

Magnetic modulation of release of macromolecules from polymers

(sustained release/drug delivery system/diabetes/hormones/controlled release)

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ABSTRACT Sustained-release systems were made by incorporating bovine serum albumin and magnetic steel beads in an ethylene-vinyl acetate copolymer matrix. When exposed to aqueous medium, the polymer matrix released the albumin slowly and continuously. Application of an oscillating magnetic field increased the release rate by as much as 100%. Intervals of 6-hr periods of magnetic exposure and nonexposure were alternated over a 5-day period, resulting in corresponding increases and decreases in release and establishing a pattern of modulated sustained release.

Modern pharmaceutical techniques of sustained release of drugs from implanted polymers began in 1964 with the demonstration that a variety of low molecular weight compounds can diffuse through silicone rubber (1). Subsequent methods of sustained release have generally been limited to low molecular weight compounds ($M_r < 1000$). In 1976, we reported a new method for the sustained release of proteins and other macromolecules (up to $M_r 2,000,000$) from implantable polymers, such as an ethylene-vinyl acetate copolymer (2). This method permits the continuous release of drugs such as insulin for periods of 30 days or more from a single small implant (3, 4).

However, a problem central to the field of sustained-release technology is that all vehicles so far developed display drug-release rates that are either constant or decay with time. There has been no way to change or modulate the release rate on demand, once release has commenced.

In this paper, we report the development of a polymeric system capable of delivering macromolecules at increased rates on demand. By embedding small magnetic steel beads in the polymer along with the drug (simulated by bovine serum albumin), release rates can be increased when desired by an oscillating external bar magnet.

MATERIALS AND METHODS

Preparation of Magnetic Sustained-Release Polymers. The procedure for preparing the magnetic sustained-release system was modified from our earlier method for preparing nonmagnetic sustained-release implants (5) (Fig. 1). The polymer casting material was a 10% (wt/vol) solution of ethylene-vinyl acetate copolymer (2, 5) in methylene chloride. Bovine serum albumin (0.5 g; Sigma) that had been sieved to contain 149- to 210- μm particles only was mixed with 10 ml of casting solution. The suspension was quickly poured onto a level glass mold (7 \times 7 \times 0.5 cm) that had been cooled by placing it on dry ice for 5 min. The mold remained on the dry ice throughout the pro-

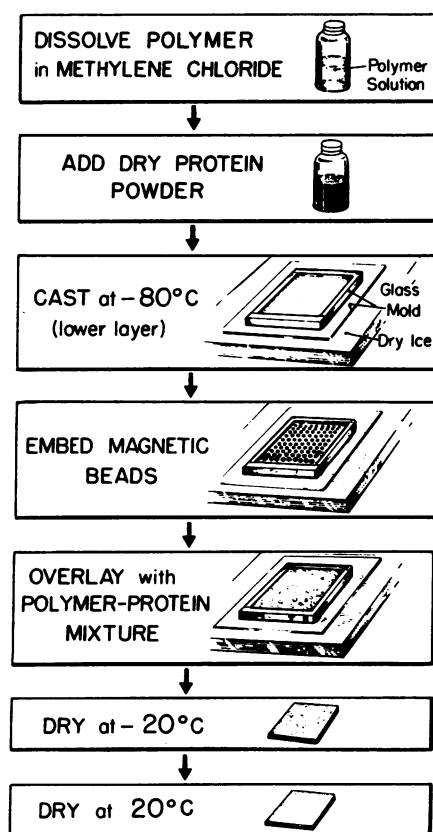


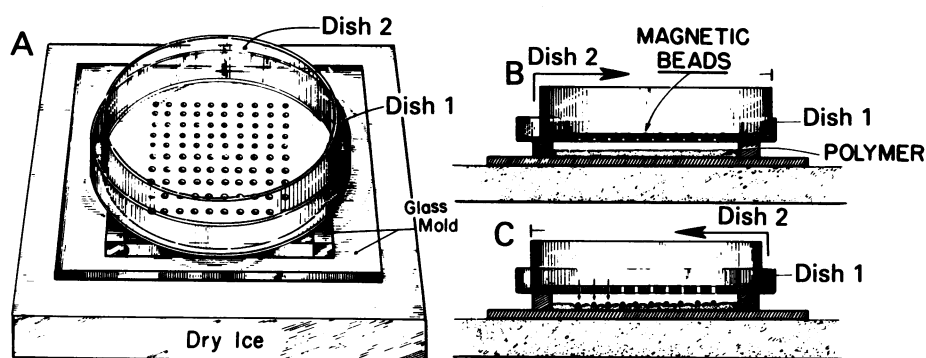
FIG. 1. Procedure for preparation of magnetic sustained-release polymers.

cedure. Immediately after pouring the polymer-albumin mixture, magnetic steel beads (79.17% iron/17% chromium/1% carbon/1% manganese/1% silicon/0.04% phosphorus/0.04% sulfur/0.75% molybdenum; 1.4 mm in diameter; from Ultrapherics, Marie, MI) were placed on the mixture by using a loading device.

