the higher PVA concentration of the continuous phase could lead to an increase in PVA molecule density at the o/w interface of the emulsion droplet, which might increase the thickness of PVA on the surface. Lee et al. demonstrated that the PVA content per weight of microparticles increases with the specific surface area as the particle size decreases [13]. They have shown that the surface PVA density (PVA content per unit surface area) of microparticles changes with the particle size in a biphasic manner. In the size range larger than 1 μm, the surface PVA density increases as the particle size decreases. However, below 1 μm, the surface density remains unchanged with the decrease in particle size.

Zambaux et al. have also reported similar increase in the amount of residual PVA with the increase in PVA concentration in external aqueous phase for PLA nanoparticles [6]. However, the amount of residual PVA associated with our PLGA nanoparticles was comparatively lower (6.15% w/w, Table 1 for dichloromethane) than that reported in the above study (13% w/w). The difference could be due to the hydrophobic nature of PLA over PLGA and/or due to the lower molecular weight of the PVA that was used in their study compared to that used in our study (13 000 to 23 000 Da vs. 30 000 to 70 000 Da).

3.2. Influence of residual PVA on pharmaceutical properties of nanoparticles

3.2.1. Size and size distribution

The mean particle size of the nanoparticles was a function of PVA concentration in the external aqueous phase (Table 2). The mean nanoparticle size decreased from 520 to 380 nm with an increase in the PVA concentration in the external aqueous phase from 0.5 to 5% w/v. A number of previous reports demonstrated that by increasing the PVA concentration in the external aqueous phase the size of the nanoparticles decreases [6,13].

This drop in the particle size with the increase in PVA concentration is probably due to the differences in the stability of the emulsions formulated with different concentrations of PVA. At concentrations lower than 2.5% w/v, PVA exists as single molecules in solution. Above this concentration, it exists in an aggregated form and has an enhanced surfactant activity. Further, the viscosity of PVA solution increases with increasing PVA concentrations (2.1 cps for 2% to 5.7 cps for 5%) (Table 2). This could result in the formation of a stable emulsion with smaller and uniform droplet size, leading to the formation of smaller sized nanoparticles with low polydispersity [6,14].

3.2.2. Zeta potential

The zeta potential values for nanoparticles pre-

Table 2
Physicochemical characteristics of the PLGA nanoparticles

PVA concentration (% w/v) used	Relative viscosity of PVA solution[a] (cps) ($n=3$)	Mean particle size (nm)[b] ($n=5$)	Polydispersity index ($n=5$)	Zeta potential S.E.M. (mV)[c] ($n=5$)	Protein loading (mg/100 mg nanoparticles)	Binding constant (K) of Rose Bengal (ml/μg) ($n=3$)
0.5	1.20	522	0.20	−15.4±0.8	19.0	0.024
1.0	1.41	468	0.20	−11.3±0.8	20.9	0.014
2.0	2.06	421	0.14	−12.5±0.4	22.3	0.013
5.0	5.70	380	0.12	−08.0±2.3	24.0	0.005

[a] Measured using Ostwald's viscometer with water as reference standard at room temperature.
[b] Mean hydrodynamic diameter measured by photon correlation spectroscopy.
[c] Measured in 0.001 M HEPES buffer, pH 7.0.

pared with different PVA concentrations are shown in Table 2. All the nanoparticles were negatively charged at pH 7, which could be attributed to the presence of ionized carboxyl groups on the surface of the nanoparticles. Fig. 2 shows the changes in the zeta potential of the nanoparticles with pH. The zeta potential–pH profiles show that the zeta potential is negative at neutral and higher pH, whilst at lower pH, there is a charge reversal and the nanoparticles acquire positive zeta potential. The above phenomenon was observed for all the formulations of nanoparticles prepared using different concentrations of PVA. However, the zeta potential values were higher for nanoparticles prepared with 0.5% PVA compared to those prepared with 5% PVA at both lower and higher pH values.

