Pharmaceutical Research, Vol. 7, No. 6, 1990

Research Article

Pulmonary Delivery of Peptide Drugs: Effect of Particle Size on Bioavailability of Leuprolide Acetate in Healthy Male Volunteers

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Received September 20, 1989; accepted December 20, 1989

Leuprolide acetate, a nonapeptide with potent luteinizing hormone releasing hormone (LHRH) agonist activity, has low oral bioavailability. Unique aerosols of leuprolide acetate were developed and particle size distribution studies were carried out. A light scattering method (Malvern Model 2600c) was compared to the impaction method (Andersen Sampler) for measuring size distribution. Results showed the Malvern method to be comparable to the impaction method with the Malvern being faster and easier to use. Absolute bioavailability of leuprolide acetate in healthy human male volunteers ranged from 4% to 18% and agreed well with particle size data. Bioavailability corrected for respirable fraction ranged from 35% to 55%, indicating that the pulmonary route may have high potential for systemic delivery of this peptide.

KEY WORDS: pharmaceutical aerosols; respirable fraction; peptide delivery; pulmonary delivery of peptide; absorption of leuprolide acetate.

INTRODUCTION

Leuprolide acetate is a potent luteinizing hormone releasing hormone (LHRH) agonist, which when introduced to the portal circulation, induces the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary (1). For this reason, systemic administration of leuprolide acetate has been suggested as an effective treatment modality for several gonadal diseases including prostatic cancer (2), endometriosis (3,4), precocious puberty, and metastatic breast cancer (ongoing studies, TAP Pharmaceuticals, North Chicago, Illinois). Leuprolide acetate is not orally active and therefore it is marketed as a 1-mg daily subcutaneous parenteral product (Lupron) and as a 7.5-mg monthly depot injectable (Lupron Depot).

Inhalation aerosols of leuprolide acetate were prepared and evaluated as potential alternatives to parenteral administration. Currently marketed pharmaceutical aerosols have been developed for localized delivery of drugs to the upper airways of the lung for treatment of bronchoconstriction. For systemic delivery a drug administered by the pulmonary route must reach the alveoli where diffusion and phagocytosis have been proposed as primary mechanisms for drug absorption (5). Because particles of 5 μm or greater in diameter deposit primarily in the upper airways (6–8), tight particle size specifications for inhalation aerosols would be required to ensure that pulmonary delivery systems deliver drugs to their intended sites within the lung. For example, in preparing typical suspension aerosols for lung and upper airway deposition, the drug powder would be finely divided by micronization techniques and sieving such that only particles between 1 and 5 μm in diameter would be used in the manufacture of the product.

Size analysis for airborne particles such as inhalation aerosols is primarily done by impaction methods (7,8). An example of these is the Andersen Sampler (Andersen Samplers Inc., 4215 Wendell Drive, Atlanta, GA), which consists of a cascade of screens with successively smaller pore diameters upon which the particles in an aerosol would collect based on their aerodynamic particle size. These methods are time intensive and require an analytical technique such as HPLC in order to quantify accurately the fractional drug deposition on the screens within the cascade.

In this paper, a light-scattering method for particle size distribution in inhalation aerosols is compared to a standard impaction technique. Aerosol formulations of leuprolide acetate are used as test materials. Data from a human bioavailability study utilizing these aerosols are reported. The *in vivo* results are examined on the basis of particle size distribution as measured by both light-scattering and impaction methods.

EXPERIMENTAL METHODS

Supplies

Three aerosol formulations were prepared, one solution and two suspensions. These formulations are described in Table I. Functional performance tests (shot weight and content uniformity) were done using validated methods. Agglomeration was evaluated using microscopy to monitor

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Table I. Composition of Inhalation Aerosols for Leuprolide Acetate[a]

Formula composition	Solution, form A, 0.5 mg/ spray	Suspension, form B, 0.5 mg/ spray	Suspension, form C, 1.0 mg/ spray
Water	1.50%		
Decanesulfonic acid, sodium salt	0.56%		
Alcohol, dehydrated, USP, 200 proof	17.30%		
Leuprolide acetate	0.5 mg	0.5 mg	1.0 mg
Sorbitan monooleate, NF	5.60%		
Sorbitan trioleate		0.5%	0.5%
Propellant 11		28.00%	28.00%
Propellant 12	q.s.	q.s.	q.s.

