Calcium Alginate Beads as Core Carriers of 5-Aminosalicylic Acid

Shun Y. Lin[1] and James W. Ayres[1,2]

Received January 23, 1992; accepted February 17, 1992

The utilization of calcium alginate beads as core carriers for delayed dissolution followed by burst release as a potential method of intestinal site specific drug delivery was investigated. 5-Aminosalicylic acid was spray-coated on dried calcium alginate beads and then coated with different percentages of enteric coating polymer and/or sustained-release polymer. Beads coated with more than 6% (w/w) methacrylic copolymer plastisized with dibutyl sebacate and triethyl citrate resisted release in 2-hr acid fluid challenge and allowed immediate dissolution upon transfer to simulated intestinal fluid. With 6% (w/w) methacrylic copolymer on top of 4% (w/w) ethylcellulose polymer, the major portion of drug did not release in 2 hr of acid treatment or the next 3 hr of simulated intestinal fluid treatment. This dosage form provides the possibility to deliver drug to the lower intestinal tract with minimal early release, followed by sustained release in the colon.

KEY WORDS: calcium alginate beads; spray coating; 5-aminosalicylic acid; acid pretreatment.

INTRODUCTION

Investigation of sulfasalazine's metabolism and distribution of its metabolites suggested that 5-aminosalicylic acid (5-ASA) is the therapeutically active portion of the drug (1). In cases of distal ulcerative colitis and proctitis, 5-ASA has been effective in the form of an enema or suppository (2–5). However, suppository and enema forms of 5-ASA are inconvenient for some patients and not likely to benefit most patients with Crohn's disease involving the ileum and ascending colon. It may also be difficult for patients to use enemas or suppositories as maintenance medication. These factors led to the development of oral forms of 5-ASA. However, when 5-ASA is given orally, most is absorbed from the proximal small bowel and inactivated by hepatic metabolism (6).

The use of controlled-release systems, such as osmotic pump devices (like hydrogel systems) and coated multiparticle preparations, may facilitate drug delivery to the colon (7). Such systems could be designed to protect the active compound from the acidic environment of the stomach, reduce the dose amount, minimize systemic absorption from the small intestine, and modulate drug release from the preparation (8). For example, a preparation which contains 5-ASA in microgranules, coated with a semipermeable membrane of ethylcellulose, was designed to release drug along the GI tract independently of bacterial flora, pH of the GI tract, and intestinal transit time (9,10). Because about 65% of administered drug was excreted in a ileostomy bag, this formulation is most suitable for treatment of disease in the jejunum and ileum rather than for drug delivery to the colon (9).

No currently available oral dosage form of 5-ASA effectively avoids major drug release in either the stomach or the upper intestine and simultaneously provides sustained release in the colon. Objectives of this study were to create a new dosage form and investigate dissolution characteristics of drug from calcium alginate beads used as a drug delivery system for 5-ASA acid, especially when such beads are coated with ethylcellulose (Aquacoat) and/or methacrylic resin (Eudragit L30D). Knowledge of such characteristics may lead to the development of a colonic specific drug delivery system.

