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PRODUCTION OF PROTEIN NANOPARTICLES BY ELECTROSPRAY DRYING

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Abstract—The feasibility of producing relatively monodisperse and biologically active insulin particles by electrospray drying is demonstrated. The process entails dissolving dry insulin in an acidic ethanol–water solution. The solution is then electrosprayed and, after solvent evaporation, dry residues can be collected on suitable deposition substrates. Particles were sized visually, using a scanning electron microscope (SEM), and aerodynamically, using an inertial impactor. When electrosprays of nearly saturated solutions were operated in the stable cone-jet mode, impactor data showed that the particle average aerodynamic diameter ranged from about 88 to 110 nm in diameter and the distributions were quasi-monodisperse with relative standard deviation estimated at approximately 10%. SEM obsevations for the same conditions showed average particle dimensions ranging from 98 to 117 nm, with predominantly doughnut shapes. Smaller particles can be generated by decreasing the insulin concentration and/or by spraying smaller liquid flow rates. Although the maximum production rate for monodisperse insulin nanoparticles from a single cone-jet is low, at about 0.23 mg h^{-1} overall production can be increased by multiplexing the device with microfabrication techniques. Increasing production rate from a single cone-jet by at least one order of magnitude can be achieved also by increasing the liquid flow rate. The resulting particles have larger sizes, on the order of 600 nm, but particle monodispersity is compromised and particle morphology is drastically modified, probably as a consequence of a different electrospray operating mode. The biological activity of the electrospray-processed insulin samples was confirmed by comparing binding properties on insulin receptors against a control sample.

INTRODUCTION

Much effort has been devoted in recent years to develop alternative drug delivery systems (DDS). The general objective of all DDSs is to optimize drug delivery: to provide the appropriate dosage at the appropriate time in the appropriate location. The problems of *targeting* (Gregoriadis *et al.*, 1986; Anderson *et al.*, 1992) and *scheduling* (Struyker-Boudier, 1986) represent the two main obstacles to practical DDSs, each of which will be discussed in turn. For the greatest efficiency in treatment and minimization of toxicity to other body organs, it is desirable to apply drugs directly to the affected region. For technological reasons, direct targeting is impractical at present in all but a handful of treatments. Conversely, the most common DDSs in use involve a "flooding" process wherein relatively massive doses of a drug are orally or parenterally delivered to the body. The drug is filtered out as it circulates through the body, usually with only a small fraction reaching the intended target.

Another key problem that has received attention is the development of a zero-order DDS, in which the drug release rate is constant in time. This area of research might be termed "the problem of scheduling." For many long-term drug treatments it is necessary to maintain drug concentration levels within a fixed range of values. Below a minimum dosage, the treatment may lose its effectiveness, and above a maximum, harmful side effects can occur (Cowser, 1974). The problem centers on the elimination of peaks in drug concentration, that is, on developing a zero-order DDS. In contrast, conventional DDSs

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show first or higher order concentration profiles, often resulting in excesses in drug dosages (Franz *et al.*, 1992). As with the targeting, an improvement in scheduling also promises to reduce cost of treatment significantly, particularly in long-term cases.

One alternate route that has been explored is the use of surgically implanted devices that release drugs slowly over a period of months or even years. They consist of a small drug-containing matrix that, for example, is surgically inserted just below the skin (Edelman *et al.*, 1996). Some matrices are designed to biodegrade slowly, gradually releasing the contained drug into the blood stream over the "lifetime" of the matrix (Ron and Langer 1992). The inherent problem with these systems is that the exposed surface area is continually varying, producing non-zero-order release profiles. Bioresistant matrices have a similar problem. They are controlled by a diffusion mechanism (Lee, 1992). As the drug is depleted from the matrix, the concentration gradient is reduced, resulting in slower diffusion rates. These difficulties aside, these devices and variations of them, including ingested or injected microspheres (Wallace and Lasker, 1993; Regan, 1991) promise significant advances in DDSs in the future. The disadvantage of most microparticulate production systems is that the produced particles are not monodisperse in size. An example along these lines is the production of sample proteins such as insulin in the 1–3 μm range, using the gas antisolvent process (Yeo *et al.*, 1995). That monodispersity would be advantageous is a reasonable premise: with a single known particle size, drug density within a matrix can be manipulated much more precisely, diffusion rates can be controlled and drug delivery rates can be tailored more readily to the desired application. With these premises in mind, we decided to explore the applicability of electrospray techniques, that are well-established for the production of monodisperse particles, to protein particle production, using insulin for demonstrative purposes.

MONODISPERSE ELECTROSPRAY AND FINE PARTICLE PRODUCTION

Electrostatic means for liquid dispersion in minute droplets are used in a variety of technological applications (Bailey, 1988). In some systems, the dispersion of the liquid is driven primarily by electric forces, so that atomization and gas flow processes are reltively uncoupled. Such systems are referred to as electrosprays. Within the electrospray class of atomizers is a particular type characterized by the additional feature of a tight control of the size distribution of the resulting aerosol. Such a system can be implemented by feeding a liquid with sufficient electric conductivity through a small metal tube maintained at several kilovolts relative to a ground electrode positioned a few centimeters away. The liquid meniscus at the outlet of the capillary takes a conical shape under the action of the electric field, with a thin jet emerging from the cone tip. This jet breaks up farther downstream into a spray of fine, charged droplets. In view of the morphology of the liquid meniscus, this regime is labeled as the *cone-jet* mode (Cloupeau and Prunet-Foch, 1989).

