Polymer Nanospheres for Improved Drug Delivery Of Protein Therapeutics and Vaccine Antigens

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ABSTRACT

Biologically adherent polymeric microsphere carriers have been shown to be effective in the oral delivery of proteins such as insulin and DNA plasmids. These microspheres are comprised of hydrophobic copolymers that appear to have a significantly longer residence time in the gastrointestinal tract as compared to conventional microspheres. While highly promising, the hydrophobic microspheres still present challenges in terms of manufacturing and formulation of clinically acceptable products.

Typically, the microspheres are produced from an organic solvent solution, which raises concerns about deleterious effects on the therapeutic proteins and residual organic solvent in the final product. These difficulties can be averted by utilizing SuperFluids™, supercritical, critical or near-critical fluids with or without polar cosolvents such as ethanol.

Conditions were established for the formation of Super-Fluids™ polymer nanospheres. These include polymer solubility, nozzle size and type, excipients and polymer/drug ratio. SuperFluids™ polymer nanospheres made with PLGA had narrow size distributions, around 200 to 400 nanometers. These nanospheres were used to encapsulate insulin and control its release into PBS. *In vivo* studies with diabetic mice indicated that Super-Fluids™ polymer nanoencapsulated insulin caused a statistical reduction in glucose levels after oral administration.

BACKGROUND

While therapeutic materials are frequently degraded or excreted before they have a chance to reach the bloodstream via the intestine, it has been recently shown that biologically adherent polymeric microsphere carriers can overcome this limitation. Specifically, effective oral delivery of dicumarol, insulin and DNA plasmids in microspheres have been demonstrated in rats [1]. The microspheres, comprised of hydrophobic copolymers, appear to have a significantly longer residence time in the gastrointestinal tract as compared to conventional hydrophilic microspheres. Conventional hydrophobic microspheres are manufactured utilizing organic solvents, which can denature the proteins that they are designed to carry and potentially leave behind toxic organic residuals.

The deleterious effects of organic solvents on proteins and viral antigens in polymer microspheres and nanospheres can be significantly reduced or eliminated by utilizing supercritical fluids to replace the organic solvents conventionally utilized in their manufacturing.

As shown by the pressure-temperature diagram in Figure 1, a pure compound enters its supercritical fluid region at conditions that equal or exceed both its critical temperature and critical pressure. These critical parameters are intrinsic thermodynamic properties of all sufficiently stable pure component compounds. Carbon dioxide, for example, becomes supercritical at conditions that equal or exceed its critical temperature of 31.1°C and its critical pressure of 7.38 Megapascals (MPa). In the supercritical or near-critical fluid region, normally gaseous substances become dense phase fluids that have been observed to exhibit greatly enhanced thermodynamic properties of solvation, penetration, selection and expansion. At a pressure of 21 MPa and a temperature of 40°C, carbon dioxide has a density around 0.8 g/mL and behaves very much like a nonpolar organic solvent. Selectivity can be modified by utilizing small quantities of polar entrainers.

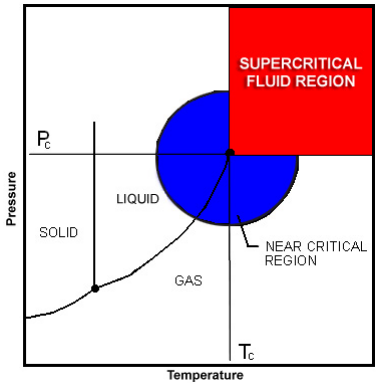

Figure 1: Supercritical Fluid Phase Diagram

A supercritical fluid solvent can simultaneously exhibit a liquid-like density and gas-like properties of viscosity and diffusivity. The latter increases mass transfer rates, significantly reducing processing times. Additionally, the ultra-low surface tension of a supercritical fluid allows facile penetration into microporous materials, increasing contact efficiency and overall process yields. Supercritical fluids, critical or near-critical solvents with/without polar cosolvents such as an alcohol are jointly referred to as SuperFluids™ [SFS].

CONVENTIONAL ORGANIC PHASE TECHNIQUES FOR MAKING POLYMER MICROSPHERES

The most commonly used bioerodable polymers are of the poly(hydroxyacid) type, in particular poly(L-lactic acid), poly(D,L-lactic acid), poly(glycolic acid) and copolymers thereof. These materials are broken down in the body to nontoxic products (lactic acid and glycolic acid) and have been approved by the FDA for use as resorbable sutures, in bone implants and as controlled release microspheres. The use of polymeric microspheres for controlled drug delivery has been the subject of a number of reviews [2].