It has been reported that the zeta potential of PLGA nanoparticles without any PVA in neutral buffer is about −45 mV [15]. This high negative charge is attributed to the presence of uncapped end carboxyl groups of the polymer at the particle surface. In several studies, a clear differentiation in the zeta potential values of coated and non-coated nanoparticles was reported, with generally highly negative zeta potential values for non-coated nanoparticles and less negative zeta potential values for coated nanoparticles. Coating of nanoparticles with some amphiphilic polymers normally decreases the zeta potential because the coating layers shield the surface charge and move the shear plane outwards from the particle surface [16,17]. Redhead et al. have reported a similar reduction in the zeta potential of PLGA nanoparticles after coating with amphilphilic polymers like poloxamer 407 and poloxamine 908 [18]. Thus, the PVA layer at the surface of the nanoparticles also probably shields the surface charge of PLGA. Since the amount of residual PVA is relatively lower in case of nanoparticles prepared with 0.5% PVA, less shielding and therefore higher zeta potential could be expected in these nanoparticles. The lower shielding effect of PVA also explains the higher zeta potential at lower or higher pH for the nanoparticles prepared with 0.5% PVA as compared to the nanoparticles prepared with 5% PVA because more carboxyl groups are available for ionization with lower amount of the nanoparticle associated PVA (Fig. 2).

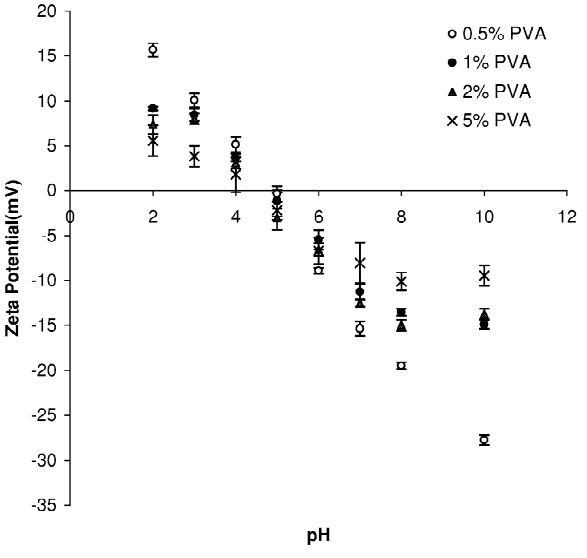

Fig. 2. Effect of pH on the zeta potential of nanoparticles formulated with 0.5, 1, 2 and 5% PVA concentration in aqueous phase, data as mean±S.E.M. ($n=5$).

Fig. 2 shows the zeta potentials of nanoparticles formulated with different concentrations of PVA. In acidic solutions (pH$<$5.0), the zeta potential of the PLGA nanoparticles becomes positive (Fig. 2). As the PVA concentration used in the formulation of nanoparticle was increased, the surface charge of nanoparticle becomes less positive in the acidic pH. The surface charge reversal can be attributed to the transfer of protons from the bulk solution to the surface of the nanoparticles [19,20]. Hydroxyl groups at the surface of nanoparticles can become $-OH_2^+$ by protonation. A similar charge reversal with the change in pH has been observed for polystyrene nanoparticles with carboxyl functional groups on the surface and was attributed to a positive charge acquired by hydrogen bonding of hydronium ions to the carboxylic group [15].

3.2.3. Surface hydrophobicity

Nanoparticles formulated with 5% PVA were found to be more hydrophilic compared to those formulated with 0.5% PVA as seen from the lower binding constants of Rose Bengal to the surface of the nanoparticles (Table 2). PVA is a hydrophilic polymer than PLGA and therefore the higher amount of residual PVA at the surface of the nanoparticles formulated with 5% PVA (Fig. 1) could account for

their higher hydrophilicity. This effect could be compared to the effect of coating a hydrophilic polymer such as polyethylene glycol (PEG) or poloxamer on to a surface of a hydrophobic polymer resulting in higher hydrophilicity of the nanoparticle surface [18].

