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Sintering Technique for the Preparation of Polymer Matrices for the Controlled Release of Macromolecules

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Abstract □ A new method for making polymeric systems for the controlled release of macromolecular drugs is described. The method consists of mixing drug and polymer (ethylene-vinyl acetate copolymer) powders below the glass transition temperature of the polymer and compressing the mixture at a temperature above the glass transition point. The macromolecule is not exposed to organic solvent during the fabrication process. Kinetic studies indicate that there is sustained release, and the bioactivity of macromolecules tested is unchanged throughout the sintering and release processes.

Keyphrases □ Copolymers—ethylene-vinyl acetate, controlled release of macromolecules □ Sustained release—macromolecules, ethylene-vinyl acetate copolymer □ Sintering technique—preparation of ethylene-vinyl acetate copolymer, controlled release of macromolecules

Previous studies (1, 2) have shown that controlled-release systems for macromolecules can be formulated by dissolution of ethylene-vinyl acetate copolymer in an organic solvent (dichloromethane), adding powdered macromolecule, casting the mixture in a mold at low temperature, and vacuum drying. However, the addition of solvent during the casting procedure may cause denaturation of certain macromolecules. In addition, the removal of the casting solvent in the drying step is time consuming and leads to shrinkage with possible shape distortion of the matrix. This report describes a 37°C sintering technique which takes advantage of the low glass transition temperature (−36.5°C) of ethylene-vinyl acetate copolymer and eliminates the need for solvent casting.

EXPERIMENTAL SECTION

Polymer Glass Transition Temperature—The glass transition temperature (T_g) of ethylene-vinyl acetate copolymer was determined with a differential scanning calorimeter[1] (3).

Matrix Preparation—Ethylene-vinyl acetate copolymer[2] was converted into a powder by one of two methods. The first method involved the dissolution of 3 g of ethylene-vinyl acetate copolymer in 20 mL of dichloromethane[3]. The solution was extruded dropwise, with a 50-mL syringe[4] fitted with a hypodermic needle[5], into a 250-mL beaker containing 100 mL of liquid nitrogen. From this point on, all instruments that came into contact with the frozen polymer solution were cooled with liquid nitrogen and, whenever possible, precooled in a freezer to minimize the quantity of liquid nitrogen needed for cooling.

The frozen droplets were ground for 5 min with a mortar and pestle. The powder was then spread evenly over three 20.3 × 20.3-cm glass sheets that were cooled to −10°C. The glass sheets were returned to a −10°C freezer for 2 h. At the end of that time, most of the solvent had evaporated, leaving a stringy powder. This powder was removed with a razor blade, bathed in a 100-mL beaker[6] with 30 mL of liquid nitrogen, and then ground to a fine powder with a mortar and pestle as described above. This powder was placed under vacuum for 2 h. The bulk powder was sieved to specific size ranges with a stack of graduated sieves[7]. Polymer powder prepared by this method is referred to here as powder I.

Powder I was produced under two aging conditions. In one set of experiments, the powder was dried under vacuum for 2 h at room temperature. This is referred to here as "fresh" powder I. In the other set of experiments, the powder was dried under vacuum for 2 h and then left in a covered petri dish for 1 week at room temperature. This is referred to here as "aged" powder I.

In the second method of powder preparation, 20 g of ethylene-vinyl acetate copolymer beads[2] were cooled in 40 mL of liquid nitrogen and placed in an electric mill[8]. The mill was set for 90-s grinding intervals. Between grindings, the polymer beads were cooled with 20-mL portions of liquid nitrogen. During the grinding process, cold nitrogen vapor was circulated around the sample chamber through the cooling ducts of the chamber. The powder collected around the outer edges of the sample chamber and could be extracted with a spatula after the second grinding and after every successive grinding. After the eighth grinding, ~4 g of frozen pellets was added to restore the sample to its original volume. This process was repeated until sufficient powder was collected to prepare the samples. The ground polymer powder was then sieved to specific size ranges with a stack of graduated sieves in an automatic sieve shaker[9] at −40°C. Polymer powder prepared by this second method is referred to here as powder II.