[a] Propellant 11 = trichlorofluoromethane (Du Pont); Propellant 12 = dichlorodifluoromethane (Du Pont). Quantities reported in percentages are in terms of % (w/v).

morphological changes in the drug particles after evaporation of the propellant system. Valve performance was examined by measuring valve delivery (dose of leuprolide acetate per spray) as a function of time and storage at various temperatures. Stability performance of formulations was investigated and found to be satisfactory for at least 1 month before initiation of the bioavailability studies.

Analytical Methods

In vitro chemical assays for leuprolide acetate were done using a previously described HPLC method (9). Bioassays for leuprolide acetate in human plasma samples were done using a radioimmunoassay technique (10).

Micromeritic Studies

Two methods were used to characterize the three preparations. The first method utilized an impaction technique similar to methods commonly used in the pharmaceutical industry to characterize airborne particles (7). The second method utilized a light-scattering technique to measure gross particle size distribution in the aerosol flume as it came out of the valve adapter.

Impaction Method for Particle Size Analysis

The Andersen Sampler, designed to mimic dosing of patients, was used. For testing, the aerosol can was placed at the valve end of the actuator, while the mouthpiece end of the actuator was sealed in a glass intake tube (throat) such that the aerosol vial was approximately 90° juxtaposed to the throat. The other end of the glass throat was attached to the top of the Andersen Sampler (Ambient Particle Sizing Chamber, Mark II, Andersen, Inc.). A schematic of the experimental setup is shown in Fig. 1 (similar to the apparatus for metered-dose aerosols shown on page 1220 of USPXXI except the Andersen Sampler is substituted for the collection chamber). Sampling of the aerosol for sizing was achieved by activating the actuator under 1 actual cubic feet per

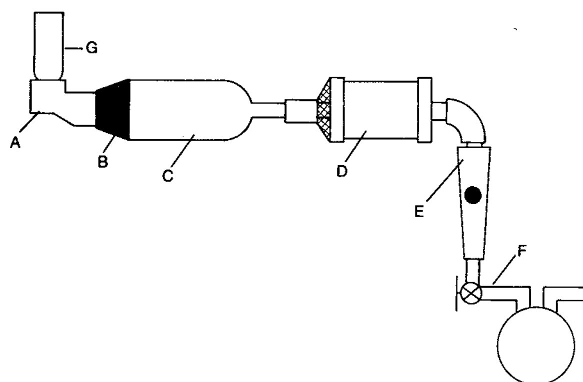

Fig. 1. Schematic diagram of the Andersen Sampler. A is actuator; B is intake adapter; C is Glass intake tube, approximately 5-cm diameter × 25.5 cm and drawn to 7 mm at one end; D is Andersen Sampler; E is Gilmont F-1500 Flowmeter; G is aerosol vial.

minute (ACFM) of air. A calibrated Gilmont-1500 Flowmeter was used to monitor airflow through the apparatus. The sample spray was allowed to travel through the actuator and glass "throat" into the Andersen Sampler, where the particles were separated onto the impact plates within the sampler according to their aerodynamic particle size (defined as the diameter of a spherical particle with unit density that settles in air at the same rate as the drug particles). Replicate samples were tested from each of five vials of formulation A, three vials of formulation B, and two vials of formulation C. Sampling constituted approximately 5 mg of leuprolide acetate, i.e., 10 sprays from each of formulations A and B and 5 sprays from formulation C. After these samples were introduced into the Andersen Sampler, drug residue from the actuator, throat, and respective impact plates were extracted into methanol and analyzed by HPLC. From these data, mean medication delivery and respirable fraction were calculated.

Light-Scattering Method for Particle Sizing

The method adapted for this study utilized a Malvern laser diffraction particle sizer (Model 2600c) with a low-power helium–neon unpolarized laser beam which was spatially filtered to form a collimated and monochromatic beam of light, 9 mm in diameter. Aerosol particles were introduced to this beam so that diffracted and transmitted light were focused by a 100-mm Fourier transform lens onto a detector placed in the focal plane of the lens. The detector consisted of 31-element, concentric, light-sensitive rings with a hole in the center. A photodiode behind the hole was used for instrument alignment prior to initiating measurements. The data were collected by sweeping all 31 rings a total of 200 times to ensure that a representative, randomly oriented sample from all size classes had been measured. Ten sprays from each of five vials were measured for each of the three formulations. Suspension aerosols were shaken prior to sampling in order to maintain sample to sample uniformity. The position of the aerosol bottle and valve adapter assembly was fixed so that the spray jet was 12.5 cm from the laser beam. Beam length, which refers to the length of aerosol flume along the path of the laser beam, was maintained at 10 mm during all sizing measurements. Under these conditions

