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Encapsulation of proteins by Electro Hydro Dynamic Atomization

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Summary: Aerosol reactor for Electro Hydro Dynamic Atomization has been used to encapsulate water soluble proteins in poly(ε-caprolactone). As a raw material w/o emulsion of BSA water solution emulsified in the organic solution of polymer was applied. Stability of the spraying process itself for particles production was investigated. Also raw material emulsion stability has been examined and emulsion stabiliser has been found. Protein release rate from produced particles was determined.

Keywords: biodegradable polymers; drug delivery systems; electrospray; emulsion; poly(ε-caprolactone)

Introduction

Microencapsulation of drugs is an important area of pharmaceutical and medical sciences. Encapsulation of an active agent in the polymer matrix protects vulnerable drugs from enzymatic degradation and increases its stability over time. Encapsulated drugs can be released in the controlled manner which helps to keep a constant therapeutic concentration in the body fluids for many hours or days from the moment of drug administration. Encapsulation of lipophilic drugs which are frequently soluble in organic solvents is fairly easy, drugs are dissolved in the organic solvent together with biodegradable polymer and spray dried or emulsified in water.[1] If drug can be dissolved in water only – like protein drugs or growth factors, problem became more complex. In this case multi step emulsification – solvent extraction/evaporation techniques are applied.[2] Electro Hydro Dynamic Atomization is one step method to produce drug loaded particles.[3–7] Application of EHDA allows producing particles of a narrow size distribution and, if compared to emulsion techniques, with high encapsulation efficiency, frequently equal 100%.[4] Protein encapsulation by EHDA has already been reported but a simplified version of the reactor collecting fresh particles in water was applied.[5,6] This can lead to premature drug release or production of porous particles.

The EHDA or electrospray refers to a process, where a liquid jet breaks up into droplets under the influence of electrostatic forces. Depending on the field strength, liquid properties (conductivity, viscosity, dielectric constant) and liquid flow rate different spraying modes will be obtained.[10] For the production of particles for drug delivery the so called cone-jet mode seems to be the most appropriate. In this mode a liquid is supplied through a nozzle at low flow rate. Applied electric field force liquid to form a stable cone from the apex of which a jet emerges, which breaks up in small droplets. Mono sized droplets from nano meters till hundreds of micrometers can be made this way.[8] Cone operating in the cone-jet mode, if observed in the back light, seams to be perfectly black. Also electric current flowing through the nozzle does not show oscillations.[9] These oscillation leads to production of different particle sizes or even spiting off

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the whole cone, which decreases encapsulation efficiency.

Process of EHDA is a very complex phenomenon and no complete theoretical model does exist. There are a few semi empirical models or scaling laws which help us to predict cone behaviour and particle size and charge in certain conditions.[11–12]

Materials and Methods

Organic solvents applied in the experimental work were purchased from Polish chemical reagent supplier POCH, they were of analytical grade. Bovine serum albumin (BSA) and surfactant (Span-80, HLB = 4.3) were purchased from Sigma, poly caprolactone was a gift from Solvay, it was of about 65 000 MW. Eudragit RS-100 used as ''water in oil'' (w/o) emulsion stabiliser was a gift from Degussa Poland. It is an aminoalkylmethacrylate copolymer of pharmaceutical grade which contains about 5% of polar amino hydrochloride groups. BSA release experiments were made in the standard phosphate buffered saline solution (PBS) with an addition of 0.1% sodium azide to prevent bacteria growth. All the components of BSA solution were of analytical grade and purchased from POCH. BSA concentration in water was estimated upon light absorption in uv-vis spectrophotometer, at 280 nm wavelength. Emulsions were made by dissolving all the components in the organic phase and mixing it with a BSA water solution (9/1 vol./vol.) and placing a vessel in the ultrasonic bath (40 kHz, 60 W) for 2 minutes. In all the cases water phase was present as 10% by volume in the emulsion. Emulsion stability was estimated based on bare eye observation.

Particle Production Setup

Particle production setup has been depictured in the Figure 1.

Setup applied in drug loaded polymer particle production consists of a glass cylinder reactor with a spraying port on one side and discharge electrode on the opposite, and it has already been described in the literature,[4] it is sometimes called a Delft type reactor since it was developed in the Technical University of Delft.