A typical copolymer used for microsphere/microparticle formation is poly(lactide-co-glycolide), abbreviated as PLGA. Conventional drug-containing PLGA microspheres are prepared by a solvent evaporation method using a double emulsion technique that involves four major sequential steps: (1) mixing of PLGA (in methylene chloride) with drug (in water) followed by sonication to obtain a water-in-oil (W/O) emulsion; (2) mixing of W/O with a second aqueous PVA solution followed by sonication to obtain (water-in-oil)-in-water emulsion; (3) solvent evaporation causing microspheres to harden; and (4) collection of microspheres by centrifugation or filtration followed by three washing steps.

An alternative process utilizes a five step process: (1) particles of the therapeutic target are first milled into a fine powder in the 1 μm to 10 μm range; (2) a suspension of the particles is made in a solution of PLGA in methylene chloride, ethyl acetate or DMSO; (3) the organic solvent/PLGA/particle mixture is injected into liquid nitrogen to form frozen microspheres; (4) the frozen microspheres are transferred into ethanol at –100°C to back-extract the organic solvent three times; and (5) the microspheres are filtered, lyophilized and packaged. This process eliminates the need for PVA to stabilize the polymer microspheres.

Large-scale production of polymeric microspheres utilizing these processing steps consume large quantities of organic solvents, and are very time consuming, costly and inefficient. In addition, the exposure of therapeutic agent to the organic solvent can adversely affect the integrity of the final product.

The uniformity and integrity as well as processing time and the cost associated with preparation of biodegradable polymer microspheres containing therapeutic products may be greatly reduced by using supercritical fluids.

SUPERCRITICAL FLUID TECHNIQUES FOR MAKING POLYMER MICROSPHERES

Two supercritical fluid techniques have been reported for making poly(L-lactic acid) (PLA) microparticles.

In the first method, PLA is dissolved in the supercritical fluid, and particles are formed as a result of rapid expansion of the supercritical fluid [3]. This process is known as rapid expansion of supercritical solution (RESS). Tom *et al.*, [3] have also demonstrated co-precipitation of the cholesterol-reducing drug lovastatin with PLA. Therapeutics must, however, be soluble in the supercritical fluid system used; most protein therapeutics and vaccine antigens are not directly soluble in supercritical fluid solvents. Also, published data [3] indicates that particles formed by the RESS process are quite large, 10 μm to 100 μm range and are quite non-uniform.

In a second method, PLA is solubilized in the organic solvent and sprayed into the supercritical fluid continuous phase [4]. Here, supercritical fluid is used as an anti-solvent that causes particle precipitation from the liquid. This method is known as gas anti-solvent precipitation (GAS). The advantage of GAS over RESS is that the therapeutic agent does not have to be soluble in the supercritical fluid, but only in a suitable organic solvent. The solubilities of protein therapeutics and vaccine antigens are low to negligible, necessitating the use of large volumes of organic solvents. The disadvantage is that organic solvent must be utilized, although the amount of organic solvent used may be considerably less than the previously described REES process.

SUPERFLUIDS™ POLYMER NANOSPHERES

In this process, a biodegradable polymer is dissolved in SuperFluids™ and decompressed through a nozzle into an aqueous solution containing the target therapeutic; as result of decompression, polymer nanospheres are formed encapsulating the therapeutics.

Alternatively, the polymer-enriched SuperFluids™ stream can be mixed with the target therapeutic in solution or as a slurry of nanoparticles at operating pressures, and the mixture decompressed into an aqueous solution, liquid nitrogen or an empty vessel (spray dryer) [5]. In addition to reduction or elimination of organic solvent usage, use of supercritical fluids for making polymer nanospheres will impart advantages of product sterility; there are a number of reports and patents demonstrating the microbicidal and virucidal effects of supercritical fluids [6].

SUPERFLUIDS™ POLYMER NANOSPHERES EQUIPMENT

An integrated SuperFluids™ polymer solubility and micro/nanospheres apparatus is shown in Figure 2.