The cone-jet mode offers the appealing feature of droplet monodispersion. It can produce droplets/particles over a wide size range, from molecular dimensions to hundreds of microns, depending on liquid flow rate, applied voltage and liquid electric conductivity. Especially in the nanometric range, the capability of producing monodisperse particles with relative ease is unmatched by any other aerosol generation scheme. Just as important is the fact that these particles are generated from capillaries with a relatively large bore (e.g., 100 μm is a typical figure), which are therefore unlikely to clog. For an extensive source of references on the electrospray, the reader is referred to *J. Aerosol Sci.* **25** (5) (Special Issue) (1994).

Within the nanoparticle context, on which this Special Issue is focused, scaling laws for the diameter of electrosprayed droplets indicate that it is possible, from highly conducting solutions, to produce directly drops with diameters as small as a few nanometers (Loscertales and de la Mora, 1993; Chen *et al.*, 1995). Alternatively, controlled and narrow distribution of sizes in the nanometric range can be produced also by dissolving some nonvolatile solute into an electrosprayable solvent. After electrospray dispersion, the solvent evaporates leaving behind nanometric residues, that can be used for specific

applications. This process is the nanoscale equivalent of spray drying that finds wide industrial applications for converting liquids into powders, even in the case of heat-sensitive materials in the biological, alimentary and pharmaceutical industry (Masters, 1991). This route was pursued in the present study to demonstrate the feasibility of producing fine particles of a sample protein, such as insulin, with a controlled and narrow distribution by electrospray drying in the cone-jet regime.

This is the first application of this device to protein processing. However, proteins or, for that matter, large macromolecules are no strangers to electrospray applications. Indeed, the leading commercial application of this device is Electrospray Ionization (ESI), as a means to introduce in the gas phase ions pre-existing in solution, including multiply charged macromolecules (Fenn *et al.*, 1989). Such ions can be analyzed in a mass spectrometer, through so-called Electrospray Mass Spectrometry (ESMS). Well over 90% of the literature on electrosprays is now devoted to ESMS of macromolecules, including proteins. Since the technique is sufficiently soft to provide no fragmentation of the macromolecules, the hope is that it is "gentle" enough even for protein processing.*

In the remainder of this article we will discuss how monodisperse insulin particles with characteristic dimensions on the order 100 nm can be generated with ease.

EXPERIMENTAL APPARATUS AND METHODS

Experiments were performed with the electrospray apparatus depicted in Fig. 1. It consisted of a gravity-controlled feed-line which was continuously adjustable to yield flow rates up to a few $\mu l\,min^{-1}$. The line was composed of approximately 1.2 m of 27-gauge Teflon tubing split with a short (a few cm) length of 76 μm ID slica tubing to increased the line pressure drop and help stabilize the flow. It was fed from 5 cm^3 disposable syringes with a $27\frac{1}{2}$ gauge needle and terminated into a 15 cm stainless steel 28-gauge (0.15 mm ID) needle with tapered end that acted as electrically charged electrode. In some experiments, the needle was mounted through a Teflon housing provided with an additional feed line for an optical gaseous co-flow to control the environment in which the spray was generated and dried. The needle was perpendicularly positioned with respect to the ground electrode. Typically, the two electrodes were kept approximately 3 cm apart, which resulted in applied voltages on the order of 5 kV for the establishment of the necessary electric field for the formation of a stable conical meniscus. During the experiment the electrospray current at the ground electrode was continuously monitored.

Insulin (bovine, Zn; Miles Inc., Pentex, Lot #211) was chosen for this study as a representative protein pharmaceutical. The insulin was received in powder form and was stored in a freezer with a silica gel desiccant. It was measured by weight using an electronic balance directly into solution vials in order to prevent any loss of material. A solution of acidic 89.1–9.9-1 ethanol–water–4% molar HCl by volume was prepared. The water was deionized and the ethanol was of HPLC grade. The addition of hydrochloric acid was necessary to lower the pH, thus enabling insulin to dissolve. Test solutions had pH ranging between 3.00 and 3.35. Their electric conductivity was measured between 1.26×10^{-2} and $1.58 \times 10^{-2}\,\Omega^{-1}\,m^{-1}$. Insulin was dissolved at a concentration of 10.0 $mg\,cm^{-3}$ of solution. This pH/concentration combination maximizes particle size (maximization of insulin concentration and minimization of solution conductivity K). The solution was sonicated for as many as 10 min to remove all traces of insulin precipitate. After complete

* As pointed out by one of the Referees, there are similarities between this work and that of Kaufman *et al.* (1996). Those authors electrosprayed very dilute protein solutions, including insulin, to form singly charged protein ions after solvent evaporation and droplet charge neutralization. To identify the dissolved protein by the mobility spectrum, they then used a differential mobility analyzer (DMA), as an alternative to the mass spectrometer of ESMS. The similarity between the two studies is only apparent. The present focus is, in fact, on protein *particle* production, whereas theirs was on the feasibility of protein identification by DMA. Furthermore, in the specific case of insulin their mobility spectra by DMA exhibited spurious peaks, that precluded the positive identification of the dissolved protein.