Studies were performed on both powders with either a specific particle size range (90–180 μm) or a mixture of sizes as described in Table I. To formulate the controlled-release system, macromolecular drug powder was sieved to a particle size range of 90–180 μm. Macromolecule and polymer powders, a total of 1.0 g, were placed in a plastic weighing boat[10], which was then

[1] Model DSC-II; Perkin-Elmer Corp., Norwalk, Conn.
[2] EVAc pellets, Elvax 40, prewashed to remove clay; DuPont Chemical Co., Wilmington, Del.
[3] Analytical reagent, Mallinckrodt, Inc.
[4] Becton, Dickenson and Co., Rutherford, N.J.
[5] 21-Gauge, 5.08 cm; Becton, Dickenson and Co.
[6] Pyrex; Corning Glass Works, Corning, N.Y.
[7] 450 90 μm; Newark Wire Cloth Co., Newark, N.J.
[8] Micromill model 550; Technilab Instruments, Pequannock, N.J.
[9] Portable sieve shaker; C. E. Tyler, Inc., Fisher Scientific Co., Philadelphia, Pa.
[10] Polyethylene, 75-mL capacity; VWR Scientific.

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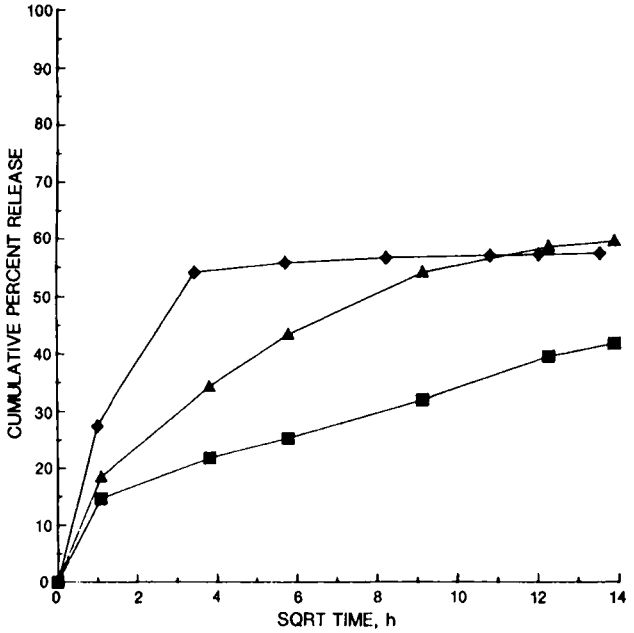

Figure 1—*Release kinetics for 25% (w/w) albumin slabs: effect of polymer powder type. Matrices were cast with a mixed particle size range (Table I) and with a formation pressure of 10.5 MPa. Key: (■) fresh powder I; (▲) aged powder I; (◆) powder II.*

transferred to a baking dish[11] containing liquid nitrogen at a depth of 1 cm. The powders were mixed in the weighing boat for 5 min with a spatula that was chilled with liquid nitrogen. After mixing, the powders were poured into a piston mold[12]. The mold was chilled in a −10°C freezer for 1 h and then chilled with 20 mL of liquid nitrogen immediately before the powder mixture was poured in. After the mixed powder was poured into the piston, the piston mold assembly was warmed to 37°C in an oven[13] for 1 h and then placed in a hydraulic press[14]. The pressure on the mold was increased over a 90-s interval from 0 Pa to the maximum pressure desired. After 30 min, the pressure was released, leaving a cohesive, heterogeneous slab.

The slab was removed from the mold with the aid of a scalpel[15] and forceps. A small amount (<2%) of the fused polymer mixture was extruded into the space between the moving piston and the jacket of the mold. The scalpel was used to trim any of the polymer mixture than may have leaked. The slab was then gently peeled from the mold with forceps.

Slabs were prepared separately at maximum pressures of 3.5, 7, and 10.5 MPa from each powder described above. Macromolecules tested for release were bovine serum albumin[16] and trypsin[17].