Droplets produced by EHDA bare high number of electric charges and to prevent they explosion during solvent evaporation they have to be discharged, which is achieved by exposing them to counter ions. Those ions are produced by corona discharge from oppositely placed discharged electrode. Usually droplets are over discharged so they finally bare a charged

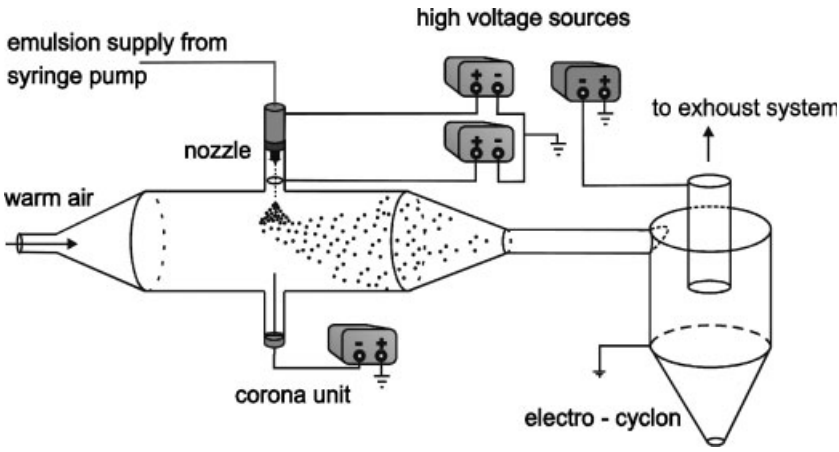

Figure 1.
Particle production setup.

opposite to the initial but corona charge process leads to much lower charge level which will not result in particle explosion. Fact that droplets are charged can be used as a collection mechanism enhancement.

In powder production stability of the spraying process is very important. Monitoring of the cone stability can be done by its observation in the back light – stable cone is perfectly black since unstable is partially translucent. Other method is a measurement of the electric current flowing through the cone by oscilloscope. In the stable cone operation the electric current is constant since in the unstable mode it shows oscillations in the range 10 Hz-500 Hz, sometimes cone vibrations are very complex.[4]

Sometimes a charged droplets deposit on the reactor walls and change electric field inside a particle production setup. This may lead to spraying process instabilities and to particle size shift. To decrease an influence of charged droplet cloud on the spraying cone a ring electrode connected to intermediate voltage is applied, but ring does note eliminate these effect completely. Because of that when we want to collect larger sample of EHDA powder constant monitoring of the process is necessary.

Additional feature of the presented system is a particle collection unit. Collection of particles in the fibrous or membrane filter is not very handy. When the membrane type filter is applied it has a very high pressure drop and gets clogged very quickly. In the case of deep bad fibrous filter it is hard to remove particles from the filter structure to collect them as powder. In the presented system cyclone aerosol precipitator with an additional electric field is applied.[13] Electric field is present between internal exhaust pipe and cyclone housing; because of that upper cover of the cyclone should be made of insulating material, in our case cyclone and all the pipes were made of aluminium and upper cover was made of PVC. Application of this additional collection mechanism is useful when particles smaller than 10–20 μm are to be collected. In the presented experiments high voltage of 5 kV was applied to the inner pipe of the cyclone. Important geometrical parameters of the cyclone were as follows: inner diameter of the cyclone 30 mm; outer diameter of the cyclone outlet pipe 15 mm; pipe walls 1.5 mm; pipe length in the cyclone chamber 25 mm; height of the cyclone 50 mm cylindrical part and 50 mm conical; externally tangent inlet pipe inner diameter was 8 mm. During the production process particles in a form of dry powder are collected in the bottom of the conical part of the cyclone which can be removed and powder can be used for future experiments.

Results

Because particles were made by atomization of BSA water solution (dispersed phase) emulsified in the organic solution of the polymer (continuous phase), emulsion stability was a crucial parameter for the process. After a series of experiment it was found that emulsions produced from polymer solution with and additive of Span-80 (0.1–1%) are not stable for more than an hour. Because particles production process usually takes 2–3 hours other solution was necessary. After a series of experiments it was found that addition of Eudragit, which is an aminoalkylmethacrylate copolymer, applied in pharmaceutical controlled release enteric coatings for tablets, results in a good w/o emulsion stability. Due to the presence of polar groups on the hydrophobic polymer chain of Eudragit, polymer adsorbs on the emulsion droplet surface and increase its stability to more than 8 hours. All the experiments were conducted with the following solutions: organic phase dichloromethane 30%, ethyl acetate 63.5%, Span-80 0.5%, Eudragit 0.5%, poly caprolactone 5%; and water phase 10% of BSA in water. Electrostatic atomization of emulsions in stable and not stable modes are shown in the Figure 2a and 2b.

In the Figure 2a we can see stable operation of the cone, liquid jet is invisible