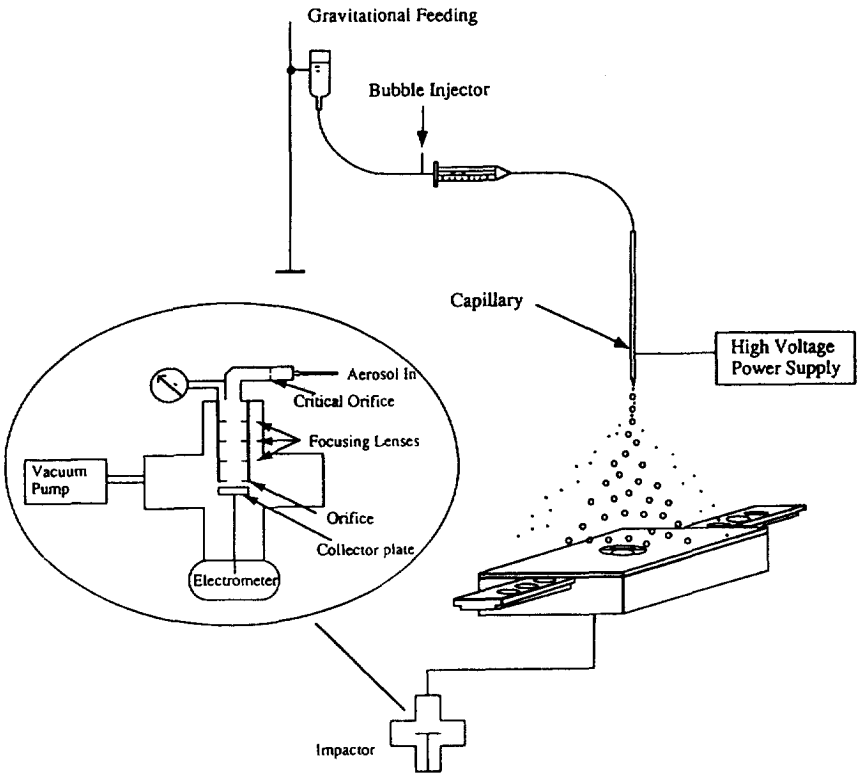

Fig. 1. Electrospray/impactor experimental system.

dissolution, it was allowed to stand for several hours to check for re-precipitation. Samples were sonicated for 3 min before being transferred into the reservoir syringe to remove any bubbles that might have caused spray instabilities.

Particle sizing technique

To examine the produced particles two techniques were used: scanning electron microscopy (SEM) and inertial impaction. SEM has the required nanometer resolution for sizing in the submicron range and is invaluable to determine the particle morphology. Computerized image analysis can also be used to access the size distribution of individual particles. This technique, however, is quite inefficient, since particles must be collected and then examined post-mortem. Potential biases because of the intrusiveness of the sampling technique and the transient phenomena associated with the insertion and retraction of the target from the test region are unavoidable. Also, the assessment of the size distribution often require the tedious analysis of several pictures to accumulate sufficient statistics, especially when automated analysis is not reliable because of particle overlap or contact.

For all these shortcomings, in addition to SEM, inertial impaction was applied as an independent on-line sizing technique. The impactor sizes an aerosol by accelerating it through a nozzle into a jet impinging perpendicularly on a collector plate. This simple scheme has the property of acting as a low pass filter, in which particles smaller than a critical diameter remain in suspension and are carried away with the flow, whereas heavier particles impact on the surface and are captured. The impactor used in this work is an automated version of the focusing impactor described by de Juan (1997), which in turn is an improved version of that developed by Fernández de la Mora (1996). The flow is accelerated through a nozzle by a mechanical pump. Varying the pumping speed changes the average jet velocity U and, thus, the Stokes number S, that is the dimensionless inertia parameter

governing the particle impaction behavior. The latter is defined as

$$S = t_p U / d_n, \tag{1}$$

where d_n is the diameter of the nozzle forming the jet and t_p is the particle relaxation time, that, for spherical particles of density ρ_p and diameter d_p, is defined as

$$t_p = \rho_p d_p^2 C_u / 18\mu, \tag{2}$$

where μ is the host fluid viscosity and C_u is the Cunninghan slip factor accounting for noncontinuum effect on the particle drag. The average jet velocity is

$$U = 4c^2 \dot{m} / (\pi \gamma d_n^2 p), \tag{3}$$

where c is the speed of sound, $\dot{m}$ is the mass flow rate passed through a critical orifice, p is the gas pressure in the plenum chamber just upstream of the nozzle and γ is the specific heat ratio of the gas (1.4 for air). $\dot{m}$ is fixed at a value that for the results reported here was $3.61 \times 10^{-3}\,\mathrm{g\,s^{-1}}$. For spherical particles in the free molecular regime, the Stokes Number S in equation (1) is given by

$$S = 0.178 \rho_p d_p \dot{m} c^3 / (p^2 d_n^3). \tag{4}$$

Thus, for a given particle, the Stokes number can be continuously varied by changing U and by monitoring such a change through the pressure p upstream of the nozzle. If the particles are charged, impaction is monitored by measuring the current I at the collctor plate. The impactor, thus, yields an aerodynamic spectrum, $I(p)$ which is closely related to the aerosol size distribution. In particular, for a quasi-monodisperse size distribution, such a spectrum exhibits a step, the steepness of which depends on the degree of monodispersity and the position of which gives the critical Stokes number.

