Controlled Release of a Contraceptive Steroid from Biodegradable and Injectable Gel Formulations: *In Vitro* Evaluation

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Received January 24, 1994; accepted November 3, 1994

Purpose. The purpose of this study was to investigate the effects of formulation factors including varying wax concentration, drug loading and drug particle size, on drug release characteristics from both pure oil and gel formulations prepared with a combination of derivatized vegetable oil (Labrafil 1944 CS) and glyceryl palmitostearate (Precirol ATO 5), using levonorgestrel as a model drug. ***Methods.*** The effects of varying drug loadings, different drug particle sizes, and wax (Precirol) concentrations on *in-vitro* drug release rates were evaluated, and the mechanisms of drug release from the gels were determined. ***Results.*** Zero-order drug release rates from the 10% Precirol gel formulations containing 0.25, 0.50 and 2.00% w/v drug loadings were lower than those observed for oil formulations containing identical drug loadings. Higher zero-order release rates were observed from formulations containing smaller drug particles suspended in both oil and gel formulations. The mechanism of drug release from gels containing less than 0.25% w/w drug was diffusion-controlled. Increasing the wax concentrations in the gels from 5% w/w to 20% w/w significantly decreased the diffusivity of the drug through the gel formulations and markedly increased the force required to inject the gels from two different sizes of needles. ***Conclusions.*** This study shows how modification of the physicochemical properties of the gel formulations by changing the drug particle size, wax concentration and drug loading, affects drug release characteristics from the system.

KEY WORDS: levonorgestrel; precirol; labrafil; controlled release; diffusion; injectable gels.

INTRODUCTION

Considerable progress has taken place in the field of parenteral drug delivery systems and the emphasis to date has been on injectable microspheres, which are typically prepared with biodegradable polymers such as polylactic acid, polylactic-co-glycolic acid, polycaprolactone, and other polyesters (1–9). Several investigators have also proposed injectable gels prepared from natural and synthetic polymers, waxes and metallic stearates as vehicles for parenteral controlled-release drug delivery systems (10–13).

Recently, gels prepared from combinations of a vegetable oil and glyceryl esters of fatty acids have been used to control the release of methionyl-Human Growth Hormone by formation of a viscous, semisolid depot at the site of intramuscular injection. Once injected, these hydrophobic formulations are believed to erode slowly upon contact with aqueous biological fluid (14). Even though the gels were found to control the release of a polypeptide, formulation factors affecting drug release from these gel formulations have not been thoroughly examined. Hence, the purpose of the present study was to investigate the effects of formulation factors including varying wax concentration, drug loading and drug particle size, on drug release characteristics from both pure oil and gel formulations prepared with a combination of derivatized vegetable oil and glyceryl palmitostearate, using levonorgestrel as a model drug.

EXPERIMENTAL

Determination of the Saturation Solubility of Levonorgestrel in the Oil Formulation

A 100 mg sample of levonorgestrel was weighed in triplicate and placed in 15 mL screw top polyethylene test tubes. To each test tube, 10 mL of polyglycolyzed apricot kernel oil or Labrafil 1944 CS (Gattefosse, Saint Priest, France) was added. The tubes were tightly capped and shaken in a Dubnoff metabolic shaking incubator—water bath at 37°C (GCA/Precision Scientific, Chicago, IL). Tubes containing only Labrafil (without drug) were also shaken with the test samples as controls. After 48 and 72 hours, the test tubes were centrifuged in a Beckman centrifuge, Model J-6 at 2000 rpm for 30 minutes at 30°C, and the supernatant was filtered through a filter paper (4.25 cm, Whatman, No 1). The last 4 mL portion of filtrate was collected, and a 10 μL aliquot was removed and diluted to 10 mL with acetonitrile/H_2O (60/40) containing 0.3% v/v Tween 80. A 1 mL portion of the internal standard (progesterone 3.33 μg/ml) was mixed with a 2 mL sample of the diluted drug solution in an autosampler vial. The mixture was vortexed for 10 seconds, and 50 μl was injected onto the HPLC column. Blank samples were also assayed in the same manner as the experimental samples. Standard curves prepared over a concentration range of 0.1–4.0 μg/ml were concurrently run in triplicate along with the experimental samples.

