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RESEARCH PAPER

20-Hydroxyecdysone Release from Biodegradable Devices: The Effect of Size and Shape

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ABSTRACT

20-Hydroxyecdysone (20-OH) is a natural compound with many demonstrated effects on the physiological functions of vertebrates, particularly increased protein synthesis. Our study sought a suitable dosage form with continuous release of the drug lasting several weeks for implantation into agricultural animals. Biodegradable microparticles and implants of poly(L-lactic) and poly(DL-lactic) acids were prepared. Oligomers of these materials were synthesized, and a method of melting the binary mixture of the oligomer and 20-OH was employed. The particles were prepared simply by grinding the solidified block of the melt and sieving. Implants were prepared by extruding the melt into silicone tubes, removing the solidified content, and cutting into cylinders of 2 mm diameter and various lengths. A new method of preparation of hollow cylinders by aspirating air into silicone tubes filled with the melt was developed. The experiments demonstrated stability of 20-OH during heat treatment. Release of the active ingredient was tested in static in vitro conditions, analogous to those at the site of implantation, and prolonged drug release was obtained with both types of implant. The hollow implants gave release rates nearest to ideal zero-order kinetics and would appear most appropriate for testing in vivo.

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Key Words: *Controlled release; 20-Hydroxyecdysone; Implants; Polylactic acid; Protein synthesis stimulation.*

INTRODUCTION

20-Hydroxyecdysone, [ecdysterone; 2,3,14,20,22,25-hexahydroxy-(2β,3β,5β,22*R*)-=cholest-7-en-6-one] (1), is a natural compound occurring in insects, higher plants, and ferns. Its European source is primarily *Leuzea carthamoides* DC, Asteraceae (2). In vivo studies demonstrated effects on many physiological functions, particularly increased protein synthesis (3). With ''overthreshold'' doses in male mice, a direct relationship between the dose and response (in terms of increased protein content and protein synthesis) was not demonstrated, but prolongation of the period of increased protein synthesis was observed (4). This compound does not show the side effects of anabolic steroids, behaving more like a xenobiotic drug. It has minimal toxicity; its LD_{50} (mice) is about 2000 $mg \cdot kg^{-1} \cdot day^{-1}$ maximally (5). Currently, it is administered either by frequent intramuscular dosing (leading to animal stress) or by oral dosing, for which comparatively large amounts are required to produce a therapeutic effect.

Most biodegradable carriers of therapeutically active substances are polymers derived from aliphatic α-hydroxyacids, particularly lactic and glycolic acids. Dosage forms containing these materials can be either multiple or unit dose. Active ingredients are most frequently released by the mechanism of diffusion/dissolution in a gradually degrading carrier (6). The rate of drug release can be modified by altering the device size (7–9) or shape (10).

At present, there are several products marketed containing 20-OH (11), although the effect of long-term continuous therapy has not been investigated. Our goal was to complete a basic study to formulate a biodegradable implantable preparation characterized by the release of the active ingredient for a period of several weeks for use in agricultural animals, for which increased protein synthesis is of great economic importance.

EXPERIMENTAL

Materials

20-OH was isolated from the roots of *Leuzea carthamoides* DC, obtained from the Horticultural Co-operative ADAVO (Velký Osek, Czech Republic) as described by Píš et al. (12). The content of the compounds in the substance was checked using a high-performance liquid chromatography (HPLC) method (12), and it was minimally 96% of the declared substance.

Polylactic acid oligomers were synthesized and characterized at our laboratory as described by Dittrich and Melichar (13). Polycondensation reactions of lactic acid (Aldrich, Prague, Czech Republic) were conducted at 160°C and 500 Pa for 72 hr. The strongly acidic cation exchanger Dowex 50W (Sigma, Prague, Czech Republic) was used as the catalyst. All other reagents and chemicals were analytical grade.

Molecular weights were evaluated by a gel permeation chromatography (GPC) method with an ultrastyragel linear column, differential refractometer R 401 (Waters) and Integrator SP 4200 (Spectra-Physics, Darmstadt-Kranichstein, Germany). Polystyrene standards were used for calibration. The thermal behavior of materials was evaluated by a Calvet Calorimeter C 80 D (Setaram, Freiberg, Germany). Samples of about 200 mg were heated at a rate of 1°C/min.

Methods

Assay and Drug Stability Studies

The total drug content of the melt was determined using a direct ultraviolet (UV) spectrophotometric method. Solutions of concentration of 0.69×10^{-4} $mol \cdot L^{-1}$ in methanol, ethanol, water, and Tris buffer pH 7.4 were prepared to find the absorption maximum of 20-OH in different solutions. Values of λ_{max} were found to be 242 nm (methanol), 243 nm (ethanol), and 247 nm (Tris buffer).