MATERIALS AND METHODS

For trapping drug inside alginate beads, 16% (w/v) 5-ASA was mixed with 3% sodium alginate solution (low viscosity; Sigma, St. Louis, MO) and extruded into 0.5 *M* calcium chloride solution (Sigma). For "blank" alginate beads, 3% sodium alginate dissolved in deionized water was pumped through a manifold to produce drops, which were allowed to fall into 0.5 *M* calcium chloride solution. The manifold contained 24 hypodermic needle outlets and an air compressor to supply air pressure up to 20 psi. Calcium alginate beads were oven-dried at 55°C for 2 days before use. For loading drug on top of dried blank calcium alginate beads, 16% (w/v) 5-ASA was suspended in 95% ethanol solution which contained 1.6% (w/v) hydroxypropylcellulose (Klucel, Aqualon, Wilmington, DE) and 1.6% (w/v) polyvinylpyrrolidone (PVP-K30, Aldrich, Milwaukee, WI) as binders. The 5-ASA suspension was spray-coated on the dried blank calcium alginate beads at 30°C in the dark. These 5-ASA-loaded alginate beads were sieved and only beads with diameters of 1.5 to 2 mm were used for further coating with Aquacoat (FMC Corp., Philadelphia, PA) and/or Eudragit L30D (Röhm Pharma, GMBH, Weiterstadt). Both coating polymers were plasticized with 15% (w/w) dibutyl sebacate (Sigma) and triethyl citrate (Aldrich). Formulations of polymer coatings on these 5-ASA-loaded alginate beads are shown in Table I. A STREA-1 spray coater with wurster column (Aeromatic Inc., Columbia, MD) was used for coating, attached to a modified high-speed fluid bed dryer (Labline Instruments, Inc., Melrose Park, IL) with a peristaltic pump (Minipuls 2, Gilson, Middleton, WI) to supply coating solution into the coating chamber. Coating was applied in the dark under the following conditions: fluid inlet, 4 ml/min; atomizing air, 10–15 psi; temperature, 30°C; blower, 50% of maximum; and nozzle size, 1.2 mm for drug coating and 0.8 mm for polymer coating. The low coating temperature was used as 5-ASA is reported to oxidize in the presence of water and light (12). Coated beads were postdried in the chamber for 30 min at 30°C for both drug-loaded and polymer(s)-coated beads. These polymer(s)-coated beads were kept in plastic screw-lock containers and stored in a drawer at room temperature.

Coated 5-ASA-loaded alginate beads were allowed to dissolve in a USP dissolution apparatus using the paddle method at 50 rpm and 37°C. Each formulation was pretreated

[1] College of Pharmacy, Oregon State University, Corvallis, Oregon 97331.

[2] To whom correspondence should be addressed.

0724-8741/92/0900-1128$06.50/0

Table I. Formulation of Coated 5-ASA "Layered" Alginate Beads

Formula	Code[a]	Aquacoat (%, w/w)	Eudragit (%, w/w)
1	EU2	0	2
2	EU4	0	4
3	EU6	0	6
4	EU8	0	8
5	AQ4	4	0
6	AQ4 EU2	4	2
7	AQ4 EU4	4	4
8	AQ4 EU6	4	6
9	EU6 AQ2	2	6
10	EU6 AQ4	4	6
11	EU6 AQ6	6	6

[a] The order of polymer coated on 5-ASA/alginate beads is indicated. For example, AQ4 EU2 means that the inner coat is 4% Aquacoat and the outer coat is 2% Eudragit L30D.

with simulated gastric fluid without pepsin (pH 1.4 ± 0.1) for 2 hr before challenging with simulated intestinal fluid without pancreatin (pH 7.4 ± 0.1) and assayed at 225 nm using a Beckman UV 34 spectrophotometer (Beckman, Irvine, CA). Different concentrations of 5-ASA dissolved in simulated intestinal fluid were prepared for studying the effect of solution aging. Solutions were kept in 50-ml volumetric flasks and stored on the shelf for up to 9 days at room temperature. Some 5-ASA-loaded alginate beads were treated with simulated gastric fluid for different times (0.5 to 2 hr) to study the effect of acid pretreatment time on release of drug after transfer into simulated intestinal fluid.

RESULTS AND DISCUSSION

5-ASA has a solubility of about 1 mg/ml in water, with pK_a values of 3, 6, and 13.9 (12). Classically, 5-ASA is assayed spectrofluorimetrically (13). It was desirable to use a rapid assay of 5-ASA for this *in vitro* study. Figure 1 shows the standard curve and reproducibility of 5-ASA in phosphate buffer solution (simulated intestinal fluid). Using a UV spectrophotometer to detect 5-ASA concentrations in phosphate buffer solution (pH 7.4) at a wavelength of 225 nm gave a reliable and consistent reading for up to 9 days at room temperature. Because 5-ASA is sensitive to light in the presence of water, it is not clear at this time whether there exists a light-degraded form of 5-ASA in the standard solution which also shares the same intensity of absorbance at 225 nm. However, the UV absorbance assay selected remains a useful indicator of relative dissolution rate from the formulations investigated.