The resolution of the impactor was improved by two crucial features. First, prior to particle impaction, a series of aerodynamic lenses focus the particles so that they are concentrated in the center of the stream, reducing losses and increasing the instrument resolution (Lui *et al.*, 1995). Second, deposition of particles at subcritical Stokes number by noninertial mechanisms such as Brownian diffusion or image charge is removed by "electrostatic blowing" (de Juan *et al.*, 1997). Further details are given in the next section.

The ground electrode in the electrospray apparatus was designed with a dual purpose, to accommodate microscope grids for particle collection and subsequent SEM analysis, as well as to interface the electrospray with the aerodynamic impactor for on-line particle sizing (Fig. 1). The collection device was a sliding metal strip equipped with indentations for the grids (Formvar Support Film Grids, copper 400 mesh) that were slid underneath the spray, where they collected insulin for a specified exposure time, typically between one and three minutes. The grids were immediately stored in a grid case, enclosed in an airtight bag, after collection. Then, they were examined in the SEM (ISI SS-40) facility, after being sputter-coated with gold/palladium. All photographs were recorded at 30 kV, with magnification of either 13 kx or 69 kx. One of the indentation on the sliding metal strips was drilled through to allow for the gas-particle flow to be drawn into the impactor. Thus simple sliding of the metal strip could allow for impactor sampling and SEM grid collection with minimal disturbance to the electrospray.

In some of the impactor experiments the metal capillary from which the spray was produced was replaced with a quartz capillary (0.05 mm ID) whose tip had been metallized. The capillary was encapsulated in a stainless steel tubing that provided the necessary rigidity for connection with the high voltage line. Use of this capillary had virtually no effect on the particle production as sampled by SEM, but was deemed essential in the impactor experiment because it improved the stability of the electrospray current at the ground electrode, which was necessary for proper analysis of the impaction data. A possible explanation as to why small capillaries help stabilize the current is that they lead to the formation of smaller conical menisci, thereby reducing evaporation effects over the reduced residence time of the liquid in the cone. As a result, insulin precipitation in the cone, with the potential of ensuing instabilities, may be either reduced or altogether avoided (Fernández de la Mora, 1997).

RESULTS AND DISCUSSION

The highest flow rate at which a stable monodisperse electrospray could be sustained was $0.38\,\mu l\,min^{-1}$, which corresponds to a production rate of about $0.23\,mg\,h^{-1}$. At larger flow rates, on the order of $2.2\,\mu l\,min^{-1}$, it was still possible to stabilize the conical meniscus but, as shown below monodispersity was compromised. Still higher feed rates resulted in unstable behavior of the conical meniscus and were not examined.

A number of samples were collected and examined after being exposed to the electrospray over a time interval of typically one minute, resulting in relatively sparse coverage. The matrix structure of impacted particles on the collector grid showed virtually no evidence of pre-impact clustering, as expected in view of the mutually repulsive electric force acting on the charged particles. Figure 2 shows at two different magnifications particles produced at a liquid flow rate of $0.17\,ml\,min^{-1}$ and a current of 64 nA. They are typically shaped as doughnuts. Some of them exhibit an horse-shoe morphology. Figure 2a was digitized and processed by an image analysis software (Image-Pro Plus, Media Cybernetics, MD). A few chains and agglomerates, that might have been due to close impaction of individual particles or might have been an artifact of the insertion and extraction of the microscopic grid, were excluded from the analysis. The remaining 557 particles were analyzed and yielded an average equivalent diameter of 98 nm, based on the projected area without accounting for the doubly connected morphology of many of the particles, and a standard deviation of 19 nm. Analysis of a similar picture obtained at a liquid flow rate of $0.38\,\mu l\,min^{-1}$ and a current of 100 na yielded an average diameter of 117 nm and a standard deviation of 27 nm.

Confirmation of the narrow distributions of the generated aerosol was provided by the impactor measurements. Figure 3 shows the collection efficiency $\eta(p)$ for different electrospray conditions, corresponding to (top → bottom) flow rates of 0.17, 0.24 and $0.38\,\mu l\,min^{-1}$ and currents of 64, 80 and 100 nA, respectively. $\eta(p)$ is a normalized collector current defined as

$$\eta(p) = \frac{I(p) - I_{max}}{I_{max} - I_{min}}, \tag{5}$$

where I_{max} is the maximum current of the spectrum, I_{min} is the minimum (offset) current of the electrometer and $I(p)$ the measured collector current as a function of pressure. For each electrospray condition, different curves are plotted as a function of repulsive voltage, V_r. As V_r increases, the steps become sharper, and the subcritical deposition, that is observed at $V_r = 0$ and is responsible for the long tail, is completed removed for $V_r > 1.8$ V. However, an increase in V_r also shifts the steps to lower pressures and one must account for this effect in order to size the particles properly.

de Juan *et al.* (1997) showed that at values V_r large enough to eliminate the tails in the aerodynamic spectra, the effect of V_r on S^*, the Stokes number at 50% collection efficiency, is only a function of the dimensionless parameter £, that is $S^* = S^*(£)$. £ is defined as

$$£ = \frac{qV_r}{kT}\,Sc^{-1}\,Re^{-1}, \tag{6}$$

where q is the particle charge, k is the Boltzman constant and T is the stagnation temperature in the plenum chamber. Re is the Reynolds number at the nozzle based on the average velocity U and in the present experiments was 72. Sc is the Schmidt number for spherical particles in the free molecular regime. Thus, the charge q of the particles must be known in order to size charged aerosols.