Preparation of Oil and Gel Formulations Containing Varying Drug Particle Size

Gel formulations were prepared by mixing oil in previously heated and molten glyceryl palmitostearate or Precirol ATO 5 (Gattefosse, Saint Priest, France) in a water bath. An appropriate quantity of levonorgestrel (Sigma Chemical Company, St. Louis, MO) was added to the molten wax-oil mixture with constant stirring. The temperature of the mixture was maintained at 55°–60°C for 30 minutes. The resulting hot mixture was withdrawn into 1 mL syringes (Becton Dickinson & Co., Rutherford, N.J.), and cooled to room temperature. The resulting gel that was formed inside the syringe was stored at ambient temperature for at least 4 days prior to its use. Formulations of levonorgestrel in oil alone were prepared by an identical procedure.

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0724-8741/95/0600-0857$07.50/0

Preparation of Oil Formulations Containing a Similar Drug Particle Size

An oil formulation containing 0.25% w/w levonorgestrel was first prepared in the same way as described above. After the oil formulation was stored at room temperature for 7 days, half of the formulation was filtered through a filter paper. The resulting filtrate was collected and the drug concentration in the filtrate was determined by the HPLC method. Appropriate quantities of pure drug were added to the filtrate in order to obtain the oil formulations containing 0.25%, 0.5%, and 2.00% w/w drug loading.

Characterization of Oil and Gel Formulations

Injectability Test. Blank gel formulations (without drug) containing varying Precirol concentrations were loaded into 1 mL syringes and the maximum forces (in Newtons) required to inject 1 mL of different formulations through 20, 22 and 26 gauge needles were determined at a crosshead speed of 200.0 mm/min using an Instron (Instron Corporation, Canton, MA).

Drug Particle Size Determination. A light microscope fitted with a calibrated micrometer was employed to determine the size distribution of pure drug and drug crystals suspended in the oil and gel formulations containing various drug loadings. Particle size analysis of original levonorgestrel crystals was performed by suspending 10 mg of drug in 5.0 mL of distilled water containing one drop of Tween-80 as a wetting agent. One drop of the resulting drug suspension was placed on a standard microscope slide. Care was taken to ensure that the drug particles were uniformly dispersed. A glass cover slip was laid on the specimen and pressed down to ensure that all the particles lay in a thin layer in the focal plane of the microscope. The preparation was systematically scanned from low power to high power and the whole area of the specimen was examined. Five hundred particles were counted, and the Martin's diameter (15) was used to determine the size of the drug crystals. For determination of particle size distribution of the drug in the drug-loaded oil and gel formulations, approximately 0.02 mL of each sample was injected onto a microscope slide through a syringe and at least 300 particles of the drug crystals were measured in the same way as described earlier for pure drug particles.

In Vitro Drug Release Studies

The release of levonorgestrel from both oil and gel formulations was studied using the rotating bottle apparatus, (Vankel Industries, Edison, N.J.,). One milliliter of drug-loaded oil or gel was transferred from a syringe into 1000 molecular weight cutoff (MWCO) dialysis membrane tubing (Spectra/Por 7, 1.8 cm width, Spectrum Medical Industries Inc., Houston, Texas). The surface areas of dialysis membrane tubing were 7.0–7.5 cm^2 and 10.0–10.5 cm^2 for the oil and gel formulations, respectively. The ends of the dialysis tubing were securely clamped and then placed in glass bottles containing 90 mL of the dissolution medium consisting of 20% of propylene glycol and 80% of Sorenson's buffer (pH 7.4). The bottles were capped with Teflon-lined lids and rotated end-over-end using a rotating bottle apparatus at 10 rpm in a constant temperature water-bath maintained at 37° ± 0.5°C. Every 24 hours, all of the dissolution fluid in the bottles was decanted and replaced immediately with fresh dissolution medium. Drug concentrations in the withdrawn samples were analyzed using HPLC. Drug release studies were performed in triplicate.

Analytical Method

The HPLC system (Waters Associates) consisted of a model 590 pump, a model 441 detector with 248 nm filter set, and a 710B WISP autosampler, and a Novapak C_{18} column (3.9 × 300 mm). The HPLC mobile phase was prepared by adding deionized water (40% v/v) to acetonitrile. The mobile phase was filtered through a 0.45 μm Nylon filter (Rainin Instrument Co., Woburn, MA). The mobile phase flow rate was 2.0 mL/minute.

Statistical Analysis

The unpaired t-test was used to statistically analyze the data.

