Preparation of Small Gelatin and Albumin Microparticles by a Carbon Dioxide Atomization Process

Levent Öner[1,2] and Michael J. Groves[1,3]

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INTRODUCTION

Depending on the size and nature of the materials employed, microparticulate drug delivery systems have been advocated as dosage forms for oral, parenteral, and other routes (1). Nanoparticles, with sizes (diameters) below 1 μm, have been suggested as ideal for intravenous administration since they readily pass through blood capillaries and, in some situations, can cross the capillary walls into surrounding tissues (2,3). Therefore, submicron particles are attractive as drug delivery systems for antitumor agents (4,5).

Micron-range particulate systems prepared from gelatin or albumin have been made by an emulsification process in which an aqueous solution of the protein is emulsified in oil, the protein denatured by chemical cross-linking agents or by heat and collected by removing the oil phase with a solvent (6–8). Environmentally undesirable solvents such as diethylether or chloroform are required to remove the oil phase of the emulsion, and the resulting microparticulates often contain residual surfactants used as emulsifiers. Industrial scale-up of these processes does not appear to have been attempted, perhaps for these reasons.

Recently, we have observed that gently stirring aqueous solutions of gelatin into cold (−15°C) anhydrous alcohol or acetone resulted in micron-range droplets that can be cross-linked with glutaraldehyde (9). The process has proved to be simple and highly reproducible in that the average diameter of the resultant microparticles was controlled by the properties of the gelatin, the concentrations and pH of the initial aqueous solution, and the conditions of cross-linking. Moreover, the stirring conditions were less critical, suggesting that scale-up might be more feasible, especially as environmentally acceptable solvents such as alcohol can be used. This simple process can be applied to other proteinaceous materials. A disadvantage of the process is that relatively large volumes of alcohol are required during the initial desolvation stage, with considerable volumes of aqueous solvents being used in subsequent cross-linking and washing stages. As a result, any drug with some water solubility incorporated in the initial mixing stage is washed out. The process has therefore been used to prepare gelatin or albumin microparticles that can be loaded with drug at a later stage.

The simple mixing process provided microparticles of gelatin in the range of 2–5 μm, although larger particles can be prepared by running the process at room temperature (9). We attempted to prepare submicron particles by spraying the aqueous solution as an aerosol with carbon dioxide into the dehydrating solvent using a Turbotak atomizer in closed systems. The use of carbon dioxide as an atomizing gas is inexpensive, is, for the most part, inert, enables the process temperature to be kept low, and serves as a flame retardant, thereby offering some built-in safety features.

MATERIALS AND METHODS

Materials

Gelatin (bovine skin, lime cured, Bloom strength of 225) and albumin (Human) were both from Sigma Chemical, St. Louis, MO. Ethanol (reagent grade denatured with 2-propanol, 5%); glutaraldehyde (50%, w/w, in water); sodium metabisulfite, mannitol, Isoton, and other reagents were all from Fisher Scientific, Itasca, IL, and used as received.

Preparation of Microparticles

Preliminary experimentation was sufficient to establish feasibility of the present atomization concept and define some of the critical experimental parameters, based on the earlier investigation (9). The single stage apparatus is outlined in Fig. 1 and consists of a 2-L glass filtration flask with a Turbotak atomizer (Turbotak Ltd., Waterloo, Ontario, Canada) with a 1-mm orifice and fitted tightly into the side arm with a sleeve cut from Masterflex (6411-15) tubing. The neck was attached to a 50-mm-diameter Acro 50 0.45-μm-pore size PTFE, Gelman Sciences, Ann Arbor, MI, filter in order to prevent exposure of laboratory personnel to respirable particulate.

The flask was immersed in a dish containing granules of solid carbon dioxide to maintain the 500-mL volume of ethanol at a temperature of −15°C. The outlet of the atomizer was maintained at a height of 175 mm above the surface of the ethanol. This dehydrating solvent was stirred using a rotating bar magnet at a speed of 200 rpm.

The gas inlet to the Turbotak was connected to a compressed carbon dioxide tank and the inlet pressure fixed at 20 psi. The liquid inlet was connected to a solution of 1% (w/v) of the gelatin in 0.01 *M* phosphate buffer (pH 4) or 1% (w/v) of albumin in deionized water maintained at 50°C through a syringe pump (Cole-Parmer Instrument Co., Chicago, IL).

