Ethylene–Vinyl Acetate Copolymer Microspheres for Controlled Release of Macromolecules

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Abstract □ A simple technique has been devised for preparing ethylene–vinyl acetate copolymer microspheres containing macromolecular drugs. Beads with good sphericity were formed by a simple extrusion process that can easily be repeated by any laboratory without special equipment. The extruded droplets gelled on immediate contact with cold ethanol in a dry ice–ethanol bath. The ethanol also served to remove the solvent and harden the microspheres as they warmed to room temperature. Release of bovine serum albumin followed the expected trends, *i.e.*, initial linearity with the square root of time and faster release rates with greater drug loading.

Keyphrases □ Microencapsulation—ethylene–vinyl acetate copolymer microspheres, release kinetics, sustained release systems □ Ethylene–vinyl acetate copolymer microspheres—drug delivery, release kinetics

Monolithic systems composed of ethylene–vinyl acetate copolymer have been used for the controlled delivery of macromolecular drugs such as insulin (1), heparin (2), enzymes (3), DNA (2), tumor angiogenesis factor (2) and its inhibitor (4), and has provided a basis for various bioassays (5–7) and chemotactic studies (8). In all these cases, the drug and polymer solution were mixed together and cast as a film on a precooled plate to yield a matrix device in the form of a slab (~5 × 5 cm, 0.75–1.75 mm thick), which could be further divided into 1 × 1 cm squares (9). These systems have been designed to release at decreasing rates (9), constant rates (10), and increasing rates (11).

A new procedure is described here for making nearly spherical beads of ethylene–vinyl acetate copolymer for controlled release of macromolecular drugs. Such microspheres may prove to be useful as an injectable dosage form. In addition, the simplicity of the technique may facilitate its widespread application in new areas.

EXPERIMENTAL SECTION

Microsphere Preparation—Microsphere preparation is shown diagrammatically in Fig. 1. Ethylene–vinyl acetate copolymer[1] (40% vinyl acetate) was washed in water and ethanol[2] (12) and dissolved in methylene chloride[3] to give a 10% (w/v) solution. Bovine serum albumin[4] was sieved[5] to give 106–150-μm particles. The polymer solution (2 mL) was added to a weighed amount of powder in a 5-mL glass vial[6] and the mixture was vortexed[7] to give a uniform suspension. The mixture was quickly drawn into a disposable 3-mL syringe[8] *via* a 1.27-cm 16-gauge needle[9] that had been trimmed to a blunt end with a standard machine shop grinder wheel. After vortexing again for a few seconds, the suspension was carefully extruded through the same 16-gauge needle drop by drop into 20 mL of cold unstirred absolute ethanol in a 50-mL beaker immersed in a dry ice–ethanol bath (−78°C). The mixture gelled virtually immediately on contact with the cold ethanol in the near-spherical shape of the droplets, and the hard, gelled spheres sank to the bottom of the beaker. The mixture was extruded as quickly as possible drop by drop to minimize methylene chloride evaporation and plugging of the needle. It was also found that extrusion was facilitated by conducting it outside of a fume hood. Microspheres were prepared with loadings of 10–50% protein by weight.

After 5–10 min, the beaker containing the microspheres was removed from the dry ice bath and allowed to warm to room temperature. The microspheres turned white as the solvent was extracted into the ethanol. After ≥1 h, the liquid was replaced with ~10 mL of fresh ethanol and set aside overnight in a 20-mL glass vial[6]. The ethanol was decanted and the microspheres were dried for 4–5 h in a vacuum desiccator. The duration of the various process steps were not considered critical as long as solvent and ethanol removal was largely complete. For example, removal of the microspheres from the cold ethanol immediately after extrusion caused the microspheres to agglomerate since insufficient methylene chloride had been removed for the microspheres to remain gelled at room temperature.

Release Kinetics—A 30–50-mg aliquot of microspheres was weighed and placed in a vial containing 10 mL of saline (0.154 M NaCl[10]) at room temperature (23°C). The liquid was sampled periodically and the concentration of protein was determined spectrophotometrically[11] at 280 nm. Each sample was returned to the vial after measurement. Release was monitored in duplicate for a period of 6 d; release values were within ≤10% of the mean.

Bead Characterization—Dry microspheres were sliced with a razor blade, mounted on studs using silver paint, sputter coated, and examined by scanning electron microscopy[12]. Average bead diameter was estimated with the aid of a metallograph microscope[13] at low magnification (15×).