However, for the special case of aerosols that are quasi monodisperse in diameter as well as in charge, their mean size can be obtained by the following technique. Plotting the inverse of the square of the pressure at the half-step versus V_r and extrapolating back to $V_r = 0$ (£ = 0) along the linear portion of the curve, a critical pressure p^* may be inferred. For a known critical Stokes number of impaction, that depends also on impactor geometry and

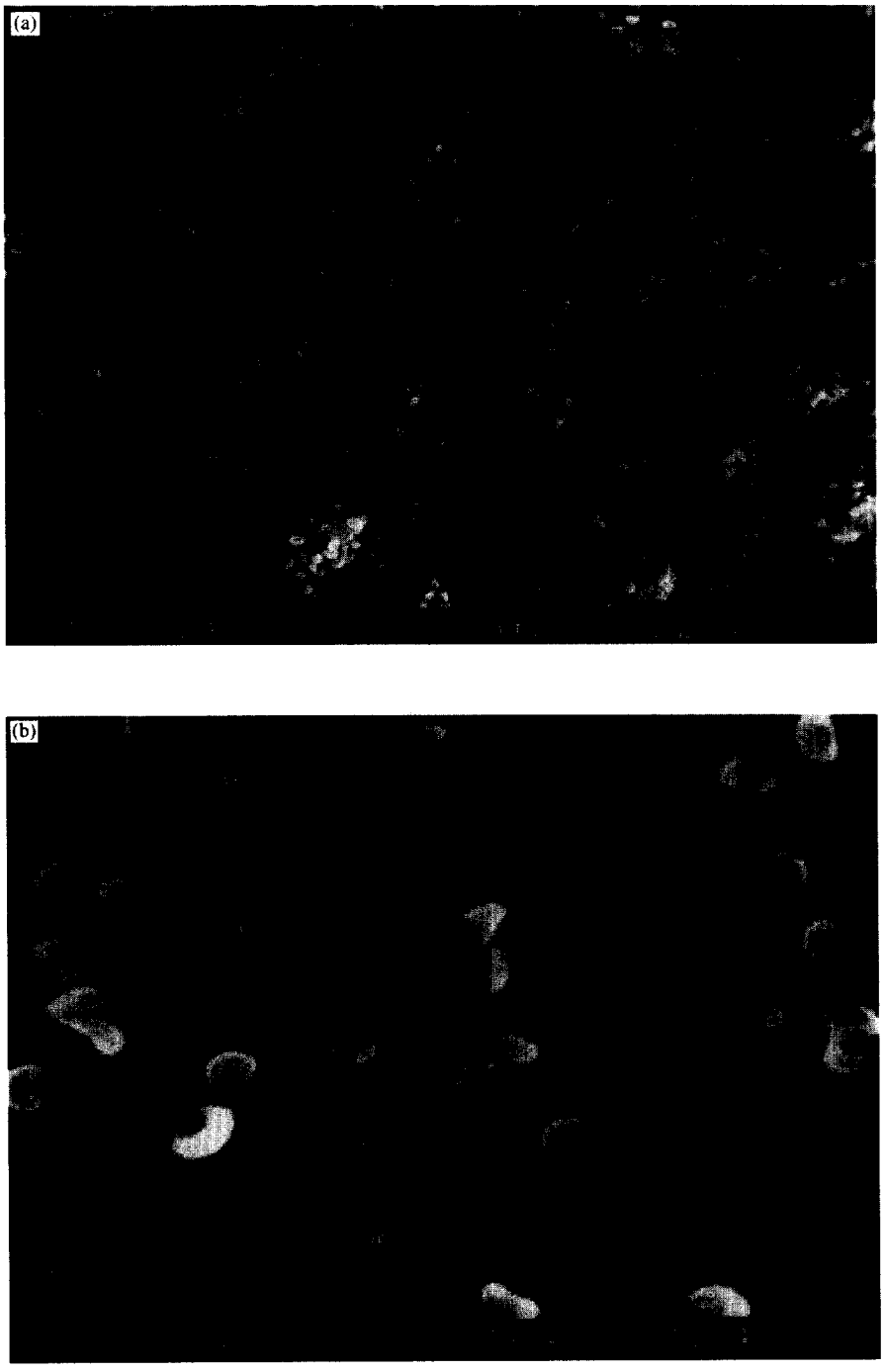

Fig. 2. SEM photographs of insulin particles at different magnification (a) 13.8 kx and (b) 69 kx). Electrospray liquid flow rate and current are 0.17 μl min^{-1} and 64 nA, respectively.