Microparticles were prepared by spraying 5 mL of the gelatin or albumin solution at 0.8 mL min^{-1} into the flask. The droplets were then hardened by adding 4% glutaraldehyde for 24 hr, at −15°C for the first hour and 5°C thereafter. Cross-linking was stopped by the addition of 1500 mL of 5% (w/v) sodium metabisulfite solution at 4°C. The suspension was vortexed before concentrating by ultrafiltration using a

[1] Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, M/C 964, 840 West Taylor, Chicago, Illinois 60607.
[2] On leave from Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.
[3] To whom correspondence should be addressed.

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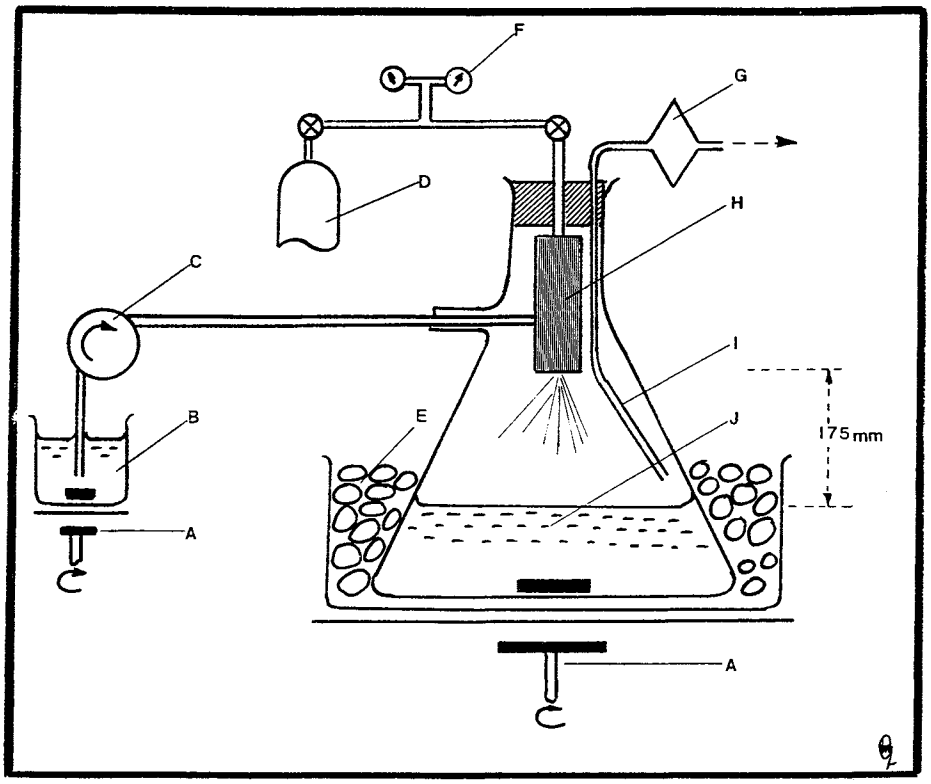

Fig. 1. Diagram of single-stage carbon dioxide activated spray system. A, Magnetic stirrer; B, hot gelatin solution; C, peristaltic pump; D, carbon dioxide tank; E, solid carbon dioxide pellets; F, gas regulator; G, 0.45-μm membrane filter venting to atmosphere; H, Turbotak; I, vent tube; J, cold stirred anhydrous ethanol.

YM100 membrane (Amicon 8200). Microparticles were washed three times in 0.01 *M* phosphate buffer (pH 7). At this point, they were suspended in deionized water containing 5% mannitol, frozen at −60°C, and lyophilized for 48 hr (Labconco Freeze Dry System). The freeze-dried product was stored at 4°C.

A two-stage device was also evaluated in which the Turbotak-generated spray was allowed to pass into an empty 2-L glass conical flask which acts as a cyclone separator (Fig. 2). The aerosol from this flask impinged directly onto the stirred cold ethanol in a second 2-L glass flask immersed in solid carbon dioxide granules similar to that shown in Fig. 1. Again, any untrapped aerosol was removed with a 0.45-μm PTFE membrane filter before venting to atmosphere.

Size Characterization of Microparticles

Microparticles were dispersed in Isoton saline and sized using a Coulter Multisizer II (upgraded to a Model II e specification) (Coulter Electronics Inc., Hialeah, FL), fitted with a 50-μm orifice tube.

Microparticles were also sized using photon correlation spectroscopy (PCS). The PCS instrument was a Malvern Zetasizer III particle electrophoresis and multiangle particle analyzer set at 90° (Malvern Instruments Ltd., UK), illuminated with a 5-mW helium–neon laser. Each microparticle sample was diluted to the appropriate concentration with deionized water. All results were calculated on the basis of a log probit plot since all materials were made by the direct mixing process and the atomization process had been found to conform to this distribution (9).

