Research Article

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Electrosprayed poly(vinyl alcohol) particles: preparation and evaluation of their drug release profile

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Abstract

Encapsulation of bioactive molecules within polymeric particles is a challenge because of several limitations, including low drug-loading efficiency, unwanted release profile, polydispersity and batch-to-batch variation in reproducibility, along with the limitations of scaling up the process. It is essential to control the morphology of pure polymer particles in the first instance, in order to obtain the desired release profile of drugs from the particles during a later stage. Here we report the preparation of electrosprayed particles from a water-soluble US Food and Drug Administration-recognized polymer, namely poly(vinyl alcohol) (PVA), as an approach towards a short-term drug delivery vehicle. Through electrospraying and varying the solvent ratios, three different sizes of particles were prepared, with sizes ranging from 500 to 2000 nm. Insulin was chosen as a model bioactive molecule, and the release profile of the drug was studied after its incorporation in the PVA particles. Fractional release plots obtained showed short-term release of insulin within the first 60 min. Release curves were analyzed according to the Ritger–Peppas model, suggesting Fickian diffusion as the predominant insulin release mechanism from the PVA particles. This work suggests electrosprayed PVA particles as an innovative drug delivery system for short-term administration of drugs.

Keywords: electrospraying; electrospinning; drug delivery; particles; PVA; insulin

INTRODUCTION

Polymeric particles are of great interest in the biomedical field, due to their ability to function as engineered micro- and nanotechnological systems for drug delivery. Various technologies have been demonstrated for the production nano- and micro-sized materials for the encapsulation of therapeutic molecules or even growth factors. Process modifications have also been performed by various researchers in an attempt to overcome the limitations of drug delivery systems (DDSs) such as the bolus delivery of molecules or bioactivity loss, especially of proteins where the harsh *in vivo* environment can cause denaturation and shortening their half-life after delivery, thus reducing the efficacy of the released molecules. Polymeric particles are therefore presented as reservoir systems, such that they are able to protect biomolecules from harsh environments, enhancing their long-term biological activity. By controlling the particle morphology and size of the polymeric matrix, these systems are able to provide tailored release rates.[1] Once in use, the propagation of drug payloads to non-targeted areas is minimized, limiting unwanted effects and allowing site-specific delivery.[1–3]

To date, emulsion-based techniques have been the commonest laboratory methods employed for particle preparation.[1] However, they have some disadvantages such as low drug-loading efficiency, limitations to scale up, polydispersity and difficulties in incorporating hydrophilic drugs. Furthermore, emulsion-based techniques imply rinsing steps which may lead to drug inactivation or degradation due to exposure to organic solvents, high shear stress, high temperature and aqueous organic interfaces.[4]

Electrospraying has emerged as a promising technique for the preparation of micro- and nanoparticles to serve as suitable DDSs. Electrospraying is based on the theory of charged droplets whereby an electric field applied to a charged liquid droplet exiting a capillary is able to deform the interface forming a Taylor cone. Eventually, the electrostatic force generated by application of a high voltage assists in overcoming the surface tension of the droplet with the subsequent ejection of particles. The main advantages of this technique over conventional encapsulating methods are higher loading efficiency, narrow particle-size distribution and ease of particle synthesis via single-step processing. This method does not require an additional particle separation procedure.[5,6]

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Previous studies have correlated the effects of key variables of electrospraying on polymer particles with mid-term and long-term release profiles, using biodegradable polymers like poly(lactic-*co*-glycolic acid) (PLGA) and polycaprolactone.[7–10] Nevertheless, in the context of drug delivery for eye or nasal applications, for example, a faster delivery system is required and poly(vinyl alcohol) (PVA) is a good choice.[11–13] PVA is a US Food and Drug Administration-recognized polymer, and various drugs have been encapsulated in PVA particles and fibers.[14–17] PVA is a non-toxic, non-carcinogenic and biocompatible polymer, which has been approved for use in several devices such as contact lenses and artificial organs.[18,19] Compared to PLGA and other polyesters, PVA has the advantage of generating a less acidic environment during drug release.[20,21] Properties such as its biocompatibility and bioadhesiveness make PVA particularly applicable for drug delivery formulations intended for alternative routes of administration. For instance, Cadinoiu *et al.* proposed chitosan–PVA for ocular delivery of pilocarpine whereas Tafaghodi *et al.* demonstrated higher efficiency of PLGA–PVA particles as nasal DDSs over other formulations.[22,23] Furthermore, the high water solubility of PVA provides additional benefits, such that it can be processed using benign preparation techniques that do not damage drugs during the production process compared to the conventional or complex drug preparation techniques. However, very few studies have investigated the production of electrosprayed PVA particles to date.[24,25] Identifying the appropriate solvents and processing variables for obtaining uniform and spherical particles using electrospraying is a challenge. The type of polymer and its properties offer individualistic characteristics to the electrosprayed particles, suggesting the complexity and interdependence of variables involved in the process.

In the study reported here, we evaluated the solution parameters for fabrication of PVA particles using the electrospraying technique. The primary objective of the work was to identify the solvents suitable for electrospraying of PVA particles, followed by method optimization to obtain reproducible particles. Insulin was chosen as a model protein drug for incorporation in the PVA particles, and the release of insulin from the PVA particles was evaluated considering the future applications of electrosprayed particle delivery systems.

MATERIALS AND METHODS

Materials

PVA of low molecular weight (L-PVA; $M_w = 20\,000–30\,000$ g mol^{-1}) and of high molecular weight (H-PVA; $M_w = 89\,000–124\,000$ g mol^{-1}), insulin from bovine pancreas, ethanol and glacial acetic acid (AA) were supplied by Sigma-Aldrich (Singapore). Pierce™ BCA protein assay kit was obtained from Thermo Fisher Scientific Inc. (Singapore).

Particle preparation

Firstly, pure PVA solutions were prepared for conducting the electrospraying experiments. A schematic of the electrospraying setup applied is shown in Fig. 1. In short, PVA was dissolved in distilled water by heating at 95 °C for 1 h and cooled to room temperature. Further to this, specific volumes of ethanol and AA were added and the obtained solutions were kept overnight with stirring. AA and ethanol addition will be discussed in the following sections. L-PVA and H-PVA were individually used to prepare solutions of different concentrations. Table 1 provides details of the L-PVA and H-PVA solutions prepared during this study. Following the optimization of the solution for preparation of reproducible PVA particles by electrospraying, encapsulation of insulin within the PVA particles was also performed. For electrospraying of insulin encapsulated in PVA particles, insulin was pre-dissolved in aqueous AA solution at a pH of 3.0, while the final ratio of PVA to insulin was maintained at 250:1.