RESULTS AND DISCUSSION

The formation of polymer microspheres by precipitation of a polymer solution in contact with a nonsolvent is a standard microencapsulation technique (13). A process based on this technique and the extrusion of the polymer solution in a dropwise manner into a nonsolvent for the preparation of controlled-release formulations was patented by Sternberg *et al.* (14). This procedure did not work for our system: the extruded droplets of methylene

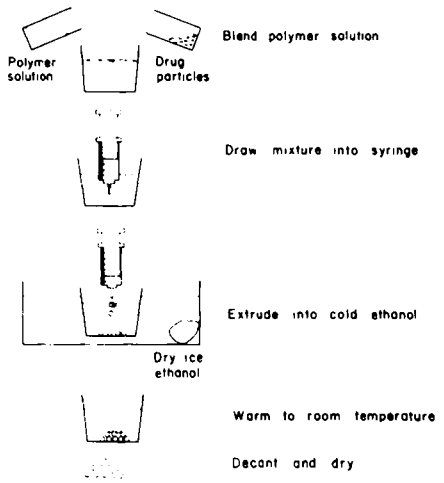

Figure 1—*Schematic illustration of microsphere preparation.*

[1] Elvax 40; DuPont Chemical Co., Wilmington, Del.
[2] U.S. Industrial Chemicals, New York, N.Y.
[3] Reagent grade; Mallinckrodt, St. Louis, Mo.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] American Standard Sieves, Nos. 40, 60, and 200; Dual Manufacturing Co., Chicago, Ill.
[6] Wheaton Scientific Co., Millville, N.J.
[7] Scientific Instruments, Springfield, Mass.
[8] Becton, Dickinson and Co., Rutherford, N.J.
[9] Perfektum, Popper & Sons, New Hyde Park, N.Y.
[10] USP grade; Mallinckrodt.
[11] Model 250; Gilford Instrument Laboratories, Oberlin, Ohio.
[12] ISI DS 130; International Scientific Instruments, Santa Clara, Calif.
[13] Reichert MEF, Austria.

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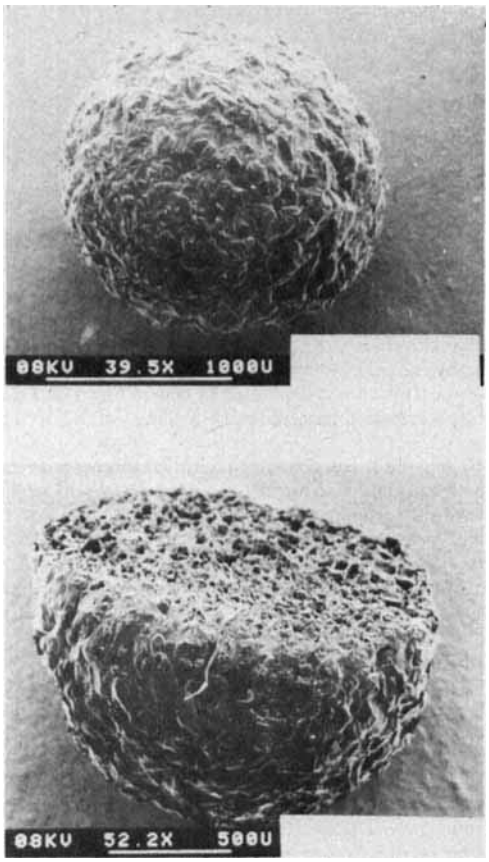

Figure 2—*Scanning electron micrographs of a whole microsphere, and a microsphere sliced with a razor blade, both containing 50% (w/w) albumin.*

chloride solution of polymer-containing dispersed protein lost their shape on contact with ethanol at room temperature; the polymer precipitated as a shapeless coagulum on the bottom of the beaker. Regularly shaped beads could be formed only by extruding into cold ethanol. Presumably, the polymer solution gelled rapidly on contact with the cold ethanol, preserving the shape of the droplets and giving time for the methylene chloride to slowly diffuse into the surrounding ethanol.

Beads prepared from 10% polymer in methylene chloride were almost perfectly spherical immediately after formation in cold ethanol. As the beads warmed and methylene chloride was removed, the polymer became softer and more easily deformed. The beads flattened slightly and became more ellipsoidal; this was accentuated for the beads with low loadings of albumin [*i.e.*, <20% (w/w) albumin]. The softness of the beads with lower albumin loadings was noted particularly when they were dry; it was difficult to handle the beads with forceps or a spatula without further changing their shape. Since these lower loading beads were also tacky (in the "dry" state), they stuck to the walls of glass or plastic containers and further increased handling difficulties. Scanning electron micrographs of beads containing 50% (w/w) bovine serum albumin are shown in Fig. 2, illustrating the sphericity of these beads.