RESULTS AND DISCUSSION

Injectability of Gel Formulations Containing Various Precirol Concentrations

The ease of injection of a gel into the subcutaneous or intramuscular tissue is an important consideration. Table I shows the effect of various Precirol concentrations on the maximum force required to inject 1 mL of oil and gel formulations through 3 different size needles. It is evident from the table that the maximum force required to inject the gel-formulations through all three gauge needles was significantly increased ($p < 0.05$) when Precirol concentration increased from 0 to 5% w/w, from 10 to 15% w/w, and from 15 to 20% w/w, respectively. Moreover, the 10% gel formulation was easily injected without much effort through a 1 mL syringe fitted with a 22 gauge hypodermic needle. In contrast, injection of gels prepared with 15 and 20% w/w Precirol through an identical syringe and needle was extremely difficult. It can thus be concluded from the injectability study that, practically, it would difficult to inject a gel-formulation

Table I. Effect of Varying Precirol Concentrations on Maximum Force Required to Inject Gel Formulations Through Different Sizes of Needles

Wax concentration (% w/w)	Mean peak force (Newtons) required to inject oil and gel-formulations through 3 different needles		
	20 gauge	22 gauge	26 gauge
0	4.59 (0.30)	8.00 (1.18)	12.41 (1.04)[a]
5	12.68 (0.65)	22.78 (3.10)	28.23 (2.34)
10	15.83 (0.79)	25.19 (2.10)	39.18 (2.80)
15	24.33 (0.76)	47.05 (5.21)	65.11 (14.80)
20	43.96 (1.42)	65.94 (2.97)	too viscous to be injected

[a] ( ) standard deviation; triplicate samples were tested for each formulation.

through a 22 gauge needle if the force of injection exceeds 45–50 Newtons.

In Vitro Drug Release Studies

A preliminary experiment was performed to ensure that the dialysis membrane did not control the release of the drug, and allowed the drug to diffuse freely through it (data not shown). Propylene glycol (20%) was added to the Sorenson's buffer in order to maintain sink conditions, and the solubility of the drug at 37°C was increased from 4.69 to 15.02 μg/mL when propylene glycol was added to the Sorenson's buffer.

Effect of Precirol Concentration on Drug Release

Figure 1 shows the cumulative amount of drug released from pure oil formulation and gel formulations containing 5, 10, 15 and 20% w/w Precirol. The amount of levonorgestrel in each formulation was 0.05% w/w. As the Precirol concentration increased from 0 to 20% w/w, the total amount of drug released at day 14 decreased from 36.2 μg/cm^2 to 19.9 μg/cm^2. No significant difference ($p > 0.05$) in the cumulative amount of levonorgestrel released from pure oil formulation and gel formulation containing 5% w/w Precirol was observed at day 14. However, the addition of 10% w/w, 15% w/w, and 20% w/w Precirol in Labrafil caused a substantial decrease ($p < 0.05$) in the cumulative amount of drug released at day 14. No significant difference ($p > 0.05$) in the cumulative amount of drug released at day 14 was observed between gel formulations containing 10% w/w and 15% w/w Precirol, whereas a significant difference ($p < 0.05$) was observed between formulations containing 15% w/w and 20% w/w Precirol.

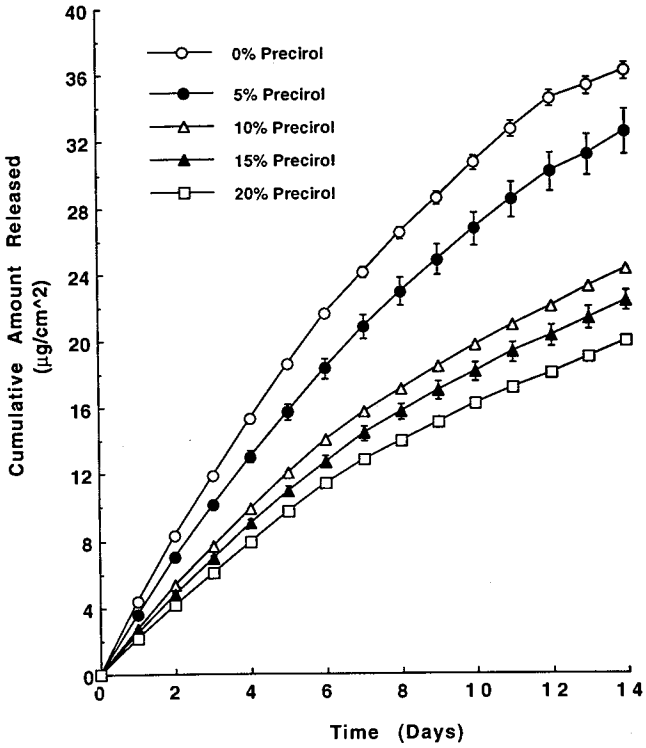

Fig. 1. Effect of varying Precirol concentrations on cumulative amount of levonorgestrel released from formulations containing 0.05% w/w drug loading. Each value represents the mean + SEM of three samples.