PVA solutions were loaded in 3 mL disposable syringes (Becton Dickinson, USA) each fitted with a 25-gauge stainless steel nozzle and a high voltage of 10 kV (Gamma High Voltage Research, USA) was applied to form a positively charged single jet, while PVA solutions were extruded through the nozzle at a constant rate of 0.30 mL h^{-1} using a syringe pump (KDS100, KD Scientific, USA). The collectors were made of standard aluminium foil and the tip-to-collector distance was set to 10 cm. Temperature and relative humidity were in the ranges 20–23 °C and 61–65%, respectively.

The morphology and size of the particles were studied as a function of the solvents used, namely water-to-AA-to-ethanol (W:AA:Eth) ratio, the molecular weight of PVA and solution concentration. The PVA particles containing insulin are referred to as PVA–insulin.

Conductivity and viscosity of polymer solutions

The conductivity of the prepared solutions was measured using a Jenway-3540 conductivity meter (UK). To measure the viscosity of the solutions, an Anton-Paar MCR 301 rheometer equipped with a plate–plate type cell was used (25 mm in diameter) at 20 °C with a shear rate range from 0 to 300 s^{-1}.

Physical characterization

The morphology and size of the electrosprayed particles (PVA and PVA–insulin) were characterized using an SEM instrument (JEOL 5600 LV, Japan) operating at a high voltage of 15 kV. The samples were sputter-coated with platinum before SEM imaging. Micrographs were obtained at low and high magnification, in order to have a detailed overview of the morphology of various batches of particles prepared during this study. In order to determine the particle size of the electrosprayed particles, the obtained images were analyzed using data and image processing software, namely OriginLab Co. (USA) and ImageJ (USA), respectively. To assess the reproducibility of the particles prepared, three replicates of each condition were produced.

In vitro release studies

For release studies, 5 mg of PVA–insulin particles were placed in Eppendorf tubes containing 1 mL of phosphate buffered saline. The release was carried out at a temperature of 37 °C, while the test tubes were shaken at 150 rpm. At designated time intervals, the samples were centrifuged at 10 000 rpm for 1 min and the supernatant was withdrawn with a micropipette. The insulin concentration at each determined time point was obtained using a Pierce™ microBCA kit following the manufacturer's instructions. The absorbance of each sample was measured at 562 nm using a microplate reader (Varioskan™ Flash Multimode Reader, Thermo Fisher Scientific Inc., USA). The fractional amount of insulin released from the PVA particles was calculated using the following equation:

$$\text{Fractional insulin release} = \frac{M_t}{M_\infty} \quad (1)$$

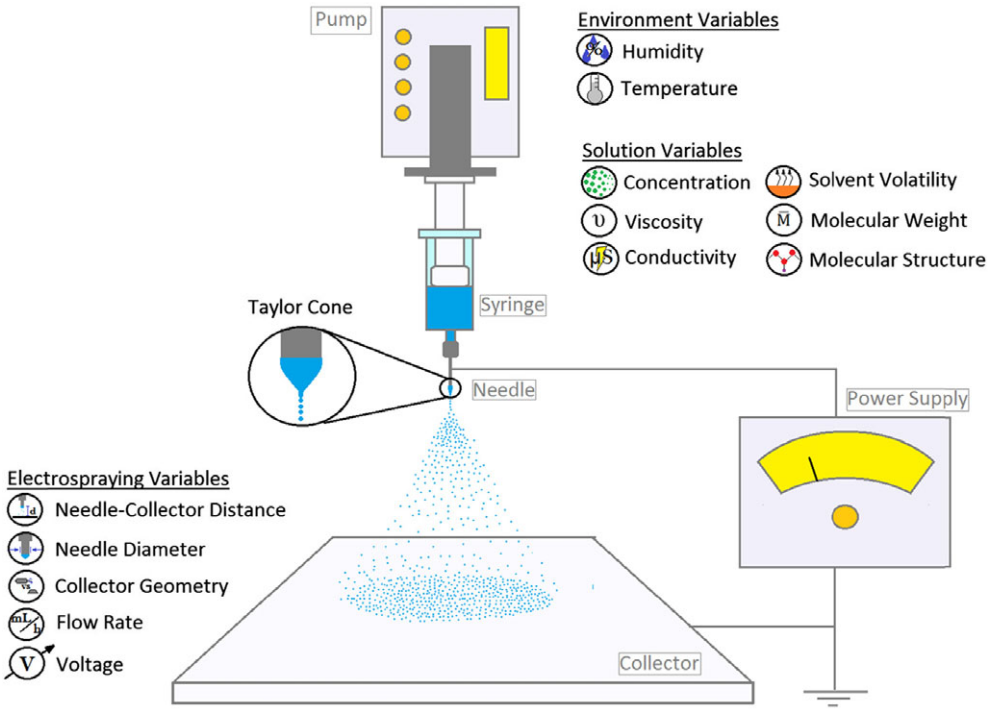


Figure 1. Schematic of electrospraying setup, with regard to environment and solution variables.

Table 1. Details of PVA and solvent ratios used for electrospraying

Molecular weight (g mol^{-1})	Solution concentration (g mL^{-1})	Solvent[a]	Solvent ratio
Solvents			
20 000–30 000	0.15	**W**	–
20 000–30 000	0.15	**W:AA**	97:3
20 000–30 000	0.15	**W:AA:Eth**	72:3:25
20 000–30 000	0.15	**W:AA:Ace**	72:3:25
20 000–30 000	0.15	**W:AA:Chl**	72:3:25
20 000–30 000	0.15	**W:AA:HFP**	72:3:25
Molecular weight and concentration			
20 000–30 000	**0.025**	W:AA:Eth	72:3:25
20 000–30 000	**0.05**	W:AA:Eth	72:3:25
20 000–30 000	**0.10**	W:AA:Eth	72:3:25
20 000–30 000	**0.15**	W:AA:Eth	72:3:25
89 000–124 000	**0.025**	W:AA:Eth	72:3:25
89 000–124 000	**0.05**	W:AA:Eth	72:3:25
89 000–124 000	**0.10**	W:AA:Eth	72:3:25
89 000–124 000	**0.15**	W:AA:Eth	72:3:25
Ethanol effect			
20 000–30 000	0.15	W:AA:Eth	**92:3:5**
20 000–30 000	0.15	W:AA:Eth	**72:3:25**
20 000–30 000	0.15	W:AA:Eth	**47:3:50**

[a] W, water; AA, acetic acid; Eth, ethanol; Ace, acetone; Chl, chloroform; HFP, hexafluoro-2-propanol.

where M_t is the amount of insulin released from the PVA particles at time t and M_∞ is the mass of drug released at infinite time period. Three individual measurements were performed for each time point. The release profiles were evaluated for particles of various sizes.