it was necessary to fix the distance of the objective lens 3 cm from the aerosol flume. A spray synchronizer, Malvern PS51, provided with Malvern PS57 infrared sensor and PS58 Rotation Trigger Sensor, was placed perpendicular to the path of the aerosol flume to automatically initiate and terminate sizing of the aerosol. The IR beam of the spray synchronizer was maintained at a distance of 4 cm from the spray jet. At this position, the laser beam of the sizer and the IR beam of the spray synchronizer were parallel to each other and 8.5 cm apart.

Sizing of each aerosol spray was performed for a duration of 15 msec, i.e., beginning from 70 msec and ending at 85 msec after interruption of the IR beam by the aerosol. Correction of the data resulting from low light scattering associated with evaporation of the propellant was not done; if desired, the instrument allows for such data correction with a "kill data" command to exclude aberrant size fractions from the results. However, adjustment for background noise prior to sizing was made using blank propellant and surfactant blends for the respective formulations. Under these conditions, droplet sizes from respective formulations were found to be reproducible based on initial and 1-month results.

Administration of Aerosol Formulations

Subjects were trained for proper technique in self-administering the aerosol on the night prior to dosing. On the morning of drug administration and immediately before dosing, subjects were seated in an upright position and made to exhale completely. With the head tilted back at an angle of approximately 45°, the mouth was opened and the mouth adapter placed so that it was completely encircled by the lips. Upon onset of inspiration and with the tongue pulled down low in the lower jaw, subjects were made to breathe in slowly through the mouth while simultaneously activating the aerosol by firmly pressing down on the actuator. At the end of inspiration, the mouth adapter was removed and subjects were made to lower the head immediately followed by a breath hold of approximately 10 sec. Subjects were then asked to exhale slowly through the nose.

Human Bioavailability Studies

Bioavailability of leuprolide acetate aerosol formulations was determined in 23 healthy human males in a single-dose, four-period crossover study. The subjects were divided into four groups and dosed according to a statistically randomized schedule. A single dose consisted of two aerosol sprays or an intravenous injection (control). A 1-week washout interval was allowed between each of the four dosing periods. Subjects were fasted overnight and served food at controlled periods on the dosing day. Five milliliters of blood was withdrawn via heparin lock at each of the following sampling points: 0, 5, 10, 15, 20, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 4.5, 6, 8, 12, and 24 hr after dosing. The blood samples were chilled at 5°C and centrifuged within 1 hr of sampling. The respective plasmas were separated and transferred to glass tubes and frozen until ready for assay.

RESULTS

Pertinent functional performance data generated to support the study (i.e., shot weight and content uniformity) are not reported in this manuscript. However, the data indicated that there were no significant changes in these parameters over the duration of the study. Microscopic examination revealed no noticeable changes in product aggregation as monitored by particle morphology throughout the duration of the study. Valve performance data as indicated by valve delivery measurements (i.e., 98.4 ± 3.1% of valve capacity, 50 μl) and unit spray leuprolide content (94.1 to 102.4% of the respective labeled amounts) were also found to be satisfactory throughout the duration of the study.

Figure 2 contains a graphical representation of fractional drug quantities collected in the Andersen Sampler following sizing by the impaction method. The total recovery of drug from the solution aerosol formulation averaged 99.5% and that from the two suspension aerosol formulations averaged 91.8 to 93.4%. Light-scattering data for particle size distribution obtained for the three aerosol products are graphically summarized in Fig. 3. The data presented in Figs. 2 and 3 show that the solution aerosol consisted largely of coarse particles, while the suspension aerosol formulations had greater fractions of particles within the respirable range (6). Results contained in this report were generated within one week after preparation of supplies. Subsequent measurements were made at 1 and 3 months after initiation of the study. These stability results were not statistically different from the initial results ($P = 0.05$).