When the cumulative amount of levonorgestrel released from pure oil formulation and gel formulations prepared with 5, 10, 15, and 20% w/w Precirol was plotted against square root of time, straight lines were obtained (correlation coefficients and slopes of the lines are shown in Table II), suggesting that the release rates of levonorgestrel from both oil and gel formulations can be described by the simplified Higuchi (16) diffusion equation (Equation 1), when the drug is completely dissolved in the vehicle.

$$Q = 2\,C_0\,(D\,t/\pi)^{1/2} \qquad (1)$$

where Q is the cumulative amount of drug released into the receptor phase per unit area of exposure, C_0 is the initial drug concentration in the vehicle, D is the diffusion coefficient of drug in the vehicle, and t is the time elapsed since the start of drug release.

The diffusion coefficients of levonorgestrel in the gel formulations containing 0.05% w/w drug and different Precirol concentrations (5, 10, 15, and 20% w/w) as shown in Table II, were plotted as a function of Precirol concentration (Figure 2). It is evident that the diffusion coefficients of levonorgestrel decreased exponentially as the wax concentration increased in the gel. This phenomenon may be attributed to both an increase in tortuosity (since the gel formulations showed presence of wax crystals in the gel-matrix upon microscopic evaluation, thus suggesting that the gel is actually a two-phase system) with increasing wax concentrations in the gel formulations (17), and an increase in viscosity of the formulations with increasing wax concentrations. A similar behavior was reported by Chi *et al.* (18) and Gilbert *et al.* (19), who observed an exponential decline in the diffusion coefficients of ketoprofen and benzoic acid derivatives with increasing Pluronic concentrations in the gels. The diffusivity of levonorgestrel through the pure oil was calculated to be 8.04×10^{-9} cm^2/second from the intercept of the least square line in Figure 2. This value is in good agreement with the drug diffusivity of 8.29×10^{-9} cm^2/second calculated from the slope of the straight line (Table II) of the pure oil, using Equation 1.

Effect of Varying Drug Loading and Drug Particle Size

Figures 3 and 4 show the effect of varying drug loading on the cumulative amount of levonorgestrel released from both oil formulation and gel formulation (10% Precirol/90% Labrafil), respectively. Increasing the drug loading from 0.25% w/w to 2.00% w/w in both oil and gel formulations resulted in a significantly greater amount of drug release.

Release rates of the drug from the oil and gel formulations with varying drug loading were calculated from the release data in Figures 3 and 4, and the results are shown in Table III. Zero-order drug release rates were obtained for approximately 4 days and 14 days from oil formulations containing 0.25% w/w and 0.50% w/w levonorgestrel, respectively, as long as the initial drug concentration (C_0) was greater than the saturation solubility (C_s). A zero-order release rate was also achieved for up to 30 days (entire duration of experiment where $C_0 > C_s$) for formulations contain-

Table II. Diffusivities of Levonorgestrel Through Oil and Gel Formulations Calculated from Slopes of Higuchi Plots

Formulation	Precirol concentration (% w/w)	Correlation coefficients	Slopes of straight lines ($\mu g \times cm^{-2} \times sec^{-1/2}$)	Diffusivity (10^{-9} $cm^2 \times sec^{-1}$)
Oil	0	0.998	12.295	8.29
Gel	5	0.999	11.012	6.56
Gel	10	0.999	8.066	3.15
Gel	15	0.999	7.474	2.70
Gel	20	0.999	6.686	2.16

ing 2.00% w/w drug loading. The release rate for the formulation containing 0.05% w/w drug loading deviated from zero order since $C_0 < C_s$.

Increasing the drug loading from 0.25% w/w to 2.00% w/w in both oil and gel formulations increased the zero-order drug release rates (Table III). Theoretically, this increase in drug release rate could have been due to both an increase in drug loading and drug particle size, since in the present study, the size of the suspended drug particles in each formulation was different for each drug loading (Table III). In order to further investigate the effect of different drug loadings on drug release rates, formulations containing identical drug particle size, but varying drug-loadings were evaluated and the results are shown in Figure 5. It is evident from the figure that as long as the particle size of the drug crystals suspended in the oil formulations with different drug loadings is similar, the same amount of levonorgestrel is released from the formulations, regardless of initial drug loadings. The release rates start to deviate from zero-order at 6 and 15 days for formulations containing 0.25% w/w and 0.50% w/w drug loading, respectively, because the drug concentration at those time points were below the saturation solubility. Similar results (i.e. little or no further changes in drug release rates with increasing drug concentrations) have been observed by Bottari *et al.* (20) when they investigated the influence of varying salicyclic acid concentrations on *in vitro* release from several ointment bases.