The release kinetics of insulin was studied using the Ritger and Peppas model, which suggests that the prevailing drug release mechanism from swellable porous hydrophilic systems such as PVA is a coupling of diffusion and macromolecular relaxation of the polymer network as a result of which the drug diffuses outward

with a kinetic behavior that is dependent on the relative ratio of diffusion and relaxation.[26] From this model, the following equation arises:

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

where M_t/M_∞ is the fractional release of insulin, k is the kinetic constant, t is the release time and n is the diffusional exponent for drug release. Further details of the model will be discussed later.

RESULTS AND DISCUSSION

AA and ethanol addition

PVA is essentially a water-soluble polymer with its solubility in water depending on its molecular weight, degree of hydrolysis and tendency to form hydrogen bonds in aqueous solutions. Nevertheless, low conductivity and high surface tension of water introduce the need for application of high voltage, such that a stable electrospraying process can be established. In fact, the voltage required to cause steady electrospraying depends on the square root of the surface tension of the liquid used for electrospraying.[27,28] Therefore, the voltage used for electrospraying aqueous PVA solution might exceed the electric breakdown threshold of the surrounding gaseous medium, usually air, and corona discharge would ensue. This phenomenon typically destabilizes the electrosprayed jet and results in the production of broad distributions of particle sizes, which are also unsuitable for drug delivery applications.[3,27,29] The most convenient method to tackle this problem is by increasing the electrical conductivity of the electrospraying solution by addition of co-solvents. In this work, first we chose to use AA as a co-solvent since AA is capable of increasing the electrical conductivity and, additionally, it is a suitable solvent when considering the incorporation of insulin.[30,31] Therefore, during this study, firstly an aqueous solution of PVA was prepared to obtain a solution pH of 3.0, using water–AA at a ratio of 97:3 (v/v). Measurements of the conductivity of the solution show an abrupt increase in electrical conductivity. PVA in water shows a conductivity of 123 μS, while the conductivity of PVA in water–AA mixture jumps to 1025 μS. However, the solution of PVA in water–AA mixture produces a highly unstable electrospraying jet. In accordance with the literature, higher conductivities lead to unstable cone-jet mode due to higher Coulombic repulsive forces.[3] Hence, the incorporation of an organic solvent like ethanol along with water and AA is required, in order to control (reduce) the electrical conductivity of the solution. At the same time, AA along with ethanol preserves insulin efficacy.[32] Studies by Zhang and Kawakami also demonstrated that ethanol not only reduces conductivity but also increases the viscosity of aqueous chitosan solution.[33] We examined such details more explicitly and the details are explained later in this paper.

Molecular weight and solution concentration

The effects of molecular weight and polymer concentration on electrospraying were assessed. Briefly, solutions of L-PVA and H-PVA at different concentrations were prepared, ranging between 0.025 and 0.15 g mL^{-1} (Table 1), while W:AA:Eth ratio was kept constant (72:3:25). Since every solution showed Newtonian behavior for the shear rate range selected, we report only one value of viscosity for every solution.

Figure 2 shows SEM micrographs of electrosprayed structures obtained from different concentrations of PVA prepared during this study. As can be seen, L-PVA produces particles at every concentration attempted. However, H-PVA is only able to produce particles below 0.05 g mL^{-1}. Further, the morphology translates from beaded fibers to stable fibers at concentrations higher than 0.10 g mL^{-1}. These morphology transitions due to variations of polymer concentration or molecular weight are common among all or most types of electrospinnable polymers, and the main underlying mechanism of this effect is the alteration of the number of chain entanglements which consequently cause changes in solution viscosity and serves as a determinant for different polymer solution regimes.[7,34–39] For electrospinning or electrospraying, the solution regimes can be distinguished as dilute, semi-dilute unentangled and semi-dilute entangled according to the critical chain overlap concentration (C^*) and entanglement concentration (Ce), also represented in Fig. 3. The former is known as the point at which the solution concentration is equal to the concentration inside the radius of gyration of every single polymer coil and marks the crossover from dilute regime to semi-dilute unentangled regime.[24,35] As the concentration is increased further, the topological constraints induced by the larger occupied fraction of the available hydrodynamic volume in the solution introduce chain entanglements so as to reach a significant critical amount. From this point, the regime is considered semi-dilute entangled and this value is known as the entanglement concentration. For electrospraying, it is necessary to work with solutions with concentrations ranging from C^* to C_e.[3,35,40] Below C^*, there are not enough chain entanglements within a drop to stabilize the particle structure formation and they lose their shape upon impinging against the collector; whereas above C_e, there is an increased number of chain entanglements which serves to stabilize the jet through jet breakup inhibition due to increased surface tension, producing beaded fibers. During this study, C^* could not be determined because a transition from amorphous particles to spherical particles was hardly observed, which also means working with very dilute solutions. It is clear that C^* for both L-PVA and H-PVA is below 0.025 g mL^{-1}. Our results show that C_e for H-PVA solutions is around 0.05 g mL^{-1}, the point at which beaded fibers start being produced until 0.10 g mL^{-1}, where the electrospinning jet is completely stabilized and fibers are produced further. On the other hand, L-PVA solutions do not show a transition point from particles to beaded fibers, and it is clear from the micrographs (Fig. 2) that these solutions are at a semi-dilute unentangled working regime at least up to 0.15 g mL^{-1}.

Several methods have been proposed to quantitatively predict the transition point from particles to beaded fibers, with respect to electrospraying. Among them, the semi-empirical equation proposed by Shenoy *et al.* is acknowledged for prediction of the transition point.[38,39] However, viscosity measurement itself serves as a common prediction technique, which is also analyzed during this work.