When a 5% solution of ethylene–vinyl acetate copolymer in methylene chloride was used to fabricate the microspheres, it was less viscous than the 10% solution and, therefore, easier to use in making microspheres with high drug loading. However, this 5% solution resulted in beads with poorly defined shapes. Beads with 50% drug loading were teardrop shaped while smaller loadings (<20%) resulted in large aggregates of irregularly shaped beads. It appeared that the low ratio of polymer to solvent in these beads resulted in a slower cold ethanol solidification process than when beads were made from a 10% polymer solution. Consequently, the beads could be deformed and changed shape while the newly formed beads sank to the bottom of the cold ethanol. The beads with 50% loading were not as easily deformed so that only a small tail was produced while the beads with lower loadings were still tacky at the bottom of the cold ethanol and stuck to the previously formed beads. The most desirable shape was obtained with the 10% solution; thus, kinetic studies were conducted with the beads made from this solution. An intermediate concentration may be a better compromise between low polymer–drug mixture viscosity and good bead shape, particularly for higher drug loadings.

Table I—Estimated Microsphere Diameter

Loading, % (w/w)	Diameter, mm[a]
10	1.11
20	1.17
30	1.43
50	1.37

[a] Average of 10–20 beads; precision = ±0.07 mm.

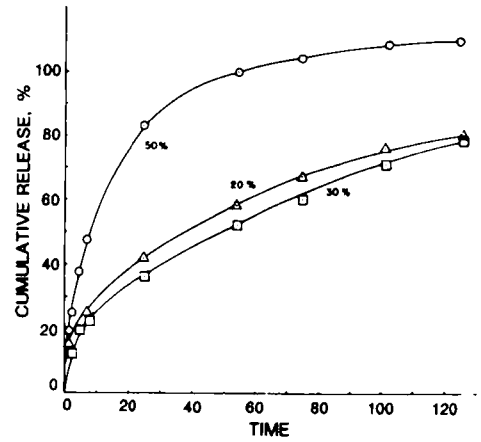

Figure 3—*Cumulative release of albumin from ethylene-vinyl acetate microspheres. Values are the mean of two measurements; release values were within ≤10% of the mean.*

The average size of microspheres made from a 10% solution are listed in Table I. As expected, the less drug in the microspheres and therefore the more polymer and solvent, the smaller (in general) the microspheres. Although the initial bead size was determined by the extruding needle diameter (16-gauge needle, 1.19 mm i.d. × 1.65 mm o.d.), the lower loading microspheres shrank more in the cold ethanol since there was more solvent to remove. The microspheres were reasonably uniform in size despite the crude nature of the extrusion process: the variation in bead size was less than the ±0.07-mm precision of the metallograph measurements. It is expected that greater microsphere uniformity could be obtained with better blending of the drug and polymer solution and by using a syringe pump to extrude the drug–polymer mixture at a constant rate.

Only to a limited extent can smaller microspheres be prepared effectively by using a smaller gauge needle, or by using a lower polymer concentration. For example, microspheres with 30% albumin were ~1.20 mm in diameter when prepared with a 20-gauge needle. However, viscosity effects and the need to grind the drug into even finer particles limit this approach, while some deterioration in bead shape may need to be tolerated if less concentrated polymer solutions are used. Smaller beads may be prepared more readily by a vibratory prilling process (15) or by blowing droplets off the end of a needle by a coaxial air stream (16). In the latter case it would be necessary to prevent the volatile methylene chloride from evaporating before the droplets leave the needle.

Typical release kinetics are shown in Fig. 3 as a plot of cumulative release against time. The release kinetics for 10% loading were not included because of the irregular shape of the beads and the difficulties in handling them.

The kinetic data is consistent with previous studies with this macromolecule-controlled release system (3). Cumulative release is generally greater at any time (*i.e.*, faster release rate) with higher drug loadings. This is presumed to result from the greater porosity of the polymer matrix at higher drug loadings (9). The seemingly faster initial release for the 20% albumin-loaded spheres compared with the 30% loaded matrices (Fig. 3) was not considered statistically significant. The release of albumin from the beads was relatively rapid with one-half of the content released in 50 h. In retrospect, such a release rate might have been predicted on the basis that a 1.0-cm thick slab of similar composition released one-half of its drug from a single face in 28 d (17). The differences in the time to release half of their content are completely attributable to the differences in the sizes and shapes of the two matrix preparations (especially the sizes).