The differences in drug particle size at each drug loading may be explained by considering the method used to prepare the oil and gel formulations. At 55° to 60°C, levonorgestrel was completely solubilized in both the oil and gel formulations with 0.25 and 0.50% w/w drug loading. However, upon cooling the formulations to room temperature, some of the

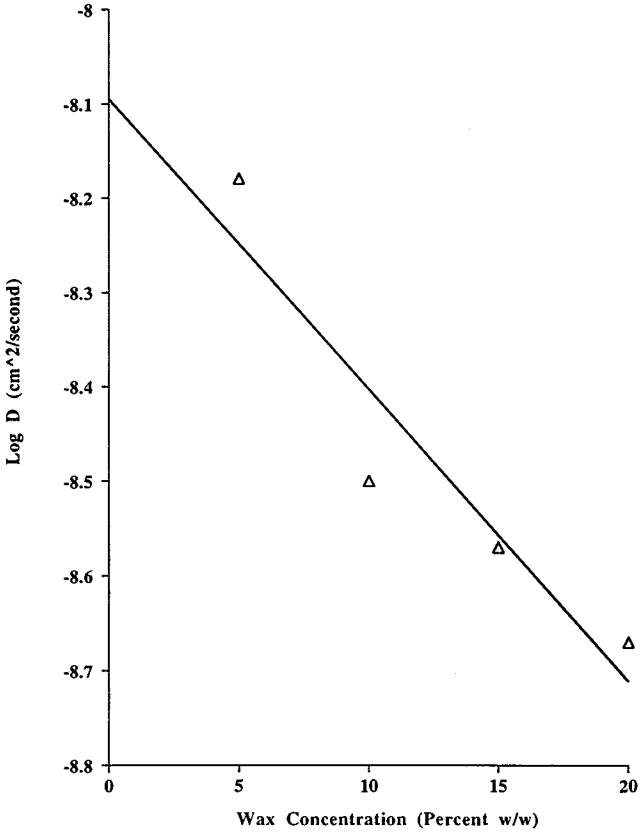

FIG. 2. Relationship between log of diffusion coefficient of levonorgestrel in the formulations containing varying Precirol concentration.

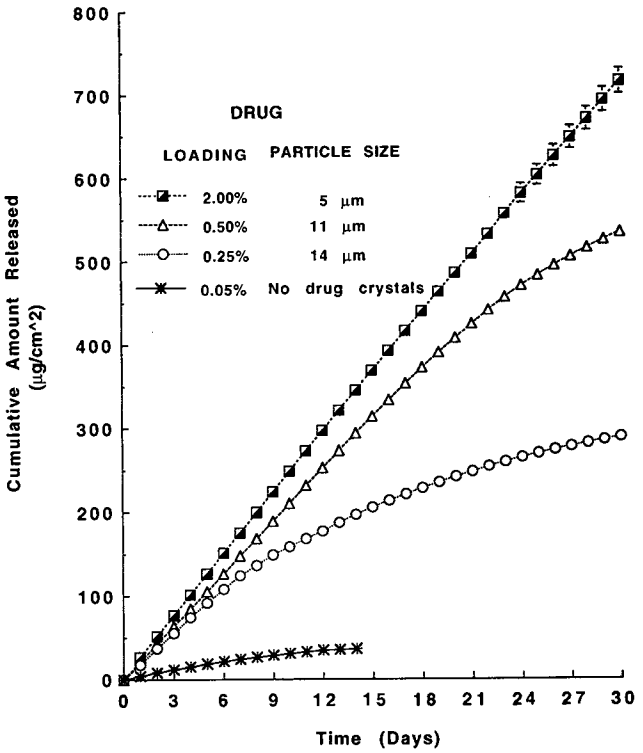

Fig. 3. Effect of varying drug loading and drug particle size on cumulative amount of levonorgestrel released from oil formulations. Each value represents the mean ± SEM of three samples.