Shenoy *et al.* developed a semi-empirical equation to predict the electrospraying regimes as a function of the number of chain entanglements.[38,39] Chain entanglements are essentially the physical interlocking of polymer chains, and are a direct consequence of polymer overlap. Chain entanglements behave in a similar manner to chemical crosslinks, although the chains can slide past one another with an influence on the viscoelastic properties.[38,39] Shenoy *et al.* proposed the following equation that models the number of chain entanglements in solution:

$$n_e = \frac{\phi M_w}{M_e} \quad (3)$$

where n_e is the number of chain entanglements in polymer solution, ϕ the polymer volume fraction, M_w the polymer

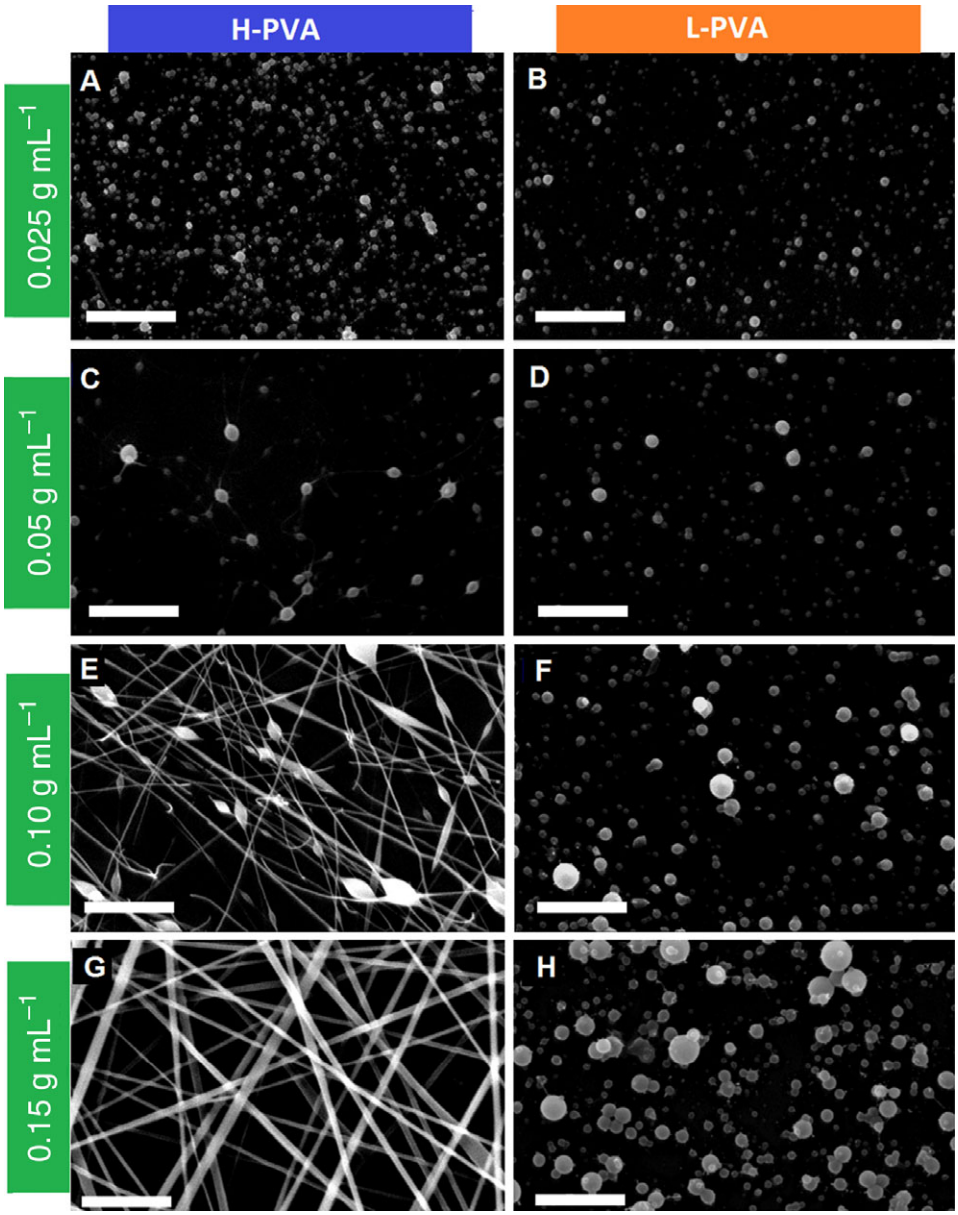


Figure 2. Effect of molecular weight and solution concentration of (A, C, E, G) L-PVA and (B, D, F, H) H-PVA as a function of the obtained electrosprayed structures.

molecular weight and M_e the entanglement molecular weight, which is the approximate molecular weight between entanglements in a polymer melt and it only depends on polymer structure.[38] This model assumes that the chain entanglements are solely responsible for viscosity variations and junction stabilization, which means that, with an increase in the number of chain entanglements and under the action of elongational stress (i.e. during electrospinning), elastic networks are created to stabilize the fiber structure formation.[35,39,41]

Following Eqn (3), Shenoy *et al.* demonstrated that $n_e = 2$ (which corresponds to one entanglement per chain since an entanglement necessarily involves two chains) is the critical number of entanglements that marks the transition from particles to beaded fibers, and, at $n_e = 3.5$, only fibers are generated. Figures 4(A) and (B) show the prediction plots of n_e *versus* concentration for high- and low-molecular-weight PVA, respectively, used in this work. H-PVA solutions show a transition from particles to beaded fibers at around $n_e = 0.75$ (i.e. at 0.05 g mL^{-1}) and from beaded fibers to fibers at $n_e = 1.55$ (i.e. at 0.10 g mL^{-1}). This difference between measured values and predicted values based on the Shenoy *et al.* equation can be explained with regard to polymer–polymer interactions. In PVA it might be that there are strong polymer–polymer interactions, such as hydrogen or ionic bonding, which might not be negligible, compared to interactions between the polymer and the solvent itself. Increased interchain interactions in these systems may serve to stabilize the chain entanglements by retarding chain disentanglement or forming additional junction points which might facilitate fiber formation at low concentrations and, consequently, lower n_e than predicted by Shenoy *et al.*, and as observed in our case. Similar results were also obtained by others.[24,38,42] This earlier transition to beaded fibers makes H-PVA a non-optimal (unsuitable) polymer for electrospraying processes. Conversely, no beaded-fibers are observed during the electrospraying of L-PVA throughout the concentration range