Kinetics—Each slab was ~1.4-mm thick. Four pieces, measuring ~7 × 7 × 1 mm each, were cut from each slab with a scalpel and tested for release as follows. Scintillation vials[18] were filled with 10 mL of physiological saline for albumin release studies and 10 mL of Tris[19] buffer for trypsin release studies. The matrix samples were attached to glass loops made from Pasteur pipets[20] that were fitted into scintillation vial caps. The samples were attached by passing a thread of 4/0 silk[21] through the sample in an orthogonal direction to its face. By tying the sample to the rod, it ensured that the sample was always bathed on all sides with fluid and provided an easy way to transfer the samples from vial to vial during the release study. Release kinetics for albumin, based on the amount of protein released, were determined by UV spectrophotometry at 220 nm on 144 samples cut from 36 25% (w/w) loaded slabs.

[11] Pyrex; Corning Glass Works.

[12] Accessory piston for Carver press (surface area, 2.54 cm²); Fred S. Carver, Inc., Menomonee, Wis.

[13] Stabiltherm constant temperature cabinet; Blue M Electric Co., Blue Island, Ill.

[14] Carver Laboratory press, model C; Fred S. Carver, Inc.

[15] Scalpel blade #22; Bard Parker, Rutherford, N.J.

[16] Catalog number T8253; Sigma Chemical Co., St. Louis, Mo.

[17] Catalog number A4503; Sigma Chemical Co.

[18] 20 mL; Fisher Scientific Co.

[19] Tris, 0.0115 M, Schwarz/Mann, Spring Valley, N.Y.; $CaCl_2$, 0.0115 M, Baker Chemical Co., Phillipsburgh, N.Y.; HCl (pH 8.0), Mallinckrodt.

[20] 13.61 cm × 7 mm, catalog number 14672-200; VWR Scientific. Loop was fashioned by heating the pipet over a bunsen burner. The shaft of pipet was fitted into the cap of a scintillation vial. A hole was drilled through the top of the cap wide enough to accomodate the shaft. The pipet was sealed into place with paraffin wax.

[21] Black braided; presoaked in saline for 1 week and dried; American Cyanamid, Pearl River, N.Y.