RESULTS AND DISCUSSION

The direct mixing of aqueous solutions gelatin or albumin with chilled anhydrous ethanol produces uniform particles with mean particle diameters below 10 μm (9,10). These observations have been confirmed under a different set of experimental conditions but, by using the atomization technique, the mean size can be reduced to 1 μm and, under some conditions, below 1 μm.

The initial evaluation of the direct mixing method enabled a number of experimental variables to be identified that influenced the size of the microparticles. In the case of gelatin the source, treatment and Bloom number of the starting material were influential (9), as were the concentration and temperature of the incoming aqueous solution. Since the lime-cured porcine gelatin with a Bloom of 225 had been found to be optimal previously (9), this material was retained for the present work.

While the effectiveness of the Turbotak atomizer as a source of uniform atomized droplets has been confirmed (10), the spray conditions required evaluation, as did the rate at which the incoming gelatin solution was pumped through the atomizer. These were therefore fixed at 20 psig and 0.8 mL/min, respectively. Higher pressures are likely to produce smaller atomized droplet sizes, but for safety reasons, 20

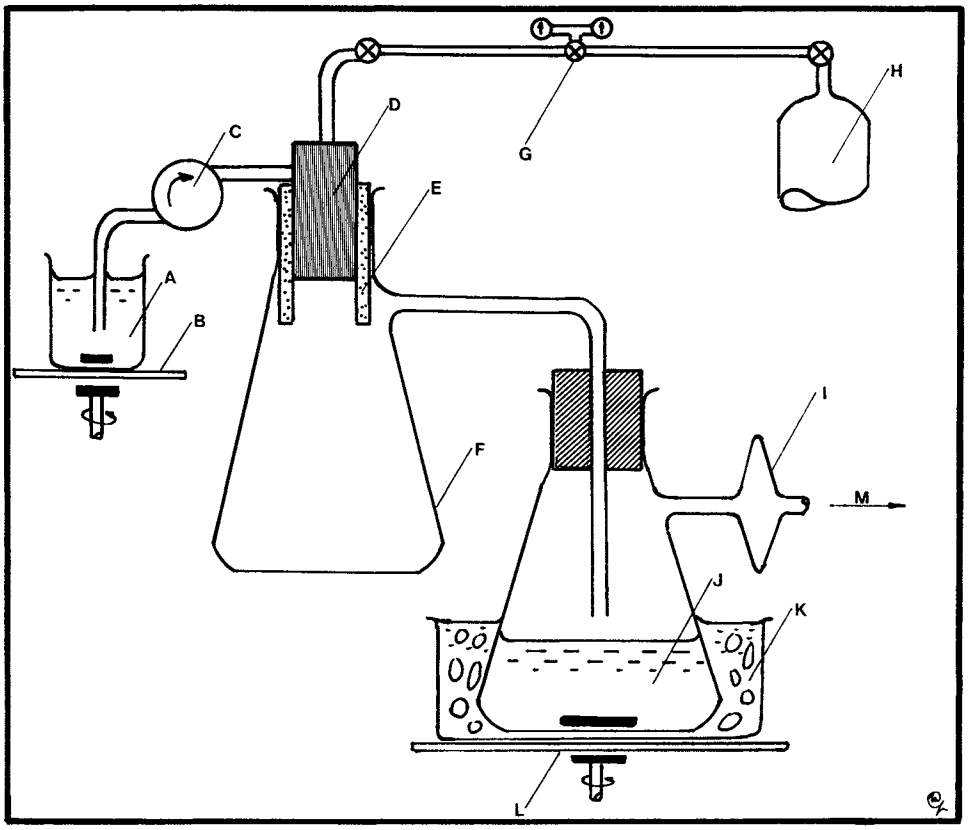

Fig. 2. Diagram of two-stage carbon dioxide-activated spray system. A, Hot protein solution; B, magnetic stirrer; C, peristaltic pump; D, Turbotak; E, sleeve cut from Masterflex tubing, 1.5 cm below base of Turbotak; F, empty 2-L glass conical flask; G, gas regulator; H, CO_2 tank; I, 0.45-μm membrane filter.

psig was considered sufficiently high for our present purpose under the constraints of the glass equipment employed.