The particle sizes of the albumin particles (106–150 μm) were ≤15% of the diameter of the microspheres themselves. It is probable that a significant portion of the albumin merely dissolved from the surface of the bead when the microspheres were placed into release media. Thus, this early burst of drug

from the microsphere can be primarily attributed to dissolution of drug from the surface with a minor contribution of matrix diffusion of albumin. To slow the release rate to levels practical for long term therapy, the beads can be coated with polymer containing no drug. A sevenfold decrease in release rate was observed after coating insulin-containing slabs with 20% ethylene-vinyl acetate solution (9).

The microsphere preparation technique can be used for other drugs and polymers other than ethylene-vinyl acetate, but it is important that the polymer-solvent solutions have glass transition temperatures > −78°C. Nonsolvents for the polymer, other than ethanol, could be used as long as they do not freeze at −78°C. In addition, the low-temperature nature of the process makes it ideally suited for thermally labile compounds that cannot be processed at the higher temperatures of conventional prilling processes [*e.g.*, 60°C for gelatin encapsulation (18)].

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Comparison of ^{99m}Tc-*N*-Pyridoxyl-5-Methyltryptophan and ^{99m}Tc-*N*-(3-Bromo-2,4,6-trimethylacetanilide)-Iminodiacetate as Hepatobiliary Radiopharmaceuticals in Rats

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Received June 13, 1983, from the **Department of Radiology, University of Colorado, Health Sciences Center, Denver, CO 80262* and the ‡*School of Pharmacy, University of Colorado, Boulder, CO 80309.* Accepted for publication March 21, 1984. §Present address: NeoRx Corporation, Seattle, WA 98119.

Abstract □ ^{99m}Tc - *N* - (3-bromo-2,4,6-trimethylacetanilide)iminodiacetate (I) and ^{99m}Tc-*N*-pyridoxyl-5-methyl-tryptophan (II) have been described as having optimal properties as hepatobiliary radiopharmaceuticals. This study compared specificity for hepatobiliary excretion, blood disappearance, rates of biliary appearance, and pharmacokinetic parameters including hepatic clearance, volumes of distribution, and mean residence times in normal and sulfobromophthalein-treated rats. The specificity of I was higher as indicated by 94% in the bile at 90 min compared to 91% for II in normal rats and a urine excretion of 0.3% for I compared with 1.9% for II. In sulfobromophthalein-treated animals, urine excretion increases were only to 0.5 and 3.0% for I and II, respectively. In control rats, blood disappearance was similar for both I and II, but II disappeared faster in treated animals. The clearance of II was 70 mL/min/kg in normal and 47 mL/min/kg in treated rats; clearance of I was 51 and 30 mL/min/kg in normal and treated rats, respectively. Volumes of distribution were larger for II. Compound I was superior in specificity while II was superior in clearance and excretion kinetics.

Keyphrases □ Hepatobiliary radiopharmaceuticals—^{99m}Tc-labeled compounds compared, sulfobromophthalein-treated and normal rats □ Radiopharmaceuticals—^{99m}Tc-labeled hepatobiliary compounds compared, sulfobromophthalein-treated and normal rats □ Sulfobromophthalein—effect on hepatobiliary radiopharmaceuticals, rats

Efforts to develop an optimal ^{99m}Tc-labeled hepatobiliary agent (I) have followed either a series beginning with ^{99m}Tc-labeled pyridoxylidene glutamate (2) or ^{99m}Tc-*N*-(2,6-dimethylacetanilide)iminodiacetate (3). These studies have resulted in improvements in hepatobiliary specificity, kinetics, and resistance to transport competition from elevated bilirubin levels. The currently "best" agents, based on animal and clinical studies, are ^{99m}Tc-*N*-(3-bromo-2,4,6-trimethylacetanilide)iminodiacetate (I) (4, 5) and ^{99m}Tc-*N*-pyridoxyl-5-methyltryptophan (II) (6).

To try to answer the question of which agent is superior, short of clinical paired comparisons in normal volunteers and patients, we have carried out detailed studies in rats. These studies were designed to compare rates of blood disappearance, rates of biliary appearance, specificity for hepatobiliary excretion, hepatic clearance of the complexes, and ability of the