3.2.4. Protein loading in nanoparticles

The effect of PVA concentration in the external aqueous phase of the multiple emulsion on the protein loading in nanoparticles is shown in Table 2. Protein loading increased with increasing PVA concentration in the external aqueous phase (19% w/w for 0.5% PVA and 24% w/w for 5% PVA). This can be related to the increasing viscosity of the PVA solution with increasing concentration of PVA leading to the resistance to the outward diffusion of BSA from the internal aqueous phase and to the better stabilization of the emulsion at higher PVA concentrations [21]. Also, higher amount of PVA at the interface of organic phase and the external aqueous phase could have contributed to the higher resistance to BSA diffusion out of the polymeric phase leading to higher protein loading in nanoparticles prepared with higher amount of PVA.

3.2.5. In vitro release of BSA

The cumulative in vitro release of BSA from PLGA nanoparticles, using 0.5% and 5% PVA as an emulsifier is shown in Fig. 3. The release profiles were biphasic for both the formulations, with an initial burst release attributed to surface associated protein, followed by a slower release phase as the entrapped protein slowly diffuses out into the release medium [21]. While the residual PVA did not affect the initial release of the protein in the first 7 days, the cumulative release of the protein was slightly higher starting at 10 days for nanoparticles formulated with 0.5% PVA. The possibility is that the higher viscosity of 5% PVA solution could have resulted in a more compact polymer matrix resulting in lowered degradation rate of the polymer and/or the slower diffusion of the encapsulated protein from the nanoparticles. Classically, the protein release from PLGA matrices is described to be diffusion-mediated in the early phases and diffusion-cum-degradation mediated in the latter phases [22]. It could be argued, therefore, that the residual PVA does not affect the amount of protein associated with the surface of the nanoparticle but possibly influences the diffusion of the protein and the degradation of the polymer matrix. PVA is a swellable, hydrophilic macromolecule that has previously been shown to sustain the release of macromolecules [23]. Hence, it is possible that that the large amounts of PVA present on the surface could form a hydrogel barrier to the diffusional release of macromolecules.

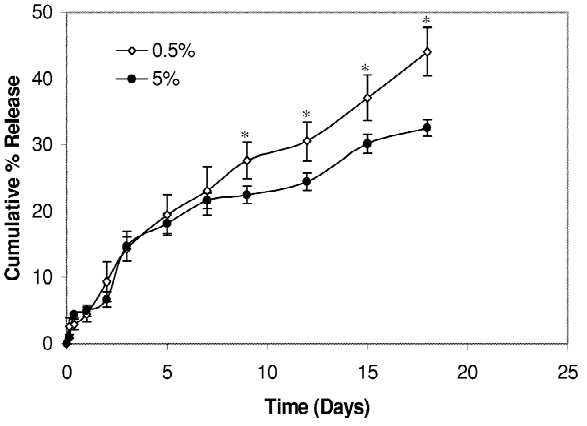

Fig. 3. Effect of residual PVA on the in vitro release of BSA from nanoparticles, data as mean±S.E.M. ($n=3$). * $P<0.05$.

3.2.6. Cellular uptake

Cellular uptake of different nanoparticle formulations in HAVSMCs is shown in Fig. 4A and B. Initial studies were carried out with nanoparticles formulated using different concentrations of PVA. There was no significant difference in the uptake of nanoparticles formulated with 0.5, 1 or 2% PVA (results not shown). However, the uptake was significantly reduced for nanoparticles formulated with 5% PVA (112.9 μg/mg of total cell protein for 2% PVA compared to 40 μg/mg of total cell protein for 5% PVA) at 100 μg/ml/well dose (Fig. 4A). This trend was observed at both the doses tested (100 and 200 μg/ml per well) and also in the presence or absence of serum. In general, nanoparticles demonstrated a dose dependent uptake at the two doses tested (Fig. 4B) and the presence or absence of serum did not affect the uptake.