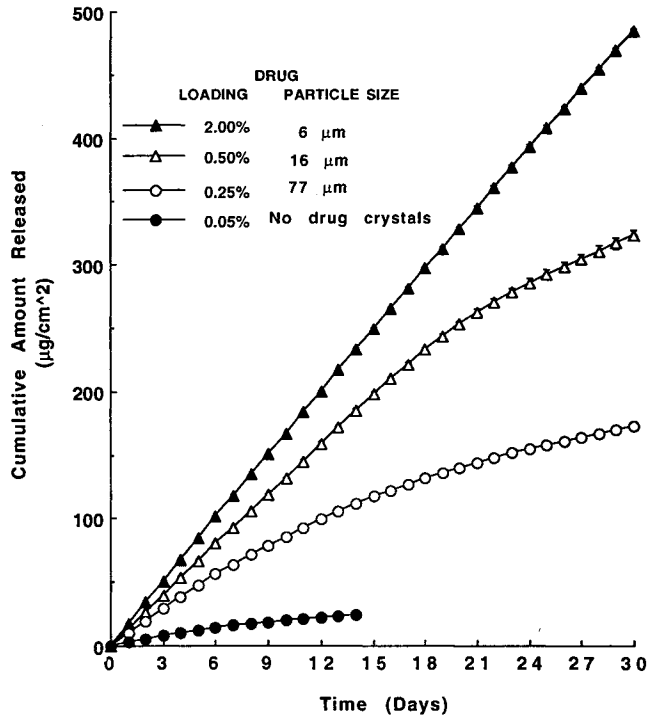

Fig. 4. Effect of varying drug loading and drug particle size on cumulative amount of levonorgestrel released from 10% Precirol gel formulations. Each value represents the mean ± SEM of three samples.

dissolved drug reprecipitated. Equilibrium solubility data show that the rate of recrystallization in the formulations with 0.25% drug loading is slower than the one with 0.50% w/w drug loading, since the amount of drug in formulations containing 0.25% w/w drug loading is less than that in formulations containing 0.50% w/w drug loading. Consequently, the size of the drug crystals in formulations containing 0.25% w/w drug is larger than that in formulations with 0.50% w/w drug. In the case of oil and gel formulations prepared with 2.00% w/w drug loading, most of the drug was in the suspended form even at 55°C–60°C. Therefore, drug recrystallization due to precipitation of the partially dissolved drug after cooling to room temperature did not have a significant influence on the overall particle size distribution of the drug. As a result, the drug particles in both oil and gel formulations with 2.00% w/w drug loading were similar in size ranges to the pure drug particles.

A linear relationship (Figure 6) was observed when the zero-order release rates of levonorgestrel from both oil and gel formulations (shown in Table III) are plotted against the specific surface area of drug crystals (calculated from the mean volume-surface diameter in Table III), indicating that the zero-order drug release rates are inversely proportional to the particle size of drug crystals suspended in both formulations. The intercept of the least-square line represents the drug release rate when $C_0 = C_s$, and the slope indicates the rate at which zero-order drug release rate increases with decreasing particle size of drug crystals.

The drug concentration in the oil and gel formulations containing 0.25% w/w and 0.50% w/w drug loadings at T_{trans}, (the time at which the zero-order release rate changes to the non-zero-order release rate), were calculated from Figures 3 and 4 using Equation (2), and the results are given in Table III.

$$C = (A_0 - A_{Ttrans})/V \quad (2)$$

where C is the drug concentration in a particular formulation at T_{trans}; A_0 is the initial amount of drug; A_{Ttrans} is the cumulative amount of drug released at T_{trans}, and V is the volume of each formulation.

The time T_{trans} was determined from the *in-vitro* drug release rate profiles based on the following statistical argument: when biphasic release rate profiles occur (zero order release followed by non-zero-order release), the time-point at which the transition between the two release profiles is derived by a method called grafted polynomials. In this method, points lying on the first phase of the release profile (zero-order release) can be identified by performing a linear regression analysis of the data. A simple mathematical equation, such as R_1 (release rate) = A_1 (constant) was generated for those data points. A second equation was obtained by performing a linear regression on the data points that belonged to the second phase of the release profile (non-zero order release), and the resulting equation is: $R_2 = A_2 - B_2 * t.T_{trans}$ is the time at which drug release rate is both zero-order and non-zero-order. Hence, at this time point, by setting $R_1 = R_2$, T_{trans} can be mathematically described by Equation 3.

$$T_{trans} = (A_2 - A_1)/B_2 \quad (3)$$

Table III. Effect of Drug Loading and Particle Size on Zero-Order Drug Release Rates

Formulations	Drug loading (% w/w)	Partizle size ($d_{v,s}$, μm)	Zero-order release rate (μg/day/cm²)	T_{trans}[a] (days)	Drug concentration at T_{trans} (μg/ml)
Oils	0.25	14	18.60	4	1700 (100)[b]
	0.50	11	21.80	14	1860 (240)
	2.00	5	26.17	—	—
Gels	0.25	77	9.64	4	1800 (200)
	0.50	16	13.03	15	1920 (120)
	2.00	6	16.12	—	—

[a] Time at which zero-order release rate changes to non-zero-order release rate.
[b] ( ) Standard deviation.
The particle size of pure drug was 5 μm.