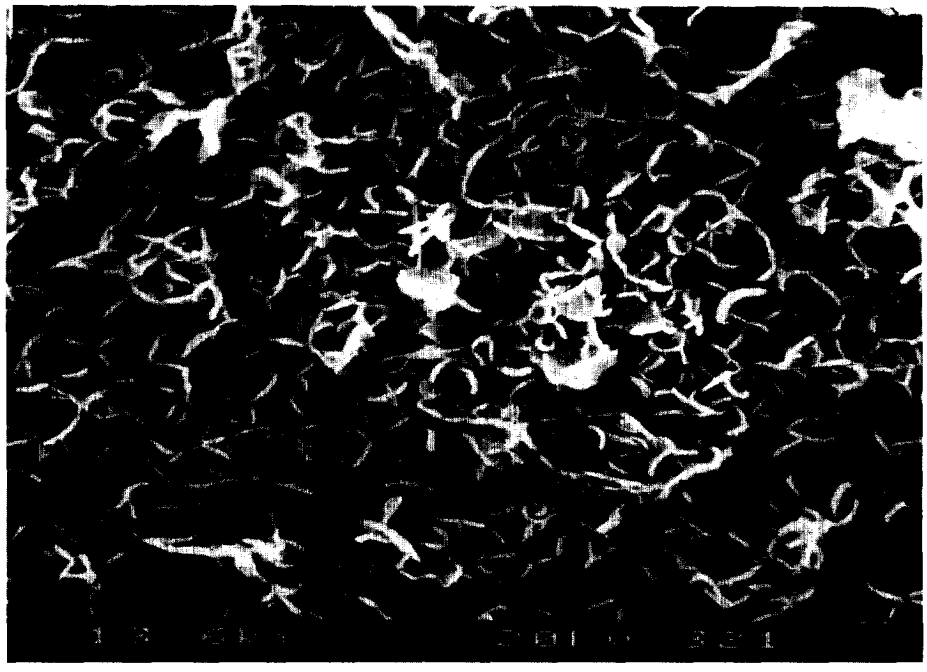

Fig. 5. SEM photographs of insulin particles at a magnification of 13.6 kx. Electrospray liquid flow rate and current are 2.26 μl min^{-1} and 211 nA, respectively.

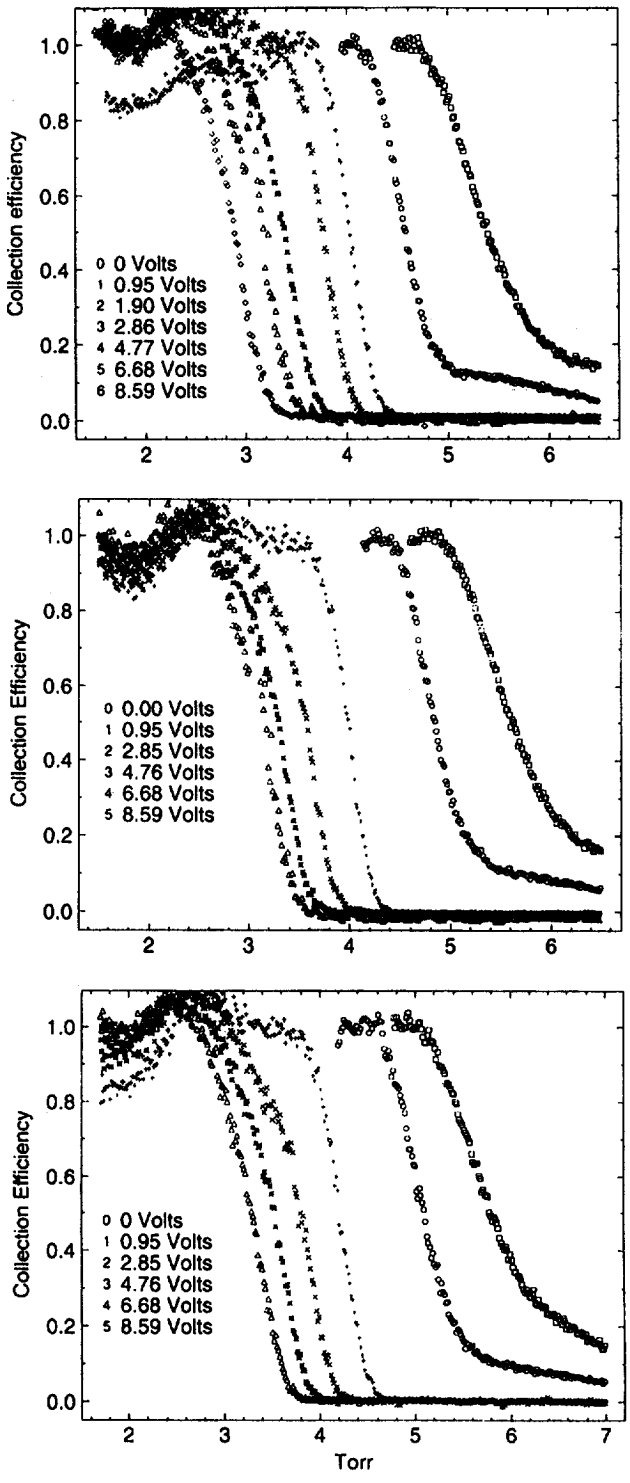

Fig. 3. Impactor aerodynamic spectra for different repulsive voltage, V_r. Electrospray conditions are detailed in Table 1.

is based on independent calibration, the particle diameter d_p may be calculated in terms of measurable quantities using equation (4) (de Juan *et al.*, 1997). Since the steps of Fig. 3 are sharp for $V_r > 1.8$ V, their size distributions are sufficiently monodisperse for this approach to be applicable.