Intracellular uptake of nano- and microparticles has previously been shown to depend on the size and the hydrophobicity of the carrier [24,25]. In general,

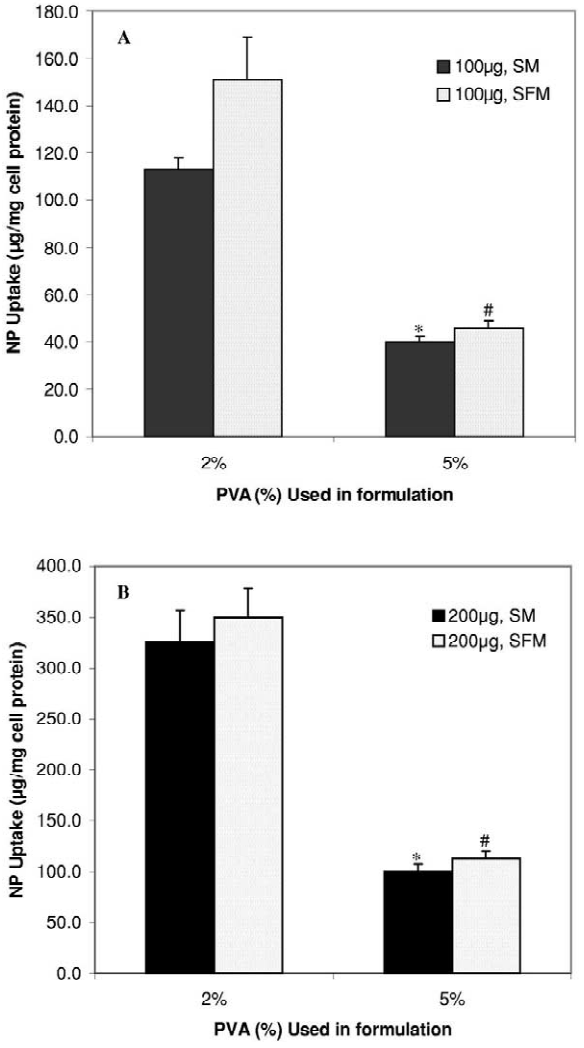

Fig. 4. Effect of residual PVA on the nanoparticle (NP) uptake in HAVSMCs. Nanoparticles were incubated with HAVSMCs at 100 μg/well (A) or 200 μg/well (B) dose in the presence or absence of serum, data as mean±S.E.M. ($n=6$). SM, serum containing medium; SFM, serum free medium; NP, nanoparticles. *,# $P<$ 0.05 compared to uptake of corresponding 2% PVA nanoparticle group.

the uptake decreases with increasing size and with increasing hydrophilicity. However, distinction has to be made in terms of the mode of intracellular uptake. Intracellular particulate uptake could either be by phagocytosis [25] or by fluid phase endocytosis [26]. A number of previous reports have demonstrated phagocytic uptake of nano- and microparticles in macrophages with a lower cut-off size for such a phagocytic uptake being about 0.5 μm [27]. For nanoparticles of lower size, the main route of intracellular entry is through fluid phase endocytosis [26]. It is also possible that the differences in the surface hydrophobicities of the two formulations (nanoparticles prepared with 5% PVA were more hydrophilic compared to those prepared with 2% PVA) could have contributed to the difference in uptake [28]. Vascular smooth cells have been reported to phagocytose other apoptotic cells and cholesterol granules in the vascular tissue [29]. However, using a phagocytosis assay kit (FcOxyBurst™, Molecular Probes, Eugene, OR), no phagocytic activity was detected even at 1000 μg/well nanoparticle dose. Lack of any effect of serum on the nanoparticle uptake further argues against a phagocytic uptake for either of the formulation [30]. Thus, the reason for the lower uptake of nanoparticles formulated with 5% PVA despite their lower size remains unclear but appears to be related to the surface hydrophobicity of the nanoparticles.