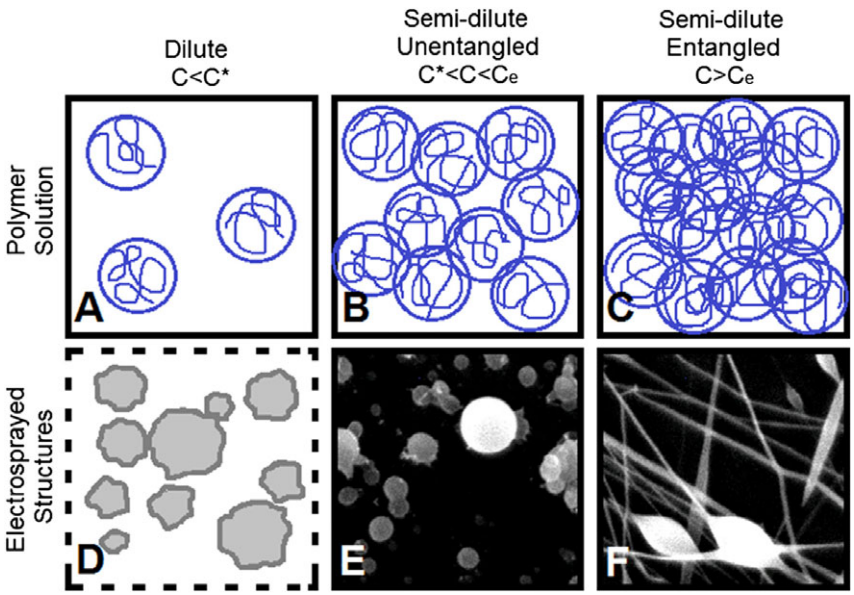


Figure 3. Physical representation of the three solution regimes. Final electrosprayed structures depend on initial solution regime used. (A) Dilute regime, (B) semi-dilute unentangled and (C) semi-dilute entangled, respectively producing (D) amorphous structures, (E) particles and (F) beaded fibers. Amorphous structures are not observed in this work.

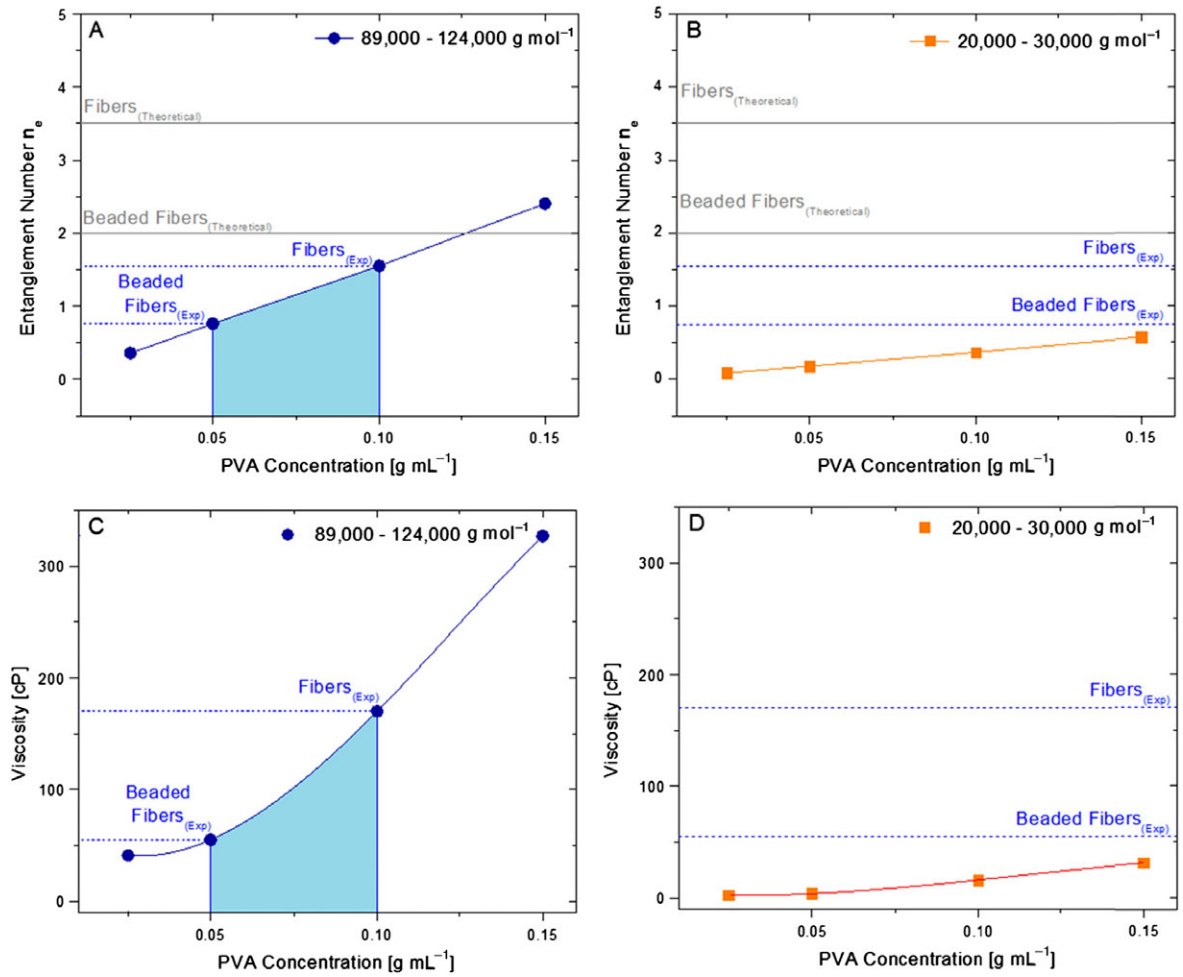
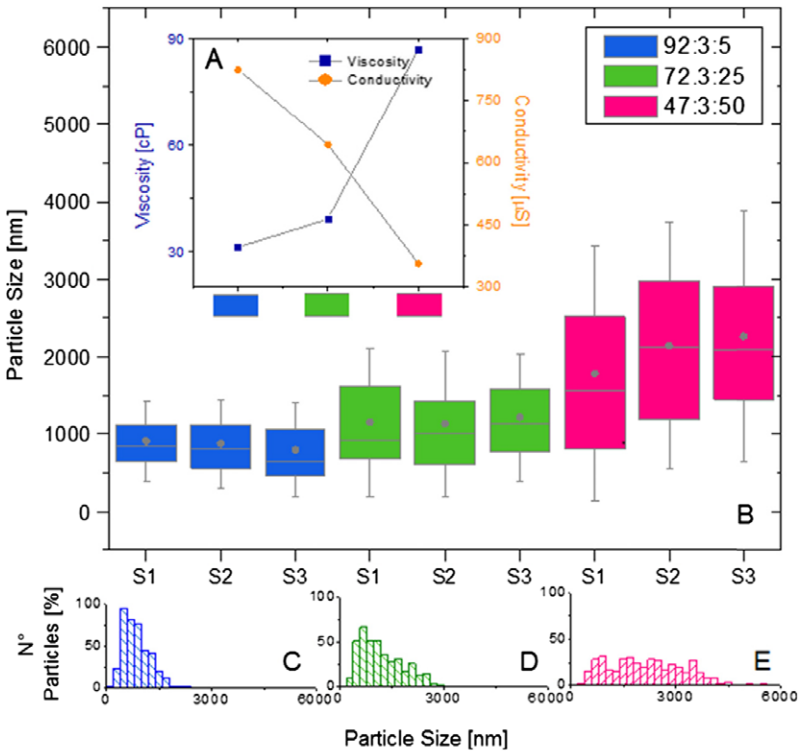


Figure 4. Entanglement number and viscosity *versus* PVA concentration for (A, C) H-PVA and (B, D) L-PVA. Plots show that H-PVA reaches semi-dilute entangled regime at $n_e = 0.75$, and produces fibers at $n_e = 1.55$ (indicated with dotted lines). Comparison was made with the Shenoy *et al.* predicted values (marked with gray lines).