In order to use equation (4), the extrapolated value of S^* at zero V_r must be obtained by calibration of the focusing impactor using particles of known size and charge. To this end, DOS oil drops were generated via standard aerosol techniques. A solution of 10^{-3} DOS in 2-propanol was nebulized in a collision atomizer, dried and neutralized before it passed through a Differential Mobility Analyzer (TSI model 3704) where monodisperse drops of known size and charge were selected. The classified aerosol entered the focusing impactor where the repulsive voltage V_r was set to 100 V to remove completely the subcritical tails. With this procedure, the extrapolated value of S^* at zero V_r was determined to be 0.162.

Figure 4 shows the inverse of the square of the critical pressure $(p^*)^{-2}$ versus V_r for the three electrospray flow rates as in Fig. 3. The straight lines are curve-fits with a linear regression of the data for $V_r > 1.8$ V. From the extrapolated values of $(p^*)^{-2}$ and the impactor calibration value of S^*, equation (4) gives the mean size of the insulin powder d_p for a unit density spherical particle. The measured values fall in the 88–110nm range. Table 1 summarizes these results for the three flow rates by listing: liquid flow rate, total current, half-step impactor pressure, particle aerodynamic diameter and Relative Standard Deviation (RSD), to be compared with average diameter and RSD from SEM image analysis in the last two columns. As regards the average values, there is surprisingly good agreement between the two techniques, the 10% discrepancy being attributable to particle morphology effects and to particle density, since the impactor measures an aerodynamic diameter and the data were processed for a unity particle density. Clearly, the trend to form larger particles at larger flow rates is captured properly and is consistent with the electrospray behavior. The initial droplet size is expected to vary with the cubic root of the liquid flow rate (Fernandez de la Mora and Loscertales, 1994). Since the insulin concentration was constant, after volatile evaporation, we expect that the residues also should scale approximately with the cubic root of the liquid flow rate, consistently with the impactor findings.

As regards the particle standard deviation, an estimate from the impactor data was made as follows. For an ideal impactor at £ = 0, the diameter standard deviation σ is

$$\sigma = 0.594/0.5R_p, \tag{7}$$

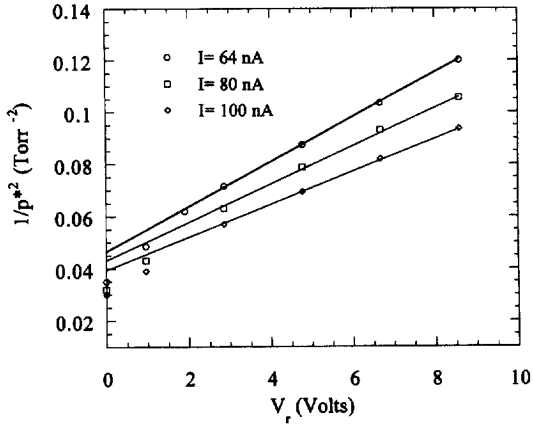

Fig. 4. Inverse of the square of the critical pressure, p^* at the half-step, as a function of repulsive voltage, V_r. Electrospray liquid flow rates and currents are the same as in Table 1.

Table 1

Q (μl min^{-1})	I (nA)	$p_{.5}$ (Torr)	d_p (nm) (Impactor)	RSD (Impactor)	d_p (nm) (SEM)	RSD (SEM)
0.17	64	4.64	88	8.7%	98	19%
0.24	80	4.82	102	9.8%		
0.38	100	5.04	110	9.5%	117	23%

when the diameter distribution is approximated to a gaussian function (de Juan, 1997). R_p is defined as

$$R_p = \frac{p^*}{p_{0.2} - p_{0.8}}, \tag{8}$$

where p_i ($i = 0.2$ and 0.8) is the pressures at collection efficiency i. As the repulsive voltage is increased and broadening effects are removed, the standard devation decreases to a minimum on the order 10% of the mean, as listed in the fifth column in Table 1, which is indicative of good monodispersity. As shown by the impactor steps in Fig. 3, the minima occur at intermediated values of the repulsive voltages. The increase of the standard deviation as the repulsive voltage is further increased may be due to particle losses in the hypersonic jet in the critical orifice at low pressures. Comparison of the impactor RSD with the SEM RSD shows a systematic overprediction of the SEM method, which is consistent with the expected artifacts of the grid insertion and removal from the spray.

Figure 5 shows a typical SEM photograph that was obtained when the electrospray was operated at a liquid flow rate of 2.26 ml min^{-1}, with a measured current of 211 nA, that is, values, much larger than for those that have been discussed so far. Clearly, the particle morphology is dramatically different from the previous pictures. The particles have typically a barnacle-like appearance. Attempts to perform impactor measurements yielded broader aerodynamic spectra. Under optical microscope examination, the liquid meniscus still appeared as a stable cone. However, it is likely that under these conditions the break-up mode is different from that typical of the cone-jet mode and lateral kink instabilities might have occurred (Rosell-Llompart and Fernandez de la Mora, 1994; Cloupeau and Prunet-Foch, 1994). Since the microscope optical resolution did not allow us to photograph the details of the liquid breakup, conclusive evidence of the relevant ES regime was not gathered.