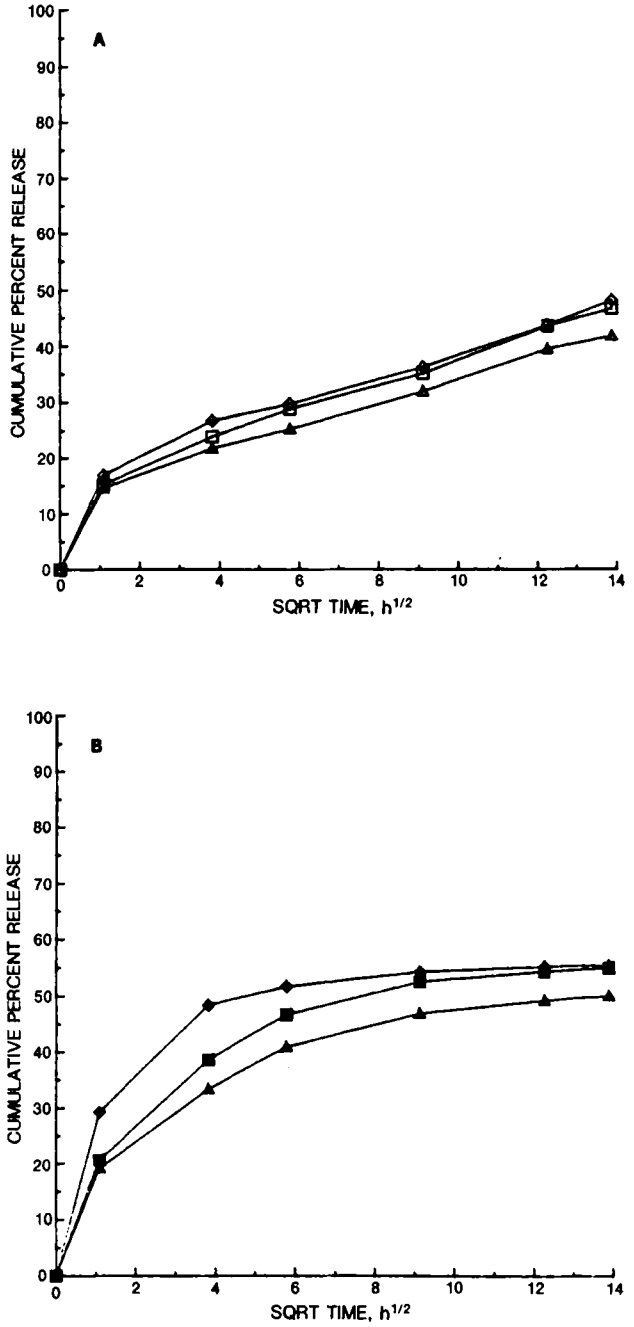

Figure 2—*Release kinetics for 25% (w/w) albumin slabs: effect of formation pressure. Key: (a) matrices cast with fresh powder I, a mixed particle size range (Table I), and with formation pressures of 3.5 (◇), 7.0 (□), and 10.5 MPa (△); (b) matrices cast with aged powder I, a fixed particle size range (90–180 μm), and with formation pressures of 3.5 (◆), 7.0 (■), and 10.5 MPa (▲).*

Trypsin release, also based on protein content, was determined by UV spectrophotometry at 220 nm on 18 samples cut from nine 18% loaded slabs.

Bioactivity—After trypsin release from the 18 samples was assayed (see above), the recovered enzyme was then diluted with Tris buffer to a concentration of 0.11 mg/mL. The enzyme turnover rate of the diluted trypsin solution on a tosyl-arginine methyl ester[22] substrate was then tested at 247 nm (4). The turnover rate of the test solution was compared with the turnover rate of a standard trypsin solution at the same concentration. The bioactivity index was taken to be the ratio of the two turnover rates.

Bioactivity was assessed at nine different times over a 310-h release period for two separate matrices cast with each powder (fresh powder I, aged powder I, and powder II) all at a pressure of 10.5 MPa. Bioactivity was also tested

[22] T4626, catalog number 1022-31; Sigma Chemical Co.