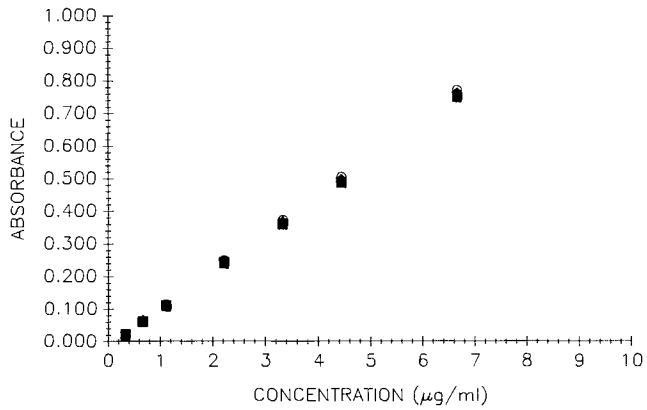

Fig. 1. Age of 5-aminosalicylic acid standard solution in simulated intestinal fluid (at wavelength 225 nm). (○) Day 1; (●) day 2; (△) day 3; (▲) day 4; (□) day 7; (■) day 8; (▽) day 9.

Calcium alginate beads have been widely used to entrap drugs (13,14) and cells (15) or enzymes (16). However, 5-ASA quite unexpectedly did not form a spherical drug alginate matrix after drying when mixed with sodium alginate solution and extruded into calcium chloride solution. Wet drug alginate gel showed a round surface, but after drying in the oven the dried drug alginate particle had an irregular shape with a wrinkled surface. A similar "unusual" behavior has been observed in other experiments in this laboratory with theophylline and aminophylline. These drug-alginate particles have more surface area and sharp ridges compared to spherical alginate beads which makes it very difficult to produce a smooth surface and desirable release rate by spray coating.

An alternative and classical way to produce 5-ASA "beads" is to "layer" the drug on top of nonpareil sugar beads. However, this approach would provide immediate drug release without coatings and only traditional release patterns with coating. A nontraditional release pattern was desired wherein drug release would be delayed for about 3 hr postdelivery into the small intestine, followed by a "burst" or sustained release, which is anticipated to deliver the major portion of the dose into the colon. Research in this laboratory has shown that dried alginate beads (unlike sugar beads) swell extensively when rehydrated and this swelling can be used to help produce a burst of drug release. Thus, 5-ASA was loaded directly on dried "blank" calcium alginate beads since trapping drug inside the beads was not successful. These 5-ASA loaded on alginate beads were spherical with a smooth surface. Drug was readily released from uncoated "layered" alginate bead surfaces during 2 hr of gastric fluid exposure.

After coating 5-ASA-loaded alginate beads with different percentages of Eudragit L30D, only beads coated with 6% (w/w) or more Eudragit L30D provided protection of 5-ASA/alginate beads against 2 hr of acid pretreatment (Fig. 2). Of course, enteric coating alone does not provide a sustained or delayed release after transfer to intestinal fluid. Transit time through the small intestine (after leaving the stomach) is about 3 to 5 hr for an oral dosage form (with diameters of 2 to 5 mm) to reach the colon (17). Thus, controlled release of 5-ASA following initial delivery to the small intestine would be more suitable than just an enteric coat. By coating another layer of polymer (Aquacoat) on the surface of 6% (w/w) Eudragit-coated beads, dissolution patterns in intestinal fluid were changed dramatically (Fig. 3). As expected, increasing the thickness of Aquacoat coating decreased the release rate of 5-ASA from coated beads. Reversing the coating order but maintaining the same percentages of polymer loading revealed a sustaining effect but a different pattern of dissolution for 5-ASA-loaded alginate beads coated with 6% (w/w) Eudragit L30D and 4% Aquacoat (Figs. 3 and 4). Aquacoat coating, 4% (w/w), did not prevent release of 5-ASA in acid solution (Fig. 4). This was

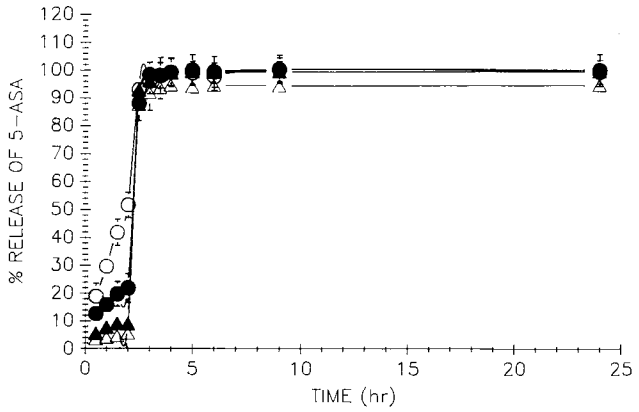

Fig. 2. Effect of Eudragit L30D coating on percentage release in simulated intestinal fluid of 5-aminosalicylic acid loaded on alginate beads. (○) 2% (w/w) coat; (●) 4% (w/w) coat; (△) 6% (w/w) coat; (▲) 8% (w/w) coat.