The loading device is made from a Petri dish (Falcon 1001), with the bottom sitting inside the inverted lid (Fig. 2A). Both the bottom and the lid have an identical arrangement of 263 holes (1.8 mm in diameter) with 3-mm spacing. With the plates arranged so that the upper and lower holes were offset, the

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upper holes were filled with magnetic steel beads (Fig. 2B), and this arrangement was placed on the polymer slab in the mold. Then, the plates were shifted so that the holes were aligned,

and the magnetic steel beads dropped onto the polymer in a uniform array (Fig. 2C).

Two minutes after the magnetic beads were added, a top layer of polymer/albumin mixture identical to the bottom layer was cast over the beads. After the entire mixture had solidified (≈ 10 min), the slab was placed in a freezer at -20°C for 48 hr and this was followed by further drying at 20°C at 600 millitorrs ($1 \text{ torr} = 1.33 \times 10^2 \text{ Pa}$) for 24 hr.

The dried magnetic polymer slab was photographed to show its appearance (Fig. 3A) and exposed to x-rays to show the arrangement of the magnetic beads contained in it (Fig. 3B).

The use of low-temperature casting and drying prevents migration of the albumin (5). The use of a three-step procedure to embed the beads between two layers of partially fluid polymer-albumin BSA mixture provides vertical homogeneity of the beads, and the device used to place the beads between the polymer layers provides horizontal uniformity.

In preliminary experiments, various sizes of magnetic steel beads were embedded in the polymer matrix, ranging in diameter from 0.5 to 1.4 mm. The polymers containing 1.4-mm-diameter beads gave the maximum release rates of bovine serum albumin. However, we do not know whether this bead size will be optimal for other molecules.

Kinetic Studies. Sixteen 1-cm^2 squares were excised from the central portion of the dried slab. Each square contained ≈ 14 beads. Each square was placed in a glass scintillation vial (Wheaton Scientific, Millville, NJ) containing 10 ml of physiological saline (0.9%). Then, the vials containing the polymer squares were placed in a plastic tray above a triggering device (Fig. 4).

The triggering device was modified from a commercial speed-controlled rocker (Minarik Electric, Los Angeles, CA) by placing a permanent bar magnet (Crucore Magnet Bar, No. RE80108, Permagan Northeast, Billerica, MA) on one end of the