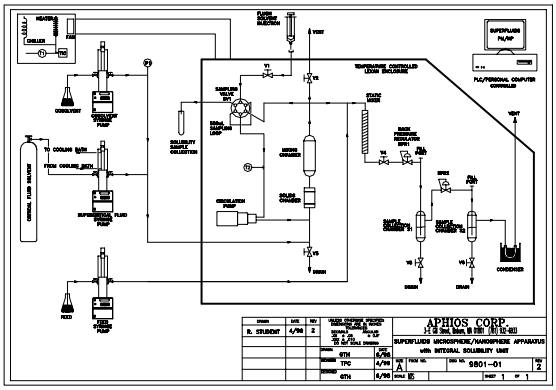

Figure 2: SuperFluids™ Nanospheres Apparatus

This apparatus consists of a mixing chamber, a solids chamber for containing the polymer, a high pressure circulation pump, a multi-port Valco sampling valve, a static in-line mixer, two back pressure regulators (BPR), two injectors and two sample collection chambers all contained in a temperature controlled chamber. External to this chamber, three syringe pumps (Isco, Inc., Lincoln, NE), are used for delivery of the supercritical fluid, cosolvent and protein solution or nanoparticles.

BIODEGRADABLE POLYMERS

Polymer nanoencapsulation experiments were conducted with various biodegradable poly(D,L-lactic glycolic acid) since the size and integrity of the polymer microspheres will depend on the polymer type and size. We investigated the use of Medisorb® biodegradable PLGA poly(D,L-lactic glycolic acid) polymers (Alkermes, Inc., Cincinnati, OH) – 5050DL2A with an inherent viscosity of 0.15 dL/g and an average MW of 12.3 KD; 5050DL2M with an inherent viscosity of 0.18 dL/g and an average MW of 17.3 KD; and 5050DL3A with an inherent viscosity range of 0.25 to 0.33 dL/g and a MW range of 20 to 28 KD. "A" indicates that the polymers contain a free carboxylic acid group on the carboxyl end of the polymer chain; "M" indicates an ester end group. We also investigated Resomer® RG 502 PLGA (Boehringer Ingelheim KG) with an inherent viscosity of 0.16-0.24 dL/g and glass transition temperature range of 40-55°C.

EXPERIMENTAL RESULTS

Conditions for the optimal polymer nanoencapsulation depend on polymer SuperFluids™ solubility, nozzle size and type, rate of bubble formation (dependent on operating pressure and rate of decompression), characteristics of the receiving media and polymer to protein ratios.

Optimum polymer solubilization in a SuperFluids™ stream depends on several parameters including the composition and molecular weight of the bioadhesive polymer, SuperFluids™ type, pressure and temperature, and for nonpolar fluids such as carbon dioxide, cosolvent type and concentration. Selected data on the solubilities of PLGA polymers in different SuperFluids™ are listed in Table 1.

Table 1: Solubilities of Resomer® RG 502 PLGA in Supercritical Fluid Solvents (20.8 MPa and 30°C)

SFS	Formula	T_C (°C)	P_C (MPa)	Dipole Moment (Debyes)	Solubility (mg/ml)
Carbon Dioxide	CO_2	31.0	7.38	0.0	0.0
Propane	C_3H_8	96.6	4.25	0.084	1.45
Freon-22	$CHClF_2$	96.0	4.88	1.4	8.98
Freon-23	CHF_3	25.9	4.73	1.6	0.20

Depending upon the type of polymer and operational conditions, narrow size distributions of nanospheres, around 200 to 400 nanometers [5] were obtained from the SuperFluids™ process with a 10-mil (internal diameter of 0.25 mm or 250 micron) capillary injection nozzle (Table 2). Polymer nanospheres were formed by injecting the polymer-rich SuperFluids™ stream into a 1% polyvinyl alcohol (PVA) solution to prevent particle agglomeration. A typical particle size distribution measured on a Coulter 4MD submicron particle size analyzer is shown in Figure 3.

Table 2: SuperFluids™ Polymer Nanospheres

Polymer	SuperFluids™	Temp (°C)	Size (nm)
DL3A	A/10 % ethanol	45	318
DL2A	A/10 % ethanol	45	292
DL2A	A/10 % acetone	45	267
DL2M	A/10 % ethanol	45	239
DL2A	B/10 % acetone	30	187
DL2A	B/3 % acetone	40	408