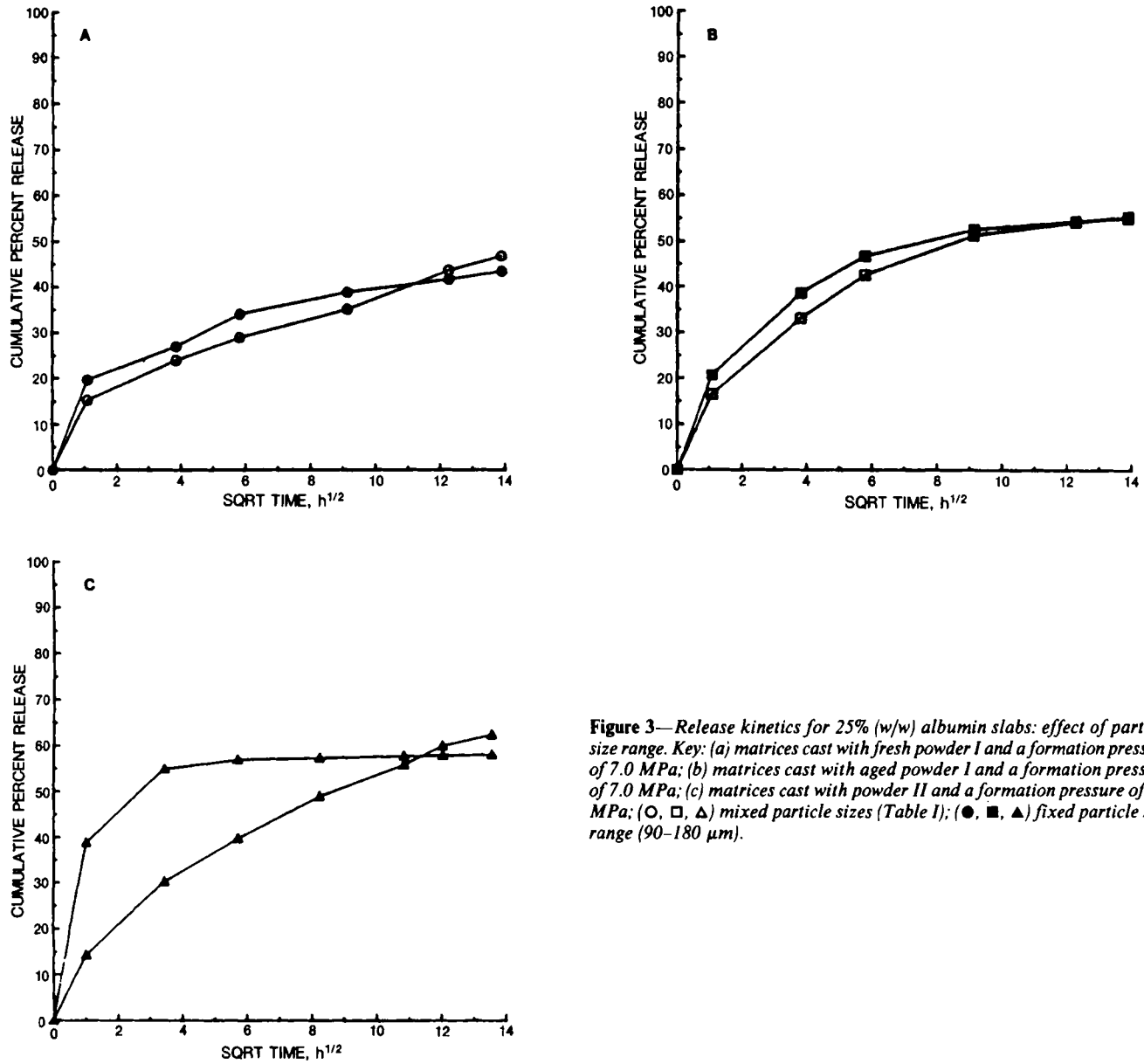

Figure 3—*Release kinetics for 25% (w/w) albumin slabs: effect of particle size range. Key: (a) matrices cast with fresh powder I and a formation pressure of 7.0 MPa; (b) matrices cast with aged powder I and a formation pressure of 7.0 MPa; (c) matrices cast with powder II and a formation pressure of 7.0 MPa; (○, □, △) mixed particle sizes (Table I); (●, ■, ▲) fixed particle size range (90–180 μm).*

for each powder on trypsin matrices cast at 3.5 and 7.0 MPa. For these matrices, bioactivity was assessed at only one time point (with release occurring between 24 and 36 h).

RESULTS

Polymer Glass Transition Temperature—The glass transition temperature was −36.5°C.

Kinetics—Figure 1 shows the release kinetics for slabs cast at 10.5 MPa with polymer powder granules with a mixed size range (Table I). Both the powder type and aging condition affect the rate of drug release. Specifically, powder II matrices release all of their releasable drug rapidly. Aged powder I matrices release drug at an intermediate rate, and fresh powder I matrices release drug at the slowest rate. Fresh powder I still releases at the same $t^{1/2}$ rate after 1 week.

In general, it was found that formation pressure has a small effect on release kinetics. When it does have an effect, it tends to be in the earliest stages of release, *i.e.*, the initial burst. Two examples of formation pressure dependence are shown in Fig. 2. The initial burst decreases with increasing pressure.

Similarly, the polymer powder particle size distribution generally does not dramatically affect release. Typical plots comparing release for the two polymer particle size distributions are shown in Fig. 3a. There is a slight tendency for matrices cast with mixed polymer particles to release more slowly and steadily than matrices cast with polymers cast with polymer particles of a single size range (90–180 μm). One case was observed in which the particle size mix had a pronounced effect (Fig. 3c).

Reproducibility between identically made slabs was within 8%. From samples cut from the same slab, reproducibility was within 6%[23].

Bioactivity—Table II shows the bioactivity index of released trypsin recovered during the time point corresponding to the release occurring between 24 and 36 h as a function of formation pressure and polymer powder type. The bioactivity index in all cases exceeded 96%. There appeared to be no difference between polymer powder types in their effect on enzyme activity. When the bioactivity index of released trypsin was measured for the amount of enzyme released from the matrix at later times, it exceeded 95% in every case.

DISCUSSION

The advantages of the sintering method used in this study, when compared with solvent casting methods used in previous studies, include (*a*) elimination of shrinkage; (*b*) elimination of the need for potentially expensive scale-up steps such as vacuum drying; (*c*) reduction of processing time [slabs were produced in 2 h[24] compared with 4 d required for solvent casting (1)]; and (*d*) lack of the necessity to expose the macromolecule to solvent.