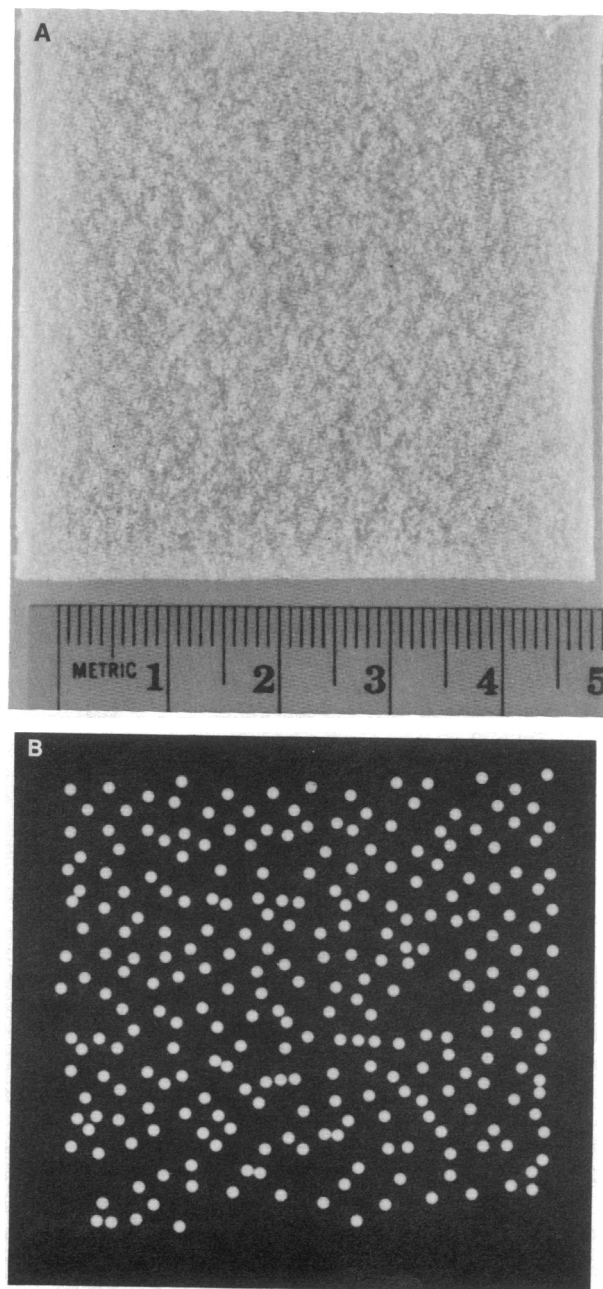


FIG. 3. (A) Photograph of the magnetic polymer. ($\times 1.4$.) (B) X-ray of the magnetic polymer. ($\times 1.4$.)

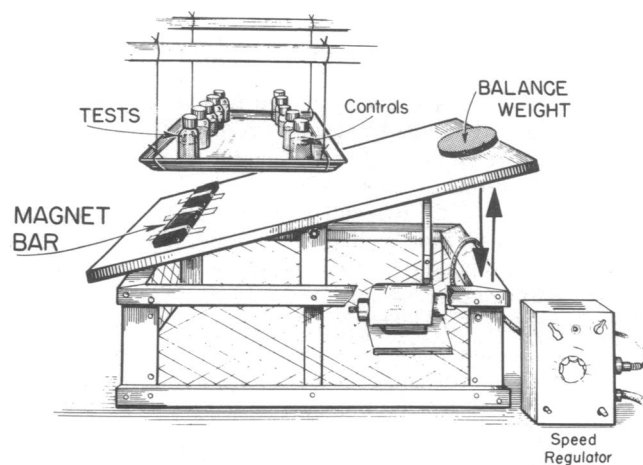


FIG. 4. Motor-driven seesaw rocker used as the triggering device.