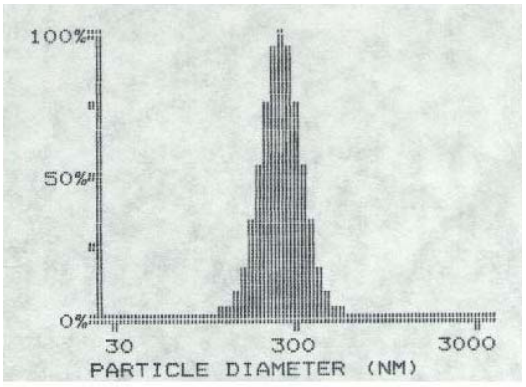

Figure 3: SuperFluids™ PLGA Nanospheres

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In vitro release characteristics of insulin from polymer nanospheres was evaluated by re-suspending dried nanospheres in phosphate buffered saline (PBS) at a pH of 7.4. Absorption of the solution was then measured at 280 nm at different time intervals. *In vitro* release characteristics of insulin from polymer nanospheres over a 5-½ hour period is shown in the Figure 4.

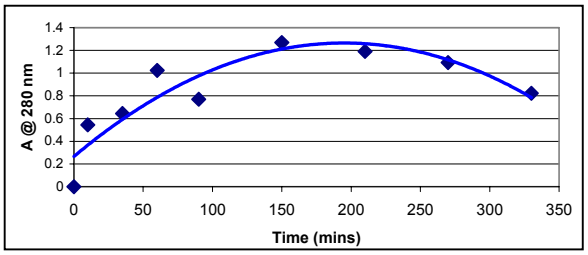

Figure 4*: In Vitro* Release of Insulin from SuperFluids™ Polymer Nanospheres

An *in vivo* study with SuperFluids™ polymer nano-encapsulated insulin was conducted in diabetic rats. Three groups of four chronic diabetic BB/Wor male rats were used to evaluate efficacy on nanoencapsulated insulin. The animals were bled prior to treatment and at 0.5, 1, 2, 4, 8, and 16 hrs after treatment. The results of this *in vivo* study showed a significant decrease in glucose levels at 0.5, 1 and 2 hours after the oral administration of SuperFluids™ polymer nanoencap-sulated insulin.

Non-encapsulated insulin given orally by gavage only showed a statistical decrease in the level of glucose at one time point, 2 hours. At all other times, oral insulin did not statistically decrease the levels of glucose in diabetic rats. The positive control, intravenously administered insulin, caused a statistical decrease in *in vivo* glucose levels at all times measured post treatment.

The integrity of viral antigens and DNA after Super-Fluids™ treatment with propane and carbon dioxide were measured; the latter by transfection efficiency using the pSV-β-galactosidase vector from Promega.

Collectively taken, DNA was not adversely affected by the treatment with near-critical propane, but was markedly affected by supercritical CO_2, most likely caused by acid hydrolysis. Sucrose helped stabilize DNA subjected to degradation-provoking conditions.

SuperFluids™ CO_2 were utilized to remove residual organic solvents from polymer microspheres with encapsulated proteins that were formed in a PEG-methylene chloride and ethyl acetate process without damaging the microencapsulated proteins or the polymer microspheres. This was achieved by processing the polymer microspheres in the absence of water that could form carbonic acid, which can denature proteins and nick DNA, and slowly decompressing the processed microspheres to prevent particle disruption [7].

CONCLUSIONS

Biodegradable polymer nanospheres for the nano-encapsulation and controlled release of therapeutic proteins and viral vaccine antigens can be manufactured utilizing specific SuperFluids™, compressed gases that exhibit enhanced thermodynamic properties of solvation, selection, penetration and expansion. The use of SuperFluids™, instead of organic solvents such as ethyl acetate and methylene chloride, reduces exposure of encapsulated therapeutic proteins and vaccine antigens to potentially denaturing organic solvents. The use of SuperFluids™ also eliminates the potential and/or need to remove any residual organic solvents in the final drug product. SuperFluids™ biodegradable polymer nanospheres can be used for the oral delivery of therapeutic proteins and the subcutaneous controlled release of vaccine antigens.

Biodegradable polymer nanospheres will protect vaccine antigens from proteolytic degradation improving *in vivo* stability, controlling release and enhancing the capability of nanoencapsulated vaccine antigens to induce the production of protective and neutralizing antibodies.

Other benefits include adjuvant stimulation of the immune system by the nanospheres, and improved shelf stability of the vaccine preparation.

SuperFluids™ biodegradable polymer nanospheres technology has the capability to control and improve the delivery of therapeutic proteins and subunit vaccine antigens as well as different types and combinations of HIV or influenza vaccine candidates including inactivated virions and DNA plasmids.

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