To compare lung deposition efficiency of the three aerosol formulations, respirable fractions were calculated from the micromeritic data. Respirable fraction was defined as a measure of the quantity of particles below 4.7 μm in diameter and was presumed to be the fraction of the aerosolized dose penetrating to the alveoli (5–8). In this investigation, respirable fractions, based on total drug sprayed, were calculated according to the following equation:

$$\mathrm{RF} = \frac{\text{mg drug, <4.7-}\mu\text{m diameter}}{\text{total mg drug sprayed}} \times 100\% \qquad (1)$$

Respirable fraction values from the impaction method were

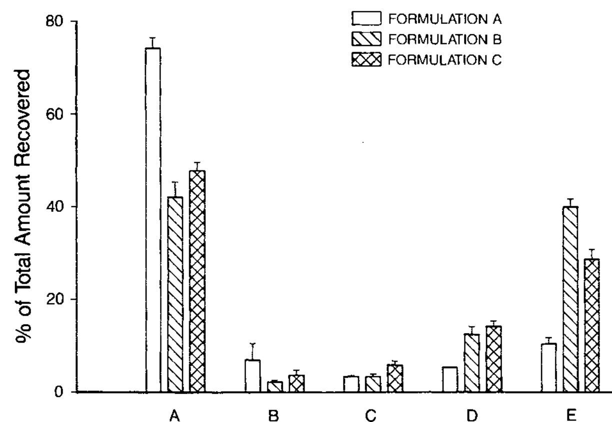

Fig. 2. Comparative size distribution of leuprolide acetate inhalation aerosols as determined by the impaction method. Lower scale designations are as follows: A represents deposition in the actuator; B represents deposition in the throat; C represents particles 9.0–10 μm in diameter; D represents particles 4.7–9.0 μm in diameter; E represents particles 0.43–4.7 μm in diameter.

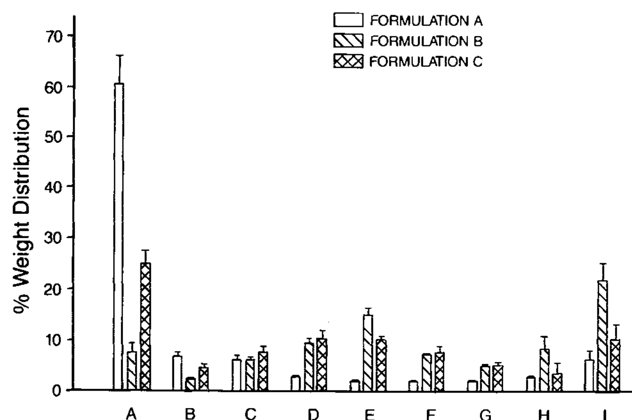

Fig. 3. Comparative size distribution of leuprolide acetate inhalation aerosols as determined by the light-scattering method. Lower scale particle diameter categories are as follows: (A) >10.1 μm; (B) 10.1–7.9 μm; (C) 7.9–6.2 μm; (D) 6.2–4.8 μm; (E) 4.8–3.8 μm; (F) 3.8–3.0 μm; (G) 3.0–2.4 μm; (H) 2.4–1.9 μm; (I) <1.9 μm.

generated from assays on recovery of drug in the Andersen Sampler. Respirable fraction data based on the light-scattering method were generated from mean volume diameters using a predetermined density of 0.956 g/cm^3 for leuprolide acetate. Results of RF values for all three aerosol formulations are summarized in Fig. 4. This graph shows that results obtained from the light-scattering method (Malvern) are comparable to data generated from the impaction (Andersen) method.

Mean plasma concentration versus time profiles following administration of the three aerosols to 23 healthy human males are provided in Fig. 5. The plasma-level data for all subjects were simultaneously fitted (11) to a two-compartment open pharmacokinetic model with first-order elimination from the central compartment (NONLIN). From the resulting nonlinear least-mean square regression curve, the primary pharmacokinetic parameters, T_{max}, C_{max}, and AUC, were estimated. These data are presented in Table II. Absolute bioavailability (BIO) of each aerosol formulation based on IV controls was calculated using the following equation:

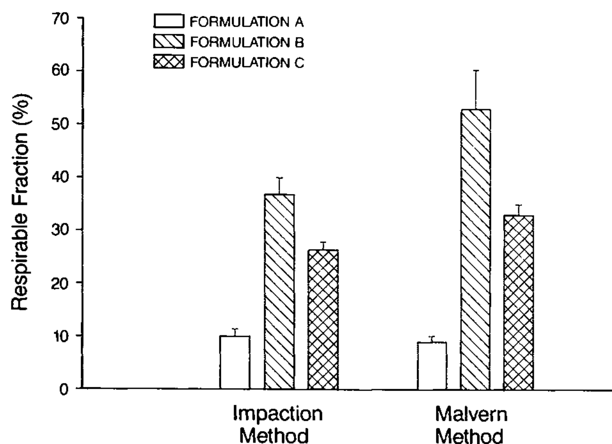

Fig. 4. Respirable fraction values of leuprolide acetate aerosols, as determined by the impaction and light-scattering methods.