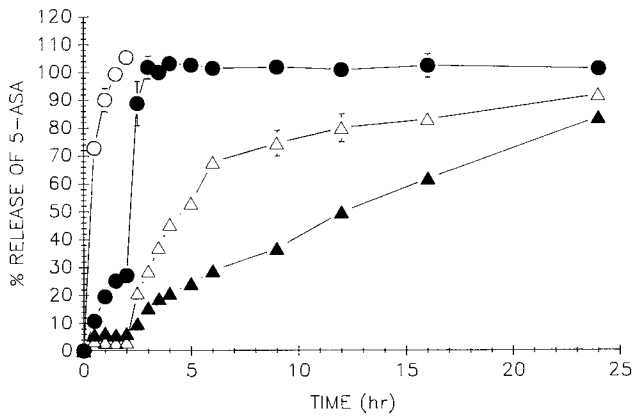

Fig. 4. Dissolution of 5-aminosalicylic acid loaded on alginate beads coated with 4% Aquacoat and variable percentages of Eudragit L30D. (○) None; (●) 2% (w/w) coat; (△) 4% (w/w) coat; (▲) 6% (w/w) coat.

caused by the small degree of swelling of dried calcium alginate beads in the acid fluid, which could rupture the Aquacoat film. After applying 4% or more Eudragit L30D on these 4% Aquacoat-coated beads, protection against 2 hr of acid treatment was established (Fig. 4). A desirable controlled release of 5-ASA was obtained with 4% (w/w) Aquacoat overlayed with 4 or 6% (w/w) Eudragit L30D (formulae 7 and 8 in Table I; also see Fig. 4). The data suggest that the majority of the dose will be released in the colon, assuming that the *in vitro* dissolution data are predictive of *in vivo* effects.

The influence of acid pretreatment time on dissolution of coated 5-ASA (loaded on alginate beads) is shown in Figs. 5 and 6. Formula 8 (with 4% Aquacoat in the inner layer and 6% Eudragit L30D in the outer layer) provides sufficient protection against 2-hr acid treatment and a more sustained release pattern compared to the others. With longer times of acid fluid pretreatment, 5-ASA tended to release only a little more rapidly after switching to simulated intestinal fluid, suggesting a general lack of sensitivity to release as a result of stomach transit time. Dissolution of two 5-ASA products, formulae 10 and 11 (see Table I), was studied following storage for 2 weeks. These products were kept in screw-lock plastic containers at room temperature without desiccant and stored in a drawer. Dissolution patterns were essentially reproducible (Fig. 7).

The pH-independent polymer (Aquacoat) remains insoluble in both acidic and intestinal fluid, and the pH-sensitive copolymer (Eudragit L30D) will dissolve when the solution pH is higher than 5. With more than 6% Eudragit L30D as an outer layer, dissolution of 5-ASA is prevented in gastric fluid and the rate of release in intestinal fluid is controlled by the inner Aquacoat film and alginate beads since the Eudragit L30D dissolves and is washed away in intestinal fluid. With Aquacoat as the outer layer, the release pattern of 5-ASA in intestinal fluid is governed by diffusion through dissolved Eudragit trapped inside the Aquacoat, if this outer membrane can remain on the surface under the stress of an osmotic gradient and expansion during hydration of the inner volume. After entering the intestinal tract, the rate of enteric-coated film dissolution is influenced by both the amount of sustained-release coat film and the order of film