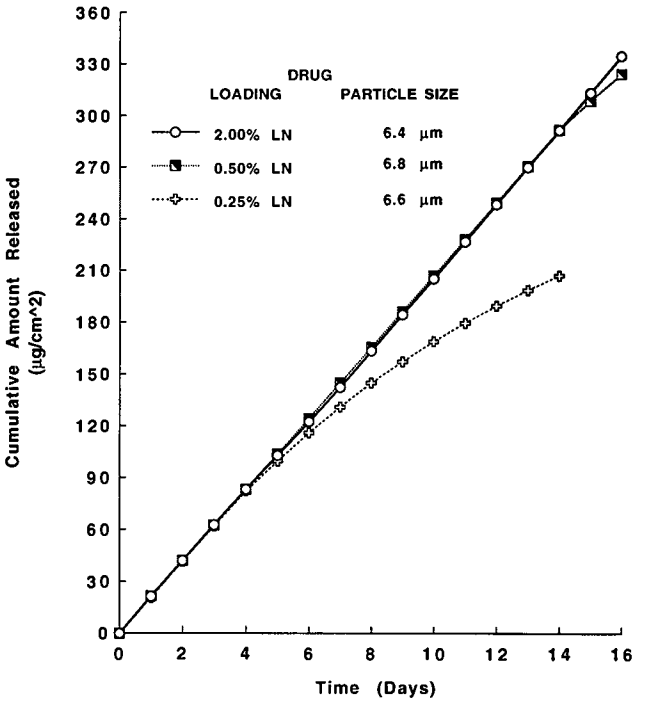

Fig. 5. Effect of varying drug loading on cumulative amount of levonorgestrel (LN) released from oil formulations containing similar drug particle size. Each value represents the mean ± SEM of three samples.

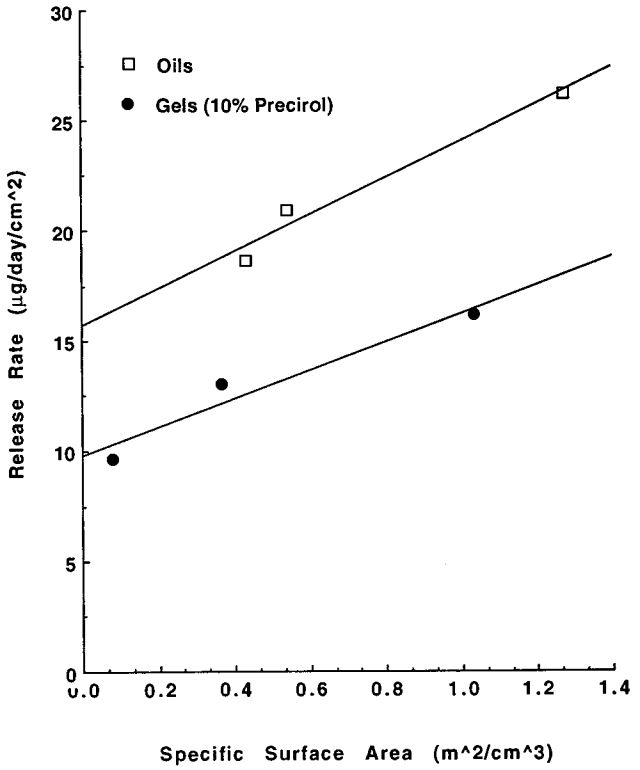

Fig. 6. Relationship between zero-order release rate and specific surface area of levonorgestrel particles suspended in both oil and gel formulations.

The concentrations of levonorgestrel in the oil formulations containing both 0.25% w/w and 0.50% w/w drug loadings at T_{trans} are similar to the experimentally determined solubility of drug in the oil formulation (C_s = 1650 mg/mL). Based on the same argument, the solubility of levonorgestrel in the gel formulations containing 0.25% w/w and 0.50% w/w drug loadings was also predicted from the *in vitro* release profiles (Figure 4) using Equation (2). The predicted solubilities of levonorgestrel in the gel formulation prepared with 10% Precirol were 1.80 mg/ml and 1.92 mg/ml at 0.25% w/w and 0.50% w/w drug loadings, respectively. Statistical analysis of the data shows that these values are not significantly different ($p > 0.05$).

CONCLUSIONS

This study shows how modification of the physicochemical properties of the gel formulations by changing the drug particle size, wax concentration and drug loading, affects drug release characteristics from the system. In addition, equations and techniques presented in this paper serve as useful tools in developing injectable gel formulations. The levonorgestrel/Precirol gel matrices formed with a 10% w/w wax concentration, may be suitable as an injectable vehicle to prolong the serum concentration of contraceptive steroids.