Water:AA:Ethanol	92:3:5	72:3:25	47:3:50
Conductivity [µS]	825	642	355
Mean [nm]	851	1217	2143
Variation Coefficient	0.43	0.49	0.53

Figure 5. Quantitative analysis of effect of ethanol concentration on particle size and distribution. (A) Viscosity and electrical conductivity as a function of ethanol concentration. (B) Box plots and (C, D, E) histograms showing mean particle size and distribution as a function of ethanol concentration. For quantitative analysis purposes, three replicates for each W:AA:Eth ratio used for L-PVA solutions were analyzed. Results are summarized in the table below the plots.

used in this study. It can be observed that the n_e value of L-PVA is always below 0.75, which is also the transition value for H-PVA and this might explain the absence of beaded fibers during the electrospraying of L-PVA.

Shenoy *et al.* also demonstrated that at the critical point where there is at least one entanglement per chain ($n_e = 2$, for good solvents and no other interactions), the solution viscosity increases abruptly and its linear dependence on concentration and molecular weight disappears.[35,39,43] Such behavior is clearly observed in Fig. 4(C) for H-PVA, where at around 0.05 g mL^{-1} (which corresponds to $n_e = 0.75$) is the aforementioned transition point from semi-dilute unentangled to entangled regime. On the other hand, such viscosity behavior is not found for L-PVA, according to Fig. 4(D). Particles produced from L-PVA differ only in their sizes, which range from 287 nm with solution viscosity of 4 cP (0.05 g mL^{-1}), up to 1217 nm when the solution viscosity is 30 cP (0.15 g mL^{-1}). These results show a clear particle size dependency on solution viscosity. Higher viscosities imply a high number of chain entanglements, which hinder drop breakup by electrical forces due to increased surface tension of the solution. In this way, larger particles are obtained with high-viscosity solutions. Therefore, L-PVA is better than H-PVA for the preparation of electrosprayed particles in a concentration range of 0.025 to 0.15 g mL^{-1}. Considering PVA particles as DDSs and taking into account that longer release profiles are preferred over shorter ones, larger particles produced with 0.15 g mL^{-1} of L-PVA were chosen for further experiments in this work.

Ethanol effect

As mentioned above, ethanol was added to PVA solutions to stabilize the electrospraying jet since ethanol can have an impact on the viscosity and conductivity of the solution, which further influence the size of the electrosprayed particles. Indeed, with electrospraying, it is possible to obtain particles over a wide spectrum of sizes, ranging from 100 nm up to 50 µm by changing specific parameters, which can be related to solution properties and electrospraying conditions (i.e. temperature, flow rate, needle diameter, voltage, etc.). In accordance with previous studies, when environmental and process parameters are kept constant, solution viscosity and conductivity are the main variables that affect the final particle size.[3,33] Therefore, 0.15 g mL^{-1} L-PVA solutions with various ethanol ratios were prepared, and this was followed by their conductivity and viscosity measurement. Viscosity and conductivity measurements were performed as a function of W:AA:Eth ratios used for PVA solution preparation. Conductivity clearly decreases with ethanol addition (an organic phase), ranging from 825 µS for 92:3:5 to 355 µS for 47:3:50 (Fig. 5(A)). On the other hand, results shown in Fig. 5(A) indicate that the viscosity of the solution increases with increasing ethanol concentration, reaching a final value of 87 cP for 47:3:50 against 31 cP for 92:3:5 of W:AA:Eth

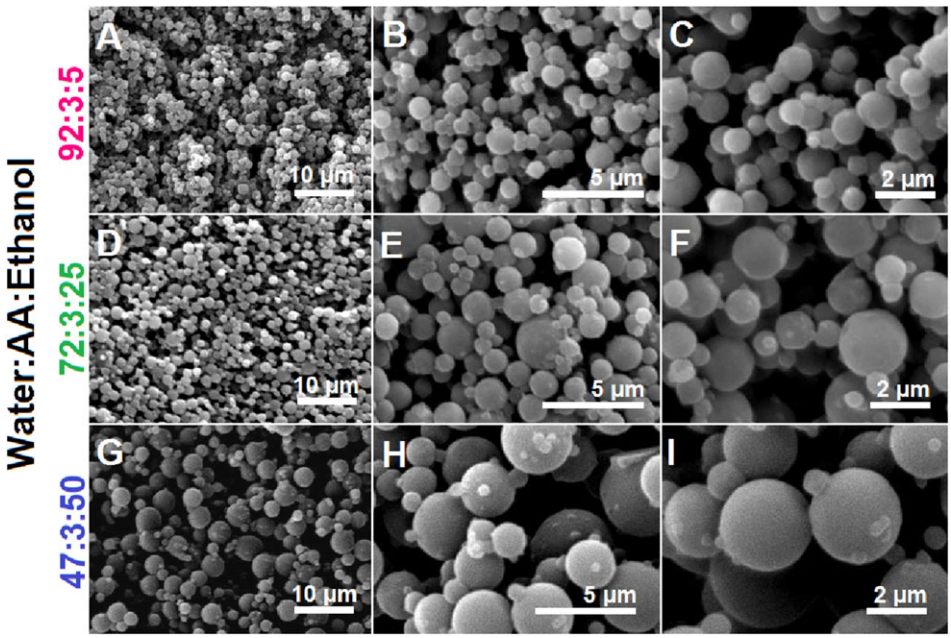


Figure 6. SEM micrographs showing the morphology of electrosprayed particles with respect to W:AA:Eth ratios: (A, B, C) 92:3:5, (D, E, F) 72:3:25 and (G, H, I) 47:3:50.

ratio. Particle size was measured after electrospraying the respective solutions. The results are presented through box plots and histograms, which are shown in Figs 5(B)–(E).