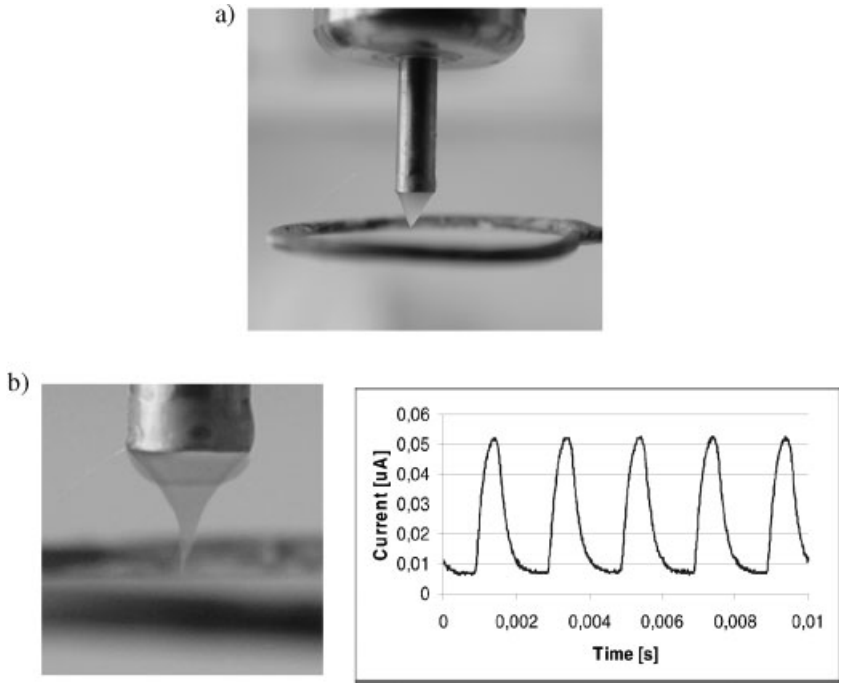

Figure 2.
(a) Electrostatic atomization of BSA water solution emulsified in the organic solution of PCL, stable operation (1 ml/h, nozzle 8.5 kV, ring 5 kV). (b) Electrostatic atomization of emulsion. Unstable operation with a corresponding electric current graph (1 ml/h, nozzle 7.2 kV, ring 5 kV).

because of the small diameter. Cone is opaque because of the emulsion particle presence; cone is illuminated from the front left side. In the Figure 2b, cone is unstable which is reflected in the electric current consumption. In this case particle production rate is very small because during reciprocal movement of the cone large droplets are spited away. Those droplet caries major flow of atomized liquid.

Stable operation of the particle production setup was observed in the area between two curves presented in the Figure 3.

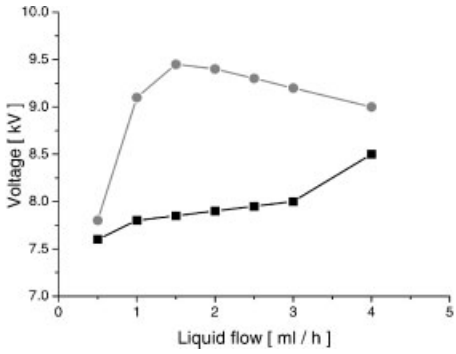

Figure 3.
Stable operation of the spraying unit, emulsion flow 1 ml/h, ring voltage 5 kV.

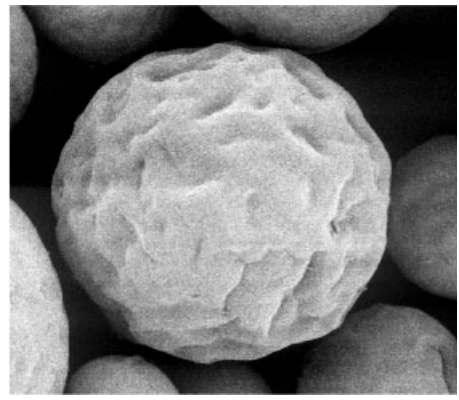

Figure 4.
Protein loaded polycaprolactone particle, 22 μm diameter.

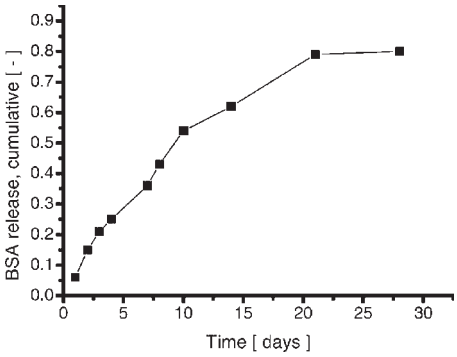

Figure 5.
BSA release rate from PCL particles.

Particles collected in the cyclone have a form of dry powder, example of such particle is shown in the Figure 4.

Surface of the particles is not smooth if compared to particles made of pure organic solution. Probably due to the evaporation of water from the vesicles inside a particles which takes place after jellification of the outer phase. BSA release rate from the presented particles to the PBS solution is presented in the Figure 5.

As can be seen in the picture BSA release rate is fairly stable and slows down after 3 weeks. Probably the rest of the BSA will be gradually released during hydrolysis of the remaining PCL. Particles of this size made of PCL usually hydrolyze completely after 6–8 weeks.

Conclusions

Electro hydro dynamic atomization when applied to w/o emulsions can be a tool for protein loaded particles production. This technique can be applied in protein drug encapsulation like vaccines, growth factors or signalling proteins. Protein encapsulated in the biodegradable polymer is slowly released to the body fluids. Continuous measurement of the electric current flowing through the spraying nozzle is a useful method to monitor stability of the EHDA process.

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