Insulin biological activity

As discussed in the introduction, the well-established Electrospray Ionization (ESI) is a technique sufficiently "soft" to provide no fragmentation of macromolecules to be analyzed by mass spectrometry. The premise of no damage to the protein integrity is comforting, but is no guarantee of preserved biological activity. A potential disadvantage of the electrospray approach is that, as the liquid evaporates in these highly charged droplets, the electric field around the particle intensifies. This field may interfere with the protein conformation, yielding denaturation and loss of biological activity, well before even more dramatic effects, such as fragmentation, occur. In favor of the electrospray approach, on the other hand, is the fact that the droplets produced are sufficiently small that complete solvent evaporation over millisecond characteristic times does not require pre-heating of the bath gas. Consequently, there is no risk of thermally damaging dissolved labile molecules.

To determine the insulin bioactivity, the assay documented in (Shapiro *et al.*, 1986) was used by comparing the insulin receptor binding properties for three different samples in a blind test. As shown in Fig. 6, the behavior of the electrospray-processed insulin was indistinguishable as compared to that of the control insulin, implying that this electrospray drying technique is sufficiently "gentle" not to hinder the insulin biological activity.

Implications for biomedical applications and scale up

The initial droplet size of the insulin solution is controlled via the liquid flow rate through the needle. Stability and range of operation, with respect to droplet size and monodispersity, are limited primarily by solution electric conductivity and solubility. Smaller particles can be produced by either decreasing flow rate or by spraying more dilute solutions. Larger particles and larger flow rates are certainly feasible for proteins and peptides that can be dissolved in less conductive solutions. As noted before, the liquid electric conductivity is, in

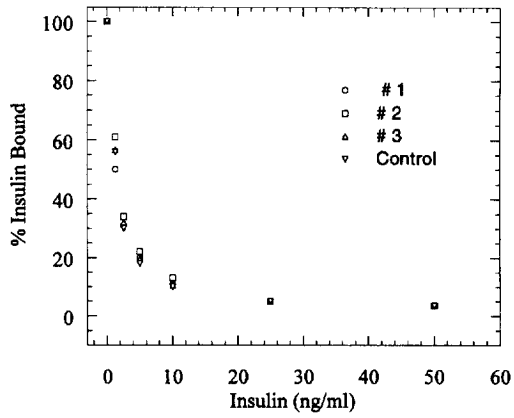

Fig. 6. Percentage of ^{125}I-insulin bound to receptors after the addition of unlabelled insulin at various concentrations.

fact, the primary physical variable to limit the liquid flow rate and the droplet size at which the monodisperse electrospray can be established.

One of the limitations that emerged from this feasibility study is that the production rate of insulin nanoparticles form a single cone-jet is limited to one fourth of one mg h^{-1}, clearly too low for any industrial applications. However, we notice that even in the case of insulin the maximum flow rate can be increased by at least one order of magnitude if strict monodispersity is not an issue and if the barnacle-like morphology of Fig. 5 is acceptable. Making bulk quantities of nanoparticles would be painstakingly slow unless many cone-jets are operated in parallel. Considerable related experience is available from early work in so-called colloidal thrusters, where the drops from a cone-jet of glycerol were electrostatically accelerated to large velocities for space propulsion (Bailey, 1988). In this case, large arrays of emitters were arranged and tested successfully under vacuum conditions. An analogous approach at atmospheric pressure would face greater difficulties because of space charge effects (Aguirre de Carcer and Fernández de la Mora, 1995). This matter has been investigated to a very limited degree (Rulison and Flagan, 1993) and the use of microfabrication techniques has been shown to be very successful in multiplying devices with similar features (Brodie and Spindtl, 1992). Thus, the production of bulk quantities of very small particles via electrospray is feasible, if space charge limitations can be overcome.

The production of protein powders by spray drying is not new (Mumumenthaler *et al.*, 1994). But, the electrospray approach brings to bear the unique capability of processing biomaterial particles to a high degree of monodispersity in the submicron size range. Drug delivery by biodegradable nanoparticles has recently been the focus of considerable interest (Couvreur and Puisieux, 1993). Applications of such products are envisioned for local drug and vaccine delivery (Song *et al.*, 1997), with the active pharmaceutical imbedded in either a biodegradable copolymer or a solid lipid (Almedia *et al.*, 1997). This feasibility study is potentially relevant also in this context, if a suitable precursor of the encapsulating material can be electrosprayed along with the protein-solvent solution.

CONCLUSIONS

The feasibility of producing relatively monodisperse and biologically active insulin particles by electrospray drying is demonstrated. Size measurements obtained by complementary techniques, via a scanning electron microscope and an inertial impactor, showed that quasi-monodisperse particles with average diameter of approximately 110 nm can be produced by operating the electrospray in the cone-jet mode. Smaller particles can be generated by decreasing the insulin concentration and/or by spraying smaller liquid flow rates. The particles have predominantly doughnut shapes. Although the maximum

production rate from a single cone-jet for monodisperse insulin nanoparticles is low, at about 0.23 mg h^{-1}, in principle overall production can be increased by multiplexing the device with microfabrication techniques. Increasing production rate from a single cone by at least one order of magnitude can be achieved also by augmenting the liquid flow rate. The resulting particles have larger sizes (on the order of 600 nm), but monodispersity is compromised and particle morphology is drastically modified, probably as a consequence of a different electrospray operating mode. The biological activity of the insulin samples was confirmed by comparing their insulin receptor binding properties against a control sample.

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