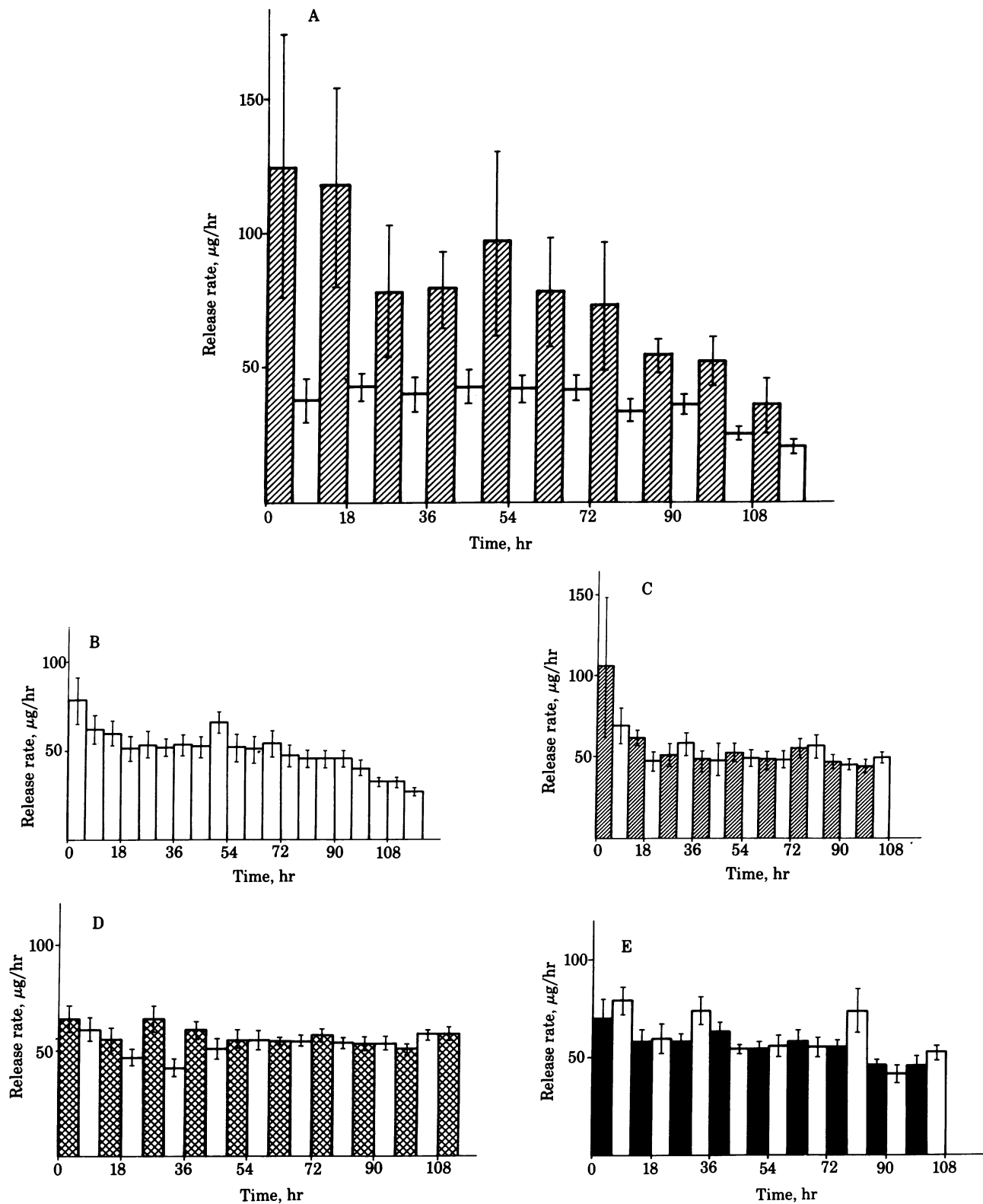


FIG. 5. Modulated sustained release of bovine serum albumin from polymeric squares. Each histogram represents the average release rate from a 1-cm² area. SDs on each histogram bar are shown. Prerelease time was 72 hr. (A) Samples were exposed to a magnetic field (see Fig. 4) for 6-hr triggering periods (▨) alternating with 6-hr nontriggering periods (□) for 5 days. (B) Control experiment in which polymeric squares were not exposed to a magnetic field. (C) Control experiment in which polymeric squares were made without magnetic steel beads and exposed to the magnetic field for 6-hr periods (▨) alternating with 6-hr nonexposure periods (□). (D) Control experiment for any minor movement of the polymers. Polymeric squares made with magnetic beads were not exposed to the magnetic field but underwent alternate periods of shaking (▩) and resting (□). These squares were subjected to movement greater than that in the experiment in A (in which the movement was almost undetectable). (E) Control experiment for the pattern of magnetic induction. Polymeric squares made with magnetic beads were exposed to a magnetic field in which the magnet bar was maintained stationary (■) at its top position (see Fig. 4) throughout the entire exposure interval alternating with an equivalent nonexposure period (□).

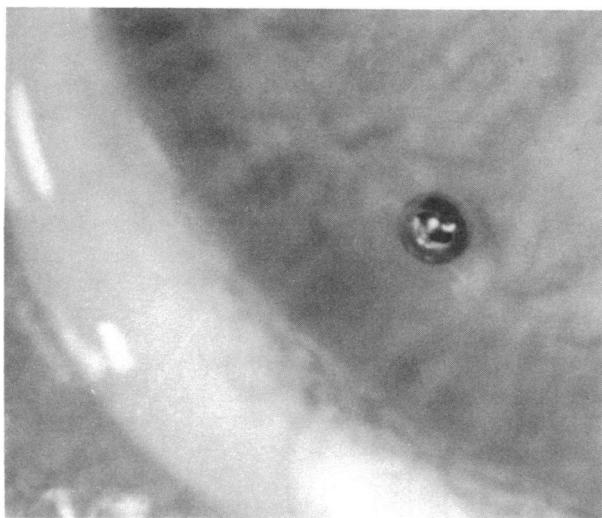


FIG. 6. Rabbit cornea implanted with polymer containing a magnetic steel bead. Note the clarity and avascularity, which indicate the absence of inflammation.

rocker and a balanced weight on the other end. The frequency of the motion was 18 cycles per min. Thus, an oscillating magnetic field ranging from 0.5 gauss (the magnetic field strength on the surface of the earth) to ≈ 1000 gauss on the surface of the magnetic bar was created for triggering.