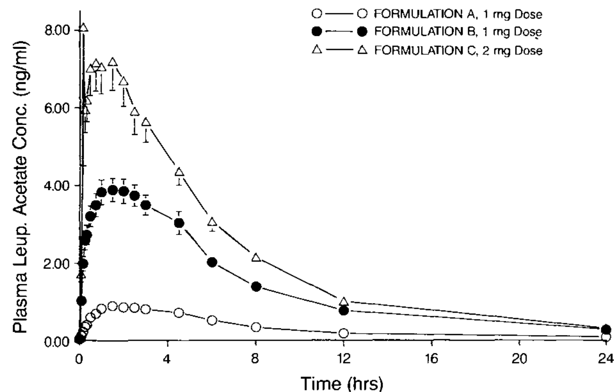

Fig. 5. Plasma concentration profiles of leuprolide acetate in healthy human males following administration of inhalation aerosol formulations. Each point represents the mean ± the standard deviation for 23 subjects.

$$\mathrm{BIO} = \frac{\mathrm{AUC_a} \times D_{\mathrm{IV}}}{\mathrm{AUC_{IV}} \times D_{\mathrm{a}}} \quad (2)$$

where AUC is the area under the curve for plasma concentration versus time (0–24 hr), D is the dose, a is the aerosol formulation, and IV is intravenous administration.

Results presented in Table II show that the suspension aerosols were significantly more bioavailable than the solution aerosol, but there was not a statistically significant difference between the two suspensions ($P = 0.05$). The results also show that C_{max} for the IV control formulation was statistically significantly greater than results for each of the three aerosol formulations ($P = 0.05$).

Respirable fraction data may be used to correct the D_a term in Eq. (1) to reflect loss of drug (nonabsorptive loss) on the aerosol device and in the mouth, throat and trachea. Drug deposited in these regions would rapidly be swallowed and virtually become unavailable for absorption into the systemic circulation (14). The relationship between the respirable fraction (RF) and the effective dose estimated to have been administered to the lung (D^*_a) is shown in Eq. (3).

$$D^*_a = D_a \times RF \quad (3)$$

The corrected dose (D^*_a) more accurately reflects the dose delivered to the deep lung, i.e., alveoli. Bioavailabilities calculated using this corrected dose may therefore better represent the apparent bioavailability of leuprolide acetate following pulmonary delivery to humans. These corrected bioavailabilities (BIO*) are presented in Table III. From these

Table II. Pharmacokinetic Parameters for Leuprolide Acetate Aerosols Following Inhalation Delivery to Humans[a]

Formulation	Dose	T_{max} (hr)	C_{max} (ng/ml)	AUC (ng · hr/ml)
Form A	1 mg	2.3 (2.2)	0.97 (0.43)	7.80 (3.94)
Form B	1 mg	1.6 (0.8)	4.38 (1.74)	33.14 (10.10)
Form C	2 mg	1.1 (0.8)	11.37 (16.65)	25.95 (10.45)[b]
IV control	1 mg	—	133.22 (7.33)	181.15 (26.92)

[a] Data presented are mean and (standard deviation) for 23 patients.
[b] Dose-corrected AUC.

Table III. Bioavailability Summary of Leuprolide Acetate Following Inhalation Delivery

Formulation	Absolute	Corrected results[a]	
		Impaction	Malvern
Form A	4.3%	43.0%	47.8%
Form B	18.3%	49.5%	34.5%
Form C	14.3%	55.0%	43.0%

[a] Corrected BIO = (absolute bioavailability/respirable fraction).

results, it may be seen that although the absolute bioavailabilities of the three aerosols ranged from 4.3 to 18.3%, the extent of pulmonary absorption of leuprolide acetate was approximately 50% (range, 43 to 55%) when corrected for medication delivery using impaction data. Corrected bioavailabilities ranged from 34 to 48% using light-scattering data. The narrow range of corrected bioavailabilities for the three formulations suggests that absorption of drug from the lung may be independent of the type of formulation (suspension or solution). Particle size distribution and the extent of lung deposition of these aerosols may be affected by the type of formulation, as demonstrated by the absolute bioavailability results.