Figure 6 shows SEM micrographs of the particles obtained. For quantitative analysis purposes, three replicates for each W:AA:Eth ratio were used for L-PVA solutions. Box plots show that higher concentration of ethanol leads to electrospraying of larger particles, with a mean particle size of 2143 nm for W:AA:Eth ratio of 47:3:50, 1217 nm for 72:3:25 and 851 nm for 92:3:5. Furthermore, as can be seen from Fig. 5(B), increased amounts of ethanol produce increased particle dispersity. To quantitatively compare the dispersity of produced particles, the coefficient of variation was used for normalization of the standard deviation of the mean particle size obtained. This coefficient is defined as

$$C_v = \frac{\sigma}{\mu} \tag{4}$$

where σ is the standard deviation and μ the mean particle size. Our results show that higher concentrations of ethanol produce a slight increase in dispersity of electrosprayed particles, being 0.43 for W:AA:Eth ratio of 92:3:5, 0.49 for 72:3:25 and 0.53 for 47:3:50.

For higher electrical conductivity, the Coulomb repulsion forces are higher and compete with the viscoelastic forces of the solution, disentangling the polymer network formed during electrospraying, producing smaller particles. Gañán-Calvo and others showed that a decrease in particle size can be obtained with an increase in the conductivity of the solution, according to the following equation:

$$d = \frac{1}{K^{1/6}} \tag{5}$$

where d and K are particle size and electrical conductivity, respectively.[3,33,44,45] Nevertheless, if only conductivity had been the parameter controlling the particle size, higher conductivity values would have produced larger particle dispersity due to Coulomb fission forces, which overcome chain entanglement forces, and therefore produce secondary droplets that are ejected from primary droplets producing increased particle dispersity.[7,36,40] Our study shows decreased dispersity with higher solution conductivity, which suggests that conductivity is not the only parameter controlling the final particle size, and viscosity should be taken into account. Rosell-Llompart and Fernández de la Mora stated that the influence of viscosity on the particle size and dispersity should not be neglected and is a main parameter for consideration.[46] High dispersity and size of particles are obtained from solution with high viscosity, and this is observed from our results as well.[36,44–46] Similar results were also observed by Zhang and Kawakami where they used W:AA:Eth solvent systems for preparation of chitosan particles by electrospraying. They reported an increased particle size and dispersity with increasing ethanol concentration.[33] In accordance with the above discussion, we demonstrate that control of the ethanol concentration is an effective method for tuning average particle size and distribution in a reproducible manner.

PVA and PVA–insulin particles

Insulin is an anabolic polypeptide hormone used medically to treat some forms of diabetes mellitus. Therapeutic insulin for diabetes is typically administered via subcutaneous injection. It has a short half-life after intravenous administration (30 min), but nevertheless can cause hypoglucemic shock.[47,48] However, its administration by parenteral route can result in peripheral hyperinsulinemia, the stimulation of smooth muscle cell proliferation and the incorporation of glucose into the lipid of arterial walls, and might therefore be the causative factor in diabetic micro- and macro-angiopathy.[49] For these reasons, other routes for administration have been explored by several research groups in order to deliver insulin to patients with type 1 and 2 diabetes such that it assists in lowering blood sugar, and allows patients to have a simpler, less invasive and more direct control of their underlying disease process. These intended routes include oral, rectal, sublingual, buccal, transdermal, vaginal, intramuscular, intrapulmonary and intranasal, which can be achieved through PVA-based

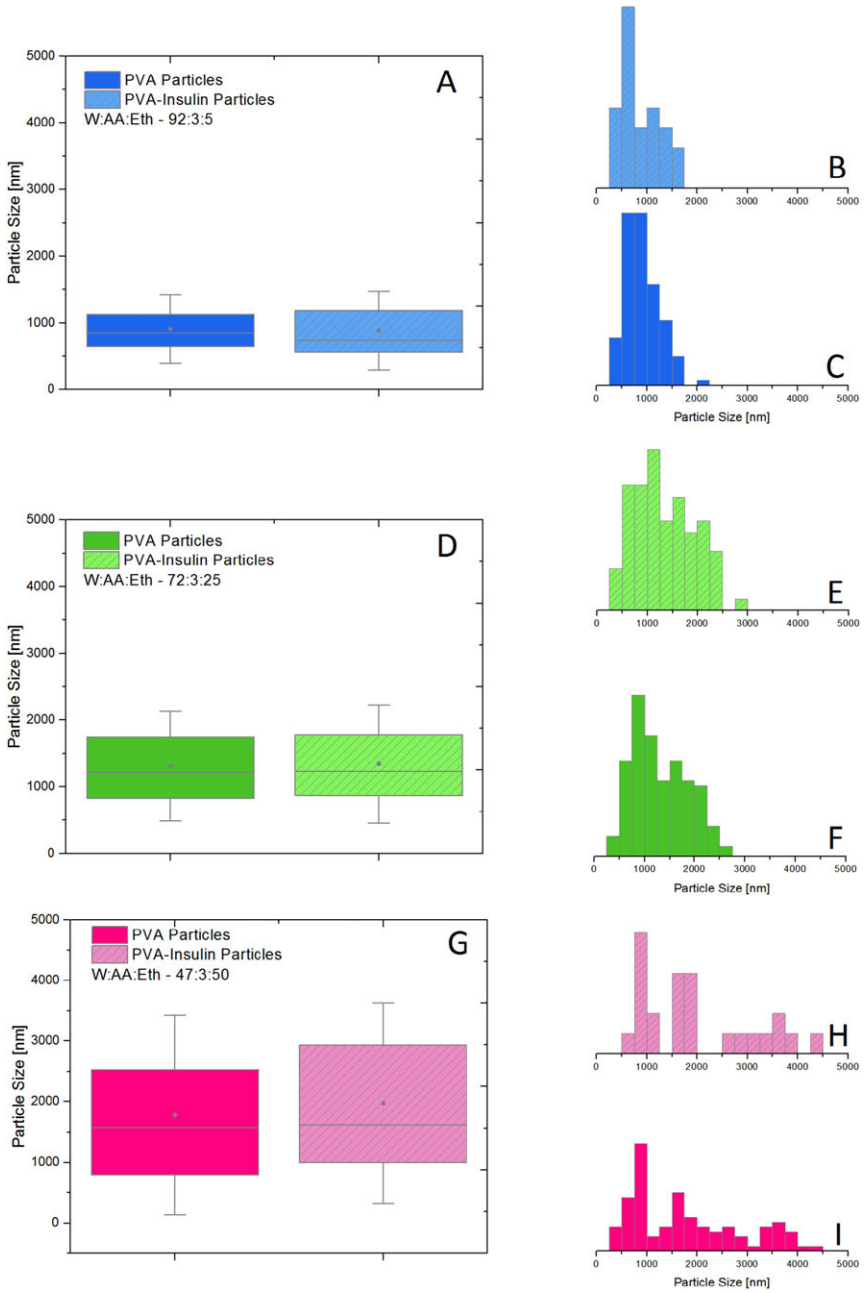


Figure 7. Box plots (A,D,G) and histograms (BC, EF, HI) showing the particle size of PVA particles and PVA-Insulin before drug release. W:AA:Eth Blue: 92:3:5, green: 72:3:25 and pink: 47:3:50.