Vials ($n = 8$) containing the polymeric squares were exposed to the oscillating magnetic field for 6-hr periods that alternated with 6-hr of nonexposure. Cyclic shifting between triggering and nontriggering was conducted for 5 days.

Four different control experiments were conducted. (i) Polymeric squares excised from the same slab as those used in the triggering experiment were subjected to the same manipulations except that the bar magnet was absent. (ii) Polymeric squares made without magnetic beads were exposed to the oscillating magnetic field. (iii) Polymeric squares containing bovine serum albumin and steel beads were exposed to intervals of shaking in the absence of the bar magnet. The shaking was done by placing the squares on a Thomas Clinical Rotator at speed 3. (iv) Polymeric squares containing bovine serum albumin and steel beads were exposed to a stationary magnetic field in which the bar magnet was maintained in apposition to the squares throughout alternating 6-hr periods.

Biocompatibility Tests. Biocompatibility was tested by implanting polymeric spheres ($1.5 \times 1.5 \times 1.5 \text{ mm}^3$) containing steel beads into rabbit corneas. We have previously shown that the cornea provides a sensitive test to judge the tissue biocompatibility of implantable polymers (6). Four polymeric spheres containing magnetic steel beads were implanted into four rabbit corneas as described (6). The corneas were observed for 2 weeks with a stereomicroscope.

RESULTS

Kinetic Studies. When exposed to the oscillating magnetic field, the polymeric squares released up to 100% more bovine serum albumin than when the magnetic field was discontinued (Fig. 5A). For example, the first 6-hr-exposure period showed an average release rate of $125 \mu\text{g/hr}$ compared with $40 \mu\text{g/hr}$ in the following 6-hr (no-exposure) period. The differential decreased with increasing time but was still significant at the end

of the experiment ($37 \mu\text{g/hr}$ versus $25 \mu\text{g/hr}$).

The validity of these findings was strengthened by the four control experiments. (i) To control for possible nonspecific effects of the magnetic steel beads, polymeric squares excised from the same slab as those used in the experiment described above were subjected to the same manipulations except that the bar magnet was absent. The average release rates (Fig. 5B) did not show the fluctuating pattern. (ii) To control for possible nonspecific effects of the magnetic field (i.e., heating), polymeric squares were made without magnetic steel beads. The fluctuating magnetic field had no effect on the release rate (Fig. 5C). (iii) To control for any minor motion of the whole polymeric square due to the effect of the magnetic field on the steel beads within the polymeric matrix, polymeric squares containing bovine serum albumin and steel beads were exposed to intervals of shaking in the absence of the bar magnet. These squares released albumin at nearly the same rate as under stationary conditions (Fig. 5D). (iv) To determine whether the *fluctuating* magnetic field was an essential factor, the bar magnet was maintained in apposition to the polymeric squares throughout alternating 6-hr periods. These squares also displayed baseline release rates rather than the fluctuating pattern displayed by the squares exposed to a moving magnet (Fig. 5E).

Biocompatibility. The magnetic sustained-release system did not cause irritation to the rabbit corneas, all of which remained completely clear (Fig. 6). Absence of inflammation was confirmed by the lack of edema, cellular infiltrate, or neovascularization and by subsequent histologic examination.

DISCUSSION

We have shown that release rates of a drug (simulated by bovine serum albumin) from a polymeric system can be increased on demand by a magnetic field. Although the first experiment was conducted for a 5-day period (Fig. 5A), only 20% of the total drug was released. Further experiments have shown that polymeric systems that have embedded magnetic beads are capable of releasing bovine serum albumin for periods of 3 weeks or more. Although the release rate was not constant with time in the resting state, we have recently shown that release kinetic patterns are a function of the geometry of the implant; methods for achieving constant release for nonmagnetic polymeric systems have been described (7).

We have previously shown that embedded proteins form channels in a polymeric matrix. These channels are as large as $200 \mu\text{m}$ in diameter and therefore permit the transport of macromolecules, such as bovine serum albumin, from inside the matrix to its surface (8). One might speculate that the beads inside the polymeric matrix cause alternating compression and expansion of the channels, thus facilitating this transport. Further kinetic studies examining the application of different magnetic field strengths and magnetic bead sizes should lead to a better understanding of the mechanism of magnetic modulation. In addition, the possible application of this modulated sustained-release system,—e.g., to the release pattern of insulin for the treatment of diabetes—needs to be further investigated.

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