ACKNOWLEDGMENTS

The authors would like to thank Gattefosse Corporation for samples of Precirol and Labrafil. The authors would also like to thank Dr. Don Beer, Dr. Jack Getz, Mr. Jim Schirmer at Schering Plough Inc., Memphis, Tennessee and Mr. David Young and Dr. Phil Hwang in the Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee, Memphis for their technical support and assistance in this work.

REFERENCES

1. K. Suzuki and J. C. Price. Microencapsulation and dissolution properties of a neuroleptic in a biodegradable polymer, poly(d,l-lactide). *J. Pharm. Sci.* 74:21–24 (1985).
2. R. K. Chang, J. C. Price and C. W. Whitworth. Control of drug release rates through the use of mixtures of polycaprolactone and cellulose propionate polymers. *Pharm. Tech.* 10:24–33 (1986).
3. M. Tsakala, J. Gillard, M. Roland, F. Chabot, and M. Vert. Pyrimethamine sustained release systems based on bioresorbable polyesters for chemoprophylaxis of rodent malaria. *J. Controlled Rel.* 5:233–242 (1988).
4. Y. Cha and C. G. Pitt. A one-week subdermal delivery system for L-methadone based on biodegradable microcapsules. *J. Controlled Rel.* 7: 69–78 (1988).
5. J. P. Benoit, F. Chabot, and M. Veillard. Biodegradable cisplatin microspheres prepared by the solvent evaporation method: Morphology and release characteristics. *J. Controlled Rel.* 7:217–229 (1988).
6. N. Leelarasamee, S. A. Howard, C. J. Malanga, and J. K. Ma. A method for the preparation of polylactic acid microcapsules of controlled particle size and drug loading. *J. Microencapsulation.* 5:147–157 (1988).
7. Y. Cha and C. G. Pitt. The acceleration of degradation—controlled drug delivery from polyester microspheres. *J. Controlled Rel.* 8:259–265 (1989).
8. F. Koosha, R. H. Muller, S. S. Davis, and M. C. Davies. The surface chemical structure of poly(β-hydroxybutyrate) microparticles produced by solvent evaporation process. *J. Controlled Rel.* 9:149–157 (1989).

9. J. M. Ruiz, J. P. Busnel, and J. P. Benoit. Influence of average molecular weights of poly(DL-Lactic Acid-Co-Glycolic Acid) copolymers 50/50 on phase separation and *in-vitro* drug release from microspheres. *Pharm. Res.* 7:928–934 (1990).
10. C. G. Pitt. The Controlled Parenteral Delivery of Polypeptides and Proteins. *Int. J. Pharm.* (Netherlands) 59(3):173–196 (1990).
11. K. A. Fults and T. P. Johnston. Sustained-release of urease from a poloxamer gel matrix. *J. Parent. Sci. Tech.* 44(2):58–65 (1990).
12. J. L. Zatz, J. P. Berry and D. A. Alderman. Gels. In H. A. Lieberman, M. M. Rieger and G. S. Banker (eds.), *Pharamceutical Dosage Forms: Dispersed Systems,* Marcel Dekker, New York, 1988, Vol. 2, pp. 495–510.
13. F. H. Buckwalter. Repository penicillin composition. U.S. Patent #2, 507, 193 (1950).
14. A. L. Thakkar and R. G. Harrison. Injectable semi-solid formulations, European Patent Application, 86306282.4, (1986).
15. T. A. Barber. Particle Population Analysis. In *Pharmaceutical Particulate Matter,* Interpharm Press, Buffalo Grove, Illinois, 1993, pp. 271.
16. W. Higuchi. Analysis of data on the medicament release from ointments. *J. Pharm. Sci.* 51(8):802–804 (1962).
17. P. C. Chen-Chow, S. G. Frank. Studies of multiple emulsions as parenteral prolonged release drug delivery systems. *Int. J. Pharm.* 8:89–99 (1981).
18. S. C. Chi and H. W. Jun. Release rates of ketoprofen from poloxamer gels in a membraneless diffusion cell. *J. Pharm. Sci.* 80(3):280–283 (1991).
19. J. C. Gilbert, J. Hadgraft, A. Bye and L. G. Brookes. Drug release from Pluronic F—127 gels. *Int. J. Pharm.* 32:223–228 (1986).
20. F. Bottari, G. Di Colo, E. Nanipieri, M. F. Saettone and M. F. Serafini. Influence of drug concentration on in vitro release of salicylic acid from ointment bases. *J. Pharm. Sci.* 63(11):1779–1783 (1974).