Conditions for the albumin particles had been optimized previously (10) and albumin particles were made under the same experimental parameters selected for gelatin. However, as shown in Tables I and II, albumin particles made under otherwise similar conditions using the single-chamber device (Fig. 1) were consistently and significantly ($P < 0.05$) larger than the gelatin particles. This is true for both the direct mixing and the atomization techniques, although the latter produced smaller particles than the former method as might be anticipated. The atomization method is also less efficient in terms of percentage yield (Table III).

Comparison of the material made by the one- and two-

Table I. The Size Characteristics of Gelatin Microparticles Prepared Using Chilled Dehydration Techniques

	Direct mixing		CO_2 atomization	
Lot no.	Mean diameter (μm)	σ^a	Mean diameter (μm)	σ
1	1.15	1.39	0.85	1.76
2	1.15	1.47	0.90	1.83
3	1.20	1.54	0.85	1.94
4	1.20	1.62	0.90	1.77
5	1.20	1.41	0.85	1.82
6	1.15	1.74	0.80	1.88
Mean	1.18	1.53	0.86	1.83
CV[b]	2.3%*	8.7%	4.4%*	3.7%

[a] Geometric standard deviation (log probit).
[b] Coefficient of variation.
* Significantly different ($P < 0.05$).

Table II. The Size Characteristics of Albumin Microparticles Prepared Using Chilled Dehydration Techniques

	Direct mixing		CO_2 atomization	
Lot no.	Mean diameter (μm)	σ^a	Mean diameter (μm)	σ
1	3.00	2.27	1.70	1.71
2	3.00	1.77	1.80	1.88
3	2.80	1.94	1.90	1.78
4	2.90	2.12	1.70	1.72
5	3.00	1.64	1.85	1.64
6	3.00	1.84	1.75	1.88
Mean	2.97	1.93	1.78	1.76
CV[b]	3.4%*	12.0%	4.6%*	5.1%

[a] Geometric standard deviation (log probit).
[b] Coefficient of variation.
* Significantly different ($P < 0.05$).

Table III. The Yield of Gelatin and Albumin Microparticles Prepared Using Chilled Dehydration Techniques

	Direct mixing (%, w/w)		CO_2 atomization (%, w/w)	
Lot no.	Gelatin	Albumin	Gelatin	Albumin
1	93	90	67	73
2	83	87	72	64
3	87	82	62	78
4	93	81	76	69
5	85	91	65	63
6	91	80	70	58
Mean	89	85	69	67
CV[a]	4.8%*	5.1%**	6.7%*	10.8%**

[a] Coefficient of variation.
* Significantly different ($P < 0.05$).
** Significantly different ($P < 0.05$).

chamber devices suggested that the two-chamber device was, if anything, less effective and the yield was significantly lower (Table IV). Micron-range material is readily made by either method and it can be concluded that removal of larger droplets on the walls of the first flask, acting as a centrifugal separator, does not apparently contribute to the efficiency of dispersion process in the ethanol at the second stage and lowers the yield of product.

We propose that the formation of the droplets of protein (gelatin, albumin or any other proteinaceous vehicle) is facilitated by droplets of the aqueous solution impacting with the anhydrous dehydrating agent and most of the water from the incoming droplet being instantly removed. The resulting anhydrous matrix is not stable when added to water. The nascent droplets redissolve if added to water again or before the subsequent denaturation process has gone to completion (Olson and Öner, unpublished observations). The denaturation process with glutaraldehyde is concentration dependent and takes significantly longer than others had suggested (9,10). This may be due to the diffusion of glutaraldehyde through the precipitated protein matrix to ensure that a water-insoluble microparticle is produced.

Table IV. Particle Diameter and Yield of Gelatin Microparticles Made Using the Two-Stage Process Shown in Fig. 2.

Lot no.	Mean particle diameter (μm)	σg	Yield (%, w/w)
1	1.9	1.78	1.1
2	1.4	1.50	8.0
3	1.9	1.32	7.4
4	1.9	1.26	5.6

The need to use relatively large volumes of alcohol and water precludes the prior addition of any drug into the initial protein solution (9). Nevertheless, the prepared microparticles can be stored in mannitol, as in this present work, or on their own, for subsequent loading with drug, as discussed by Lou *et al.* (12).

We believe that the carbon dioxide atomization technique is promising for the large-scale preparation of small gelatin and albumin microparticles as drug delivery systems, with the potential of preparing submicron material under optimized conditions. The low temperatures and inert nature of the gaseous environment are attractive when handling quantities of potentially inflammable solvents on an industrial scale.

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