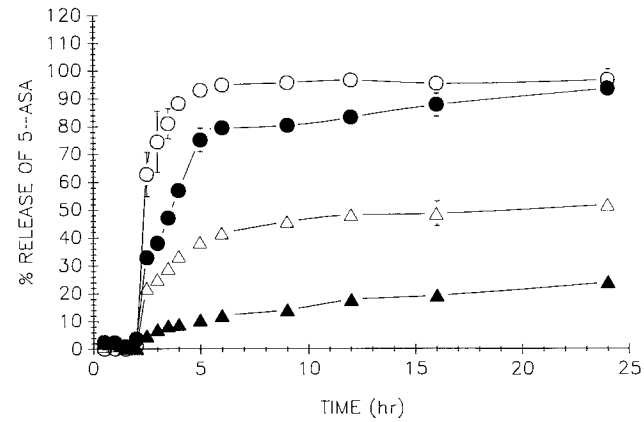

Fig. 3. Effect of Aquacoat coating on percentage release in simulated intestinal fluid of 6% (w/w) Eudragit L30D-coated 5-aminosalicylic acid loaded on alginate beads. (○) None; (●) 2% (w/w) coat; (△) 4% (w/w) coat; (▲) 6% (w/w) coat.

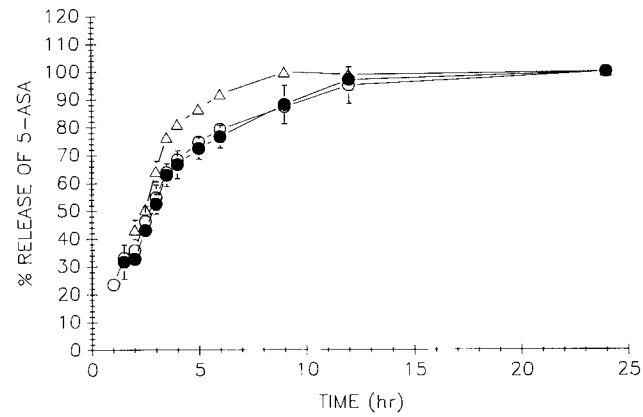

Fig. 5. Influence of acid pretreatment time (GF) on dissolution of 5-aminosalicylic acid loaded on alginate beads coated with 2% (w/w) Aquacoat on top of 6% (w/w) Eudragit L30D film. (○) 0.5 hr; (●) 1 hr; (△) 1.5 hr.

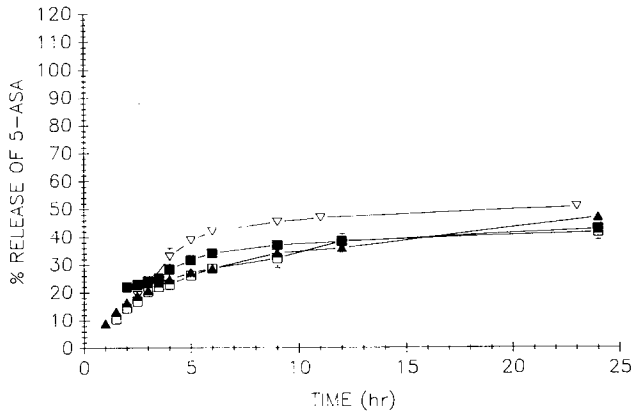

Fig. 6. Influence of acid pretreatment time (GF) on dissolution of 5-aminosalicylic acid loaded on alginate beads coated with 4% (w/w) Aquacoat on top of 6% (w/w) Eudragit L30D. (▲) 0.5 hr; (□) 1 hr; (■) 1.5 hr; (▽) 2.0 hr.

application. Calcium alginate beads swell to several times their dry size when hydrated. When drug-loaded calcium alginate beads swell sufficiently to exceed the strength of the outer coat sustained-release film, the sustaining film bursts and release of drug is facilitated. Sugar beads as cores do not swell sufficiently to produce the desired burst effect. Thus, this formulation can provide an oral dosage form designed for targeted delivery of drug to any desired site in the gastrointestinal tract, depending on the coating thicknesses and order of coat application.