Microscopic examination of the polymer particles of the two powder types reveals that they are similar in shape and are both white and translucent. They range in shape from a spherical form to an oblate spheroid form, in which the

[23] Reproducibility was determined by dividing the range of all points corresponding to a mean value by that mean value.

[24] These studies were performed on test slabs that were produced in 2 h. Since then, slabs have been produced in as little as 24 min.

Table I—Particle Size Distribution for Polymer Powder with a Mixture of Particle Sizes

Particle Size, μm	Weight, %
300-425	33
150-300	7
106-150	33
90-106	10
53-90	17

length is ~1.5 times the width. The only visually discernable differences between the powder types is surface topography. Powder I shows a roughened surface, with occasional folds and depressions. Powder II shows particles that are smoother with neither folds nor depressions. Some of the powder II particles have sharp, well-defined edges.

When stored at room temperature for 2 weeks and reexamined with a microscope, polymer powders produced by both of the methods described above showed the same surface morphology as the respective fresh powders. Neither fabrication procedure nor aging affected the adhesive quality of the polymer powder.

In the sintering method, the powdered drug and polymer were cooled to −200°C at which temperature they lose their adhesive properties. They were then mixed to give a homogenous blend. Warming the powders restored their elastic properties. Pressure applied at temperatures > −36.5°C caused the polymer particles to flow around the drug powder and merge together with other polymer particles. The result was a solid with drug powder locked into it.

Temperatures below T_g are essential for uniform mixing. Ethylene-vinyl acetate with 40% vinyl acetate by weight is a highly adhesive substance. Above T_g there are clumps of polymer powder that cannot be evenly mixed with the macromolecule. When fabrication was undertaken without cold mixing, some of the polymer particles clumped together. Substrate crystals adhered to these lumps in a nonuniform fashion. The mechanical strength of the slabs that were prepared without cold mixing was poor. Samples prepared in this way were fragile, pulled apart easily, and did not have evenly distributed substrates.

The polymer powder type seems to be the most important variable in determining release rates. The difference in the release kinetics for polymers cast with different powder types may be due to the fact that fresh powder I may contain residual solvent which might help fuse the boundaries between the polymer particles. Powder I granules also appear to have a folded surface. The extra surface area of the grains may allow for a more complete fusion to the solid matrix. Similarly, it is likely that aged powder I contains less solvent than fresh powder I, and this might explain why the fresh powder releases drug on a more sustained basis.

Increasing formation pressure often decreases the initial burst of release when both powder I and powder II are used. This is probably due to the requirement of high pressure to ensure fusion of polymer granules. At low formation pressure, the polymer matrix is grainy. The boundaries between polymer grains may provide extra channels (4) through which drug can diffuse.

Table II—Percent Bioactivity for Trypsin Release Averaged over Two Slabs for Each Formation Pressure and Powder Type

Pressure, MPa	Powder I, %		Powder II, %
	Fresh	Aged	
3.5	97.3	100	100
7.0	99.4	98.5	96.4
10.5	98.3	98.8	100

Using a mixture of varying polymer particle sizes can have the effect of prolonging release. This is probably due to the fact that the smaller particles fit into the interstitial spaces between the larger grains. The smaller particles might then help cement the larger polymer particles and thereby foster a more complete fusion. The small particles might also help fill spaces that would otherwise become small pores that would increase the release rate. It was noted, however, that there was only one case in which the particle size mixture had a significant effect on release rates (Fig. 3c). At present, we cannot ascertain whether the results shown in Fig. 3c are anomalous or whether there is some uncontrolled variable that caused the difference between the results shown in Fig. 3c and Fig. 3a and b.

It is well known (5) that diffusive release from slabs shows $t^{1/2}$ kinetics. Our results also follow $t^{1/2}$ kinetics. It has been shown that diffusive release with zero-order release kinetics is obtained from hemispheric drug–polymer pellets that are coated with an impermeable barrier, except for a small cavity in the center face (6). It is possible to form hemispheres by the sintering method presented here. Initial results with such sintered hemispheres have shown near-constant release of polypeptides such as insulin for >70 d (7).

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