PVA has been widely used in the formulation of biodegradable micro- and nanoparticles in different concentrations, the range being 0.5 to 10%. Traditionally, the main role of PVA has been that of an emulsion stabilizer. Previous studies by others have shown that the hydrophobic segment of PVA binds to the surface of the nanoparticles, which can not be removed during the washings [12]. Shakesheff et al. have demonstrated that PVA is adsorbed on the surface of PLA and PLGA microparticles by employing X-ray photoelectron spectroscopy [31]. While there are previous investigations that have reported the effect of PVA on the nanoparticle size and zeta potential [5,6], there is no detailed study on the effect of PVA on the cellular uptake and other pharmaceutical properties of nanoparticles. The fact that the residual PVA remains at the surface of the nanoparticles could enable it to control the way nanoparticles interact with that of a cell surface. Based on this hypothesis, we investigated the effect of residual PVA on the different pharmaceutical properties of PLGA nanoparticles. True enough, almost all of the pharmaceutical properties studied were affected by the residual PVA content. Importantly, the residual PVA significantly affected the intracellular uptake of nanoparticles.

A number of previous reports including that from our laboratory have shown the applications of biodegradable nanoparticles formulated from poly (D,L-lactide-co-glycolide) (PLGA) for drug and macromolecular delivery [32]. For a number of these applications including gene therapy, it is not only important that nanoparticles release the encapsulated therapeutic agent at a sustained rate but also release it intracellularly. Thus, efficient intracellular uptake of nanoparticles is critical for these applications. Since in our studies we have shown that the residual PVA present in the nanoparticles affects their intracellular uptake, it becomes important to consider this parameter more critically for applications that involve intracellular drug delivery. Fontana et al. have shown a significant difference between polyethylcynoacrylate nanoparticles prepared in the presence and absence of PEG and demonstrated that nanoparticles coated with PEG have reduced uptake by macrophages [33]. Further, the in vivo half-life of a carrier such as liposome or nanoparticle in the systemic circulation has been shown to depend on the surface hydrophobicity of the carrier [18]. Hydrophobic nanoparticles are rapidly cleared by circulating monocytes resulting in their localization predominantly in the liver. Gref et al. have used PEG modified block co-polymers of PLA and PLGA to formulate nanoparticles with hydrophilic properties in order to prolong their circulation time [34]. Since PVA also alters the surface hydrophobicity of nanoparticles, it would be interesting to determine if the amount of residual PVA alters the pharmacokinetics and the biodistribution of the nanoparticles in vivo.

While it is important to realize that the residual PVA influences different pharmaceutical properties of nanoparticles, it is equally important to derive strategies to control the extent the residual PVA associated with the nanoparticles. In this report, we have demonstrated that the residual PVA content can be altered by changing the PVA concentration in the external aqueous phase or by altering the type of organic solvent used in the emulsion. Further investigation is needed to study the influence of other possible parameters on the amount of residual PVA including the molecular weight and composition of the polymer, physical characteristics (hydrophilicity/hydrophobicity) of the drug incorporated in nanoparticles, composition of co-solvents, aqueous to organic phase volume ratio of the emulsion, and the molecular weight and composition of the PVA used.

4. Conclusions

The amount of residual PVA that remains associated with the PLGA nanoparticles can be controlled by altering the PVA concentration or the type of organic solvent used in the emulsion formation, and is an important factor that influences the cellular uptake of nanoparticles. Further, the residual PVA is an important formulation parameter that can be used to modify or alter the different pharmaceutical properties of nanoparticles.

Acknowledgements

Grant support from the National Institutes of Health (HL 57234) and the Nebraska Research Initiative, Gene Therapy Program. JP is supported by a pre-doctoral fellowship from the American Heart Association and SP by a pre-doctoral fellowship (DAMD-17-02-1-0506) from Department of Army, the US Army Medical Research Association Activity, 820 Chandler Street, Fort Detrick, MD 21702-5014. We thank Ms Elaine Payne for providing administrative support.

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