DDSs.[50,51] Here we explored the possibility of encapsulating insulin within PVA particles using the electrospraying process as a first approach for future studies concerning delivery of insulin and other proteins through alternative administration routes.

Figure 7 shows the particle size distribution of pure PVA and PVA–insulin particles (before drug release). Solvent systems of W:AA:Eth at ratios of 92:3:5,72:3:25 and 47:3:50 were used for the particle production. Three replicates of each condition were analyzed and pure PVA particles have a mean particle size of 851, 1217 and 2043 nm with C_v of 0.43, 0.49 and 0.53, respectively; whereas PVA–insulin particles have a mean particle size of 884, 1294 and 1976 nm with C_v of 0.44, 0.48 and 0.56. ANOVA test was applied to determine the differences in size measurements between PVA and PVA–insulin particles. No significant difference in size between PVA and PVA–insulin particles is observed, which means that loading of insulin does not influence the particle size and morphology.

Release studies

Release studies were performed using PVA–insulin particles of three different sizes obtained from 0.15 g mL^{-1} L-PVA solutions made using solvent with W:AA:Eth ratios of 92:3:5, 72:3:25 and 47:3:50, with final mean particle size of 884, 1294 and 1976 nm, respectively. The insulin fractional release profiles and rate of release from PVA particles of the three different sizes are shown, respectively, in Figs 8 and 9. From the curves, it can be observed that the release rate is independent of the particle size, and that the majority of the release occurs during the first 600 min (10 h).

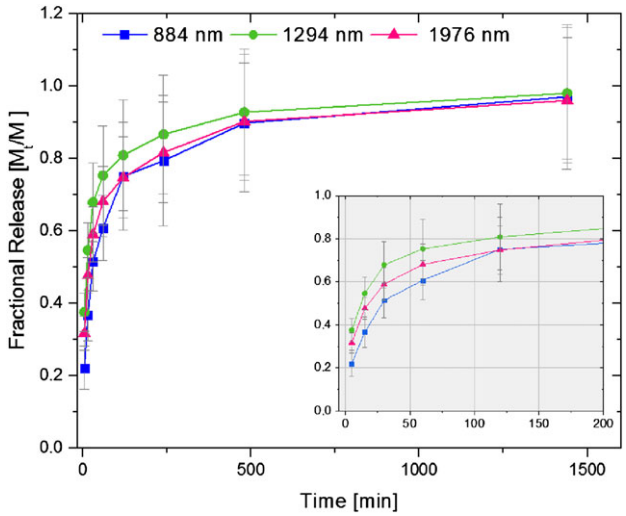


Figure 8. Insulin fractional release from PVA particles of various sizes. The inset shows an expanded view of fractional release during the first 120 min.

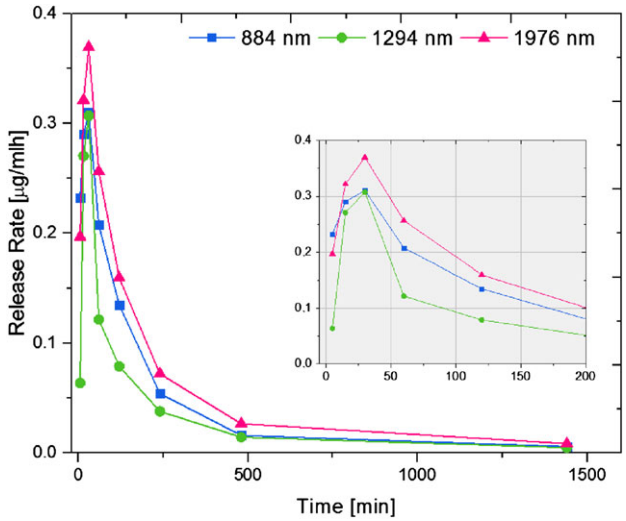


Figure 9. Rate of release of insulin. The inset shows an expanded view of release rate during the first 120 min.

This phenomenon can be explained by analyzing the mechanism of insulin release from PVA particles and the absolute amount of insulin released.

To determine the mechanism of release, a much acknowledged empirical equation proposed by Ritger and Peppas was applied (Eqn (2)).[26,52] The equation is used to model the release behavior of swelling-controlled systems which absorb solvent up to a moderate equilibrium degree and are prepared by incorporation of a drug in a hydrophilic and glassy polymer such as PVA. This equation was applied to the first 60% of the fractional drug release curve, and, subsequently, linear fitting was performed and R^2 coefficients were assessed to determine the release mechanism. R^2 coefficients are 0.96 for $t^{0.43}$, and 0.83 for $t^{0.85}$. In accordance with the Ritger–Peppas model, if $n = 0.43$ ($R^2 \sim 1$ when $x = t^{0.43}$), the release from spherical particles is governed by Fickian diffusion which means that the release occurs by molecular diffusion of the drug due to a chemical potential gradient. Otherwise, if $n = 0.85$ ($R^2 \sim 1$ when $x = t^{0.85}$), the drug release is swelling-controlled and it is associated with stresses and glass transition, swelling in water or biological fluids.[25,52,53] Therefore, our results clearly suggest that insulin release from PVA particles occurs through pure Fickian diffusion, and the non-Fickian release component is negligible.

CONCLUSIONS

Electrospraying is a simple, rapid and reproducible method for generating spherical particles of PVA by dissolving PVA in a mixture of water, AA and ethanol. The average size of the particles can be tuned by modulating the ratio of the solvents during the preparation of PVA in a water–AA–ethanol mixture. Results show that L-PVA is better than H-PVA in producing particles through electrospraying, even with the existing strong polymer–polymer interaction properties of the polymer. Insulin was encapsulated within PVA and the release of insulin was completed within a period of 1 h. Our study demonstrated a precise control of electrosprayed PVA particle size, and application of the particles for better delivery of insulin compared to traditional parenteral administration. Furthermore, PVA particles might also serve as carriers for delivery of other molecules that require high rates of release, involving future investigations.

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