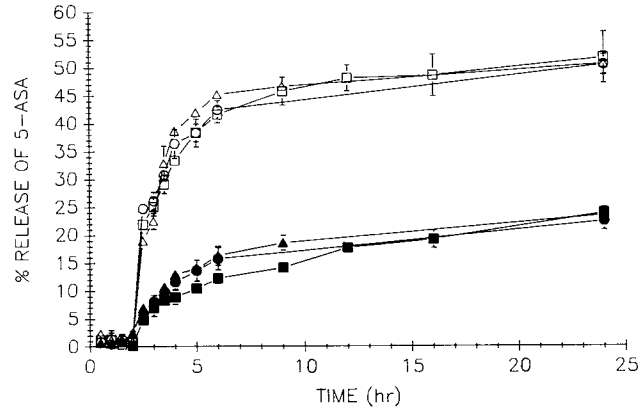

Fig. 7. Stability of 5-aminosalicylic acid loaded on alginate beads coated with 4 or 6% (w/w) Aquacoat on top of 6% (w/w) Eudragit L30D (stored at room temperature). (□) Day 1, 4% (w/w) Aquacoat coated; (○) day 7, 4% (w/w) Aquacoat coated; (△) day 14, 4% Aquacoat coated; (■) day 1, 6% (w/w) Aquacoat; (●) day 7, 6% (w/w) Aquacoat coated; (▲) day 14, 6% (w/w) Aquacoat coated.

REFERENCES

1. A. K. A. Khan, J. Piris, and S. C. Truelove. An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet* 2:892–895 (1977).
2. M. Campieri, G. A. Lanfranchi, G. Bazzocchi, C. Brignola, F. Sarti, G. Franzin, A. Battocchia, G. Labo, and P. R. D. Monte. Treatment of ulcerative colitis with high-dose 5-aminosalicylic acid enemas. *Lancet* 2:270–271 (1981).
3. L. R. Sutherland, F. Martin, S. Greer, M. Robinson, N. Greenberger, F. Saibil, T. Martin, J. Sparr, E. Prokipchuk, and L. Borgen. 5-Aminosalicylic acid enema in the treatment of distal ulcerative colitis, proctosigmoiditis, and proctitis. *Gastroenterology* 92:1894–1898 (1987).
4. J. Guarino, M. Chatzinoff, T. Berk, and L. S. Friedman. 5-Aminosalicylic acid enemas in refractory distal ulcerative colitis: Long-term results. *Am. J. Gastroenterol.* 82:732–737 (1987).
5. L. C. Karp and S. R. Targan. New enema treatments for inflammatory bowel disease. *Digest. Dis. Sci.* 33:85s–87s (1988).
6. M. A. Peppercon. Sulfasalazine and related new drugs. *J. Clin. Pharmacol.* 27:260–265 (1987).
7. J. G. Hardy, C. G. Wilson, and E. Wood. Drug delivery to the proximal colon. *J. Pharm. Pharmacol.* 37:874–877 (1985).
8. M. J. Dew, P. J. Hughes, M. G. Lee, B. K. Evans, and J. Rhodes. An oral preparation to release drugs in the human colon. *Br. J. Clin. Pharmacol.* 14:405–408 (1982).
9. S. N. Rasmussen, S. Bondesen, E. F. Hvidberg, S. H. Hansen, V. Binder, S. Halskov, and H. Flachs. 5-Aminosalicylic acid in a slow-release preparation: Bioavailability, plasma level, and excretion in humans. *Gastroenterology* 83:1062–1070 (1982).
10. G. Jarnerot. Newer 5-aminosalicylic acid based drugs in chronic inflammatory bowel disease. *Drugs* 37:73–86 (1989).
11. G. K. McEvoy (ed.). *AHFS Drug Information,* American Society of Hospital Pharmacists, Bethesda, MD, 1990, pp. 1674–1675.
12. P. N. Shaw, L. Aarons, and J. B. Houston. HPLC analysis of sulfasalazine and its metabolites. *J. Pharm. Pharmacol.* 32:67p (1980).
13. G. Bahadir and G. S. U. Munchen. Safe formulations of agrochemicals. *Chemosphere* 16:615–621 (1987).
14. W. J. Connick, Jr., J. M. Bradow, W. Wells, K. K. Steward, and T. K. Van. Preparation and evaluation of controlled release formulations of 2,6-dichlorobenzonitrile. *J. Agr. Food Chem.* 32:1199–1205 (1984).
15. F. Lim and A. M. Sun. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 210:908–910 (1980).
16. P. Sheurich, H. Schnabl, U. Zimmermann, and J. Klein. Immobilization and mechanical support of individual protoplasts. *Biochim. Biophys. Acta* 598:645–651 (1980).
17. A. Khosla, L. C. Hardy, and J. W. Davis. Gastrointestinal transit of non-disintegrating tablets in fed subjects. *Int. J. Pharm.* 53:107–117 (1989).