Airborne particles, including pharmaceutical aerosols, are affected by the high relative humidity, approximately 100%, of the respiratory tract (12,13). High humidity may accelerate the rate of aggregation of hygroscopic particles during transit to the lung. The resultant increase in "apparent diameter" based on lung humidity conditions coupled with changes in other biophysical characteristics of the inhaled particle cannot be modeled by sizing methods carried out under ambient room conditions where relative humidity is approximately 40%. Therefore, calculated respirable fractions may overestimate actual pulmonary delivery. Overestimation of delivery would result in low corrected bioavailability values. This limitation occurs with both methods used in this study, although light-scattering methods may be amenable to operation under conditions simulating the respiratory tract.

Advances in biotechnology have produced many peptide and protein molecules for which conventional oral dosing is not useful due to extensive chemical and metabolic degradation within the gastrointestinal tract (14,15). Several alternate routes of administration have been investigated for leuprolide acetate (14), all of which have shown little promise for clinical use. Oral absorption of leuprolide was 0.05% (14), while absorption following nasal or transdermal delivery (ongoing work in our laboratories) was less than 2% compared to intravenous controls. Results from this investigation show that pulmonary delivery of this peptide may be an effective alternative to parenteral administration. Bioavailability of leuprolide acetate following administration of inhalation aerosols was 4–18% and approximately 50% after correction for nonabsorptive drug loss. Future advancès in aerosol devices could improve pulmonary deposition of pharmaceutical aerosols, making them acceptable for nonparenteral delivery of peptides and proteins.

Commercial development of pulmonary aerosols would require appropriate techniques for particle size characterization. Pharmaceutical aerosols are often characterized by impaction methods which are time-intensive. Light-scattering methods often yield erroneously high intensities due to refraction from evaporation of nonhomogeneous propellant blends in formulations. The light-scattering method described in this paper may provide a satisfactory alternative during formulation screening studies. As shown by these studies, the Malvern method gave results comparable to those obtained with the impaction method. Particle size characterization by each method demonstrated differences among the three leuprolide acetate formulations which were consistent with results from the clinical studies.

ACKNOWLEDGMENTS

The authors are deeply grateful to Mr. R. Doyle and Mr. P. Moy for technical support, Mr. J. Sutherland and Dr. G. Menon (Pharmaceutical Products Division, Abbott Laboratories) for analytical support, and Dr. K. Tollman (Drug Research Center, University of Utah Medical Center, Salt Lake City, Utah) for directing the human studies.

REFERENCES

1. A. V. Schally, A. J. Kastin, and A. Arimura. *Fertil. Steril.* 22:703–721 (1971).
2. M. M. Garnick and other participants including D. T. Max from Abbott. *N. Engl. J. Med.* **311**:1281–1286 (1984).
3. D. R. Meldrum, R. J. Chang, J. Lu, W. Vale, J. Rivier, and H. L. Judo. *J. Clin. Endocrinol. Metab.* **54**:1081–1083 (1982).
4. A. Lemay, R. Maleux, N. Faure, C. Jean, and A. T. A. Fazekas. *J. Steroid Biochem.* **20**(N6B):1379 (1984).
5. D. H. Bowden. *Environ. Lung Res.* **12**:89–107 (1987).
6. D. Ganderton and T. M. Jones. *Drug Delivery to the Respiratory Tract. Ellis Horwood Series in Biomedicine* (1987), Chap. 1.
7. R. N. Dalby and P. Byron. *Pharm. Res.* **5**(1):36–39 (1988).
8. G. W. Hallworth and D. G. Westmoreland. *J. Pharm. Pharmacol.* **39**:966–972 (1987).
9. J. W. Sutherland and G. N. Menon. *J. Liq. Chromatogr.* **10**(10):2281–2289 (1987).
10. I. Yamazaki and H. Okada. *J. Endocrinol. Japon.* **27**:593–605 (1980).
11. C. M. Metzler, G. L. Elfring, and A. J. McEwen. *Biometrics* **30**:562–563 (1974).
12. P. R. Byron, S. S. Davis, M. D. Bubb, and P. Cooper. *Pestic. Sci.* **8**:521–526 (1977).
13. A. J. Hickey. *Drug Dev. Ind. Pharm.* **14**:337–352 (1988).
14. H. Okada, I. Yamazaki, Y. Ogawa, S. Hirai, T. Yashiki, and H. Mima. *J. Pharm. Sci.* **71**:1367–1371 (1982).
15. V. H. Lee. In S. S. Davis, L. Illum, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs,* Plenum Press, New York, 1986, pp. 87–104.