To evaluate the stability of these 20-OH solutions, they were stored at temperatures of 3°C and 25°C both in the dark and in daylight for up to 72 days. 20-OH stability was studied by HPLC with UV detection. The apparatus and conditions used were isocratic micropump LPC 3001 (Ecom, Prague, Czech Republic); Waters 486 UV detector set at 247 nm; Waters 746 Data Module; TESSEK Separon CGC 3 × 150 mm column, SGC C_{18}, 5 μm (Tessek, Prague, Czech Republic); flow rate 0.5 $ml \cdot min^{-1}$; mixture of methanol : water 50 : 50 eluting solvent; temperature 25°C.

The 20-OH was extracted from the formulation con-

Table 1

Characteristics of Lactic Acid Oligomers Used

Oligoester	Abbreviation	M_n	M_w	M_n/M_w	T_g(K)	T_m(K)
Poly(L-lactic acid)	PLLA	5300	10,700	2.0	48	161
Poly(DL-lactic acid)	PDLLA	4500	9750	2.2	43	—

M_n = mean molecular weight by number; M_w = mean molecular weight by mass; T_g = glass transition temperature; T_m = melting temperature; M_n/M_w = polydispersity index.

taining polylactic acid by grinding it to a fine powder, extracting into methanol, and analyzing as above.

Preparation of Microparticles

Polylactic acid powder was mixed with 20-OH in a mortar. The mixture was placed into a glass test tube, the pressure was reduced to 500 Pa by a membrane pump, and the temperature was increased to 145°C–165°C for a period of 5 min. The test tube was then cooled in cold water; the solidified block was removed and ground up using a pestle and mortar. After sieving, the 25- to 125-μm fraction was retained.

Preparations of Cylindrical Implants

A mixture of the oligoester of poly(DL-lactic acid) and 20-OH was melted in a test tube at 145°C–165°C under vacuum. The vacuum was removed, and the melt was sucked, by means of syringe, into a silicone rubber tube (2 mm inner diameter, 4 mm outer diameter). The tube was constricted with a clamp and allowed to cool. After cooling to room temperature, the tube was cut longitudinally, and the contents were removed. Cylindrical devices of the required lengths were cut using a warmed scalpel. Devices shaped as hollow cylinders were obtained by a new procedure consisting of aspiration of the air into a silicone rubber tube filled with the melt by means of a vacuum pump, then processed in a similar manner to the solid cylinders.

In Vitro Drug Release

Samples of microparticles of a total weight of 3.00 mg, or one implant of the standard size, were immersed in 10.00 g Tris buffer (0.03 mol·L^{-1} pH 7.8 at 25°C, pH 7.4 at 37°C) and made isotonic using sodium chloride in glass vials. These were incubated statically in a biological thermostat (BT 120, Laboratory Equipment, Prague, Czech Republic) at 37°C to imitate the conditions on implantation. At set time intervals, all the medium was removed, the absorbance was measured at 247 nm, and the medium was replaced with fresh buffer.

RESULTS AND DISCUSSION

The molecular and thermal characteristics of the polylactic acid oligomers are given in Table 1.

We focused on the preparation of a biodegradable dosage form characterized by continuous release of the active ingredient during a period of several weeks. The preparation was intended for in vivo evaluation in farm animals.

Unit-dose implants are more advantageous both for the study and particularly for future use on farm animals as they can be applied to the sites easily and are readily removed by a minor surgical procedure in the live animal or after slaughter.

Advantages of oligomeric compounds for the formulation of biodegradable potential preparations have been reported (14) (i.e. expense, ease of manufacture, and rapid biodegradation). In this study, a relatively unusual method of purification by nonisothermal precipitation was used as described by Dittrich and Melichar (13).

The stability of 20-OH in methanolic, ethanolic, and aqueous solutions was examined for up to 72 days. In an initial study using UV spectroscopy, a decline in the absorption of ethanolic solutions of 20-OH was observed to 86.2% after 35 days, while the aqueous and methanolic solutions showed no change. To confirm the stability of 20-OH in methanolic and aqueous solutions, an HPLC method was then used. Solutions were measured for a period of 72 days. No significant decomposition occurred at 3°C or 25°C or when the solution was exposed to daylight during this period (maximum difference 100 ± 3.0% for methanolic and 100 ± 2.5% for aqueous solutions).

On vacuum melting, the drug/polylactic acid mixture at 165°C, no degradation of the 20-OH occurred during the 15-min period. Heating of the same mixture for a period of 15 min at 180°C resulted in 15% decomposition

of the drug. This suggests that it is not possible to prepare samples from high molecular polymers of L-lactic acid, which has a temperature of melting that is higher than 180°C. Degradation of the polylactic acid carrier in the presence of nonreactive additives does not take place at temperatures below 180°C (15,16).

Melting of a mixture of the oligoester and 15% 20-OH yielded systems physically similar in appearance to the melt of the carrier alone. Melting of systems containing 20-OH in a concentration higher than 21% produced, after melting down and sintering, a compact solid mass, mechanically rugged up to 200°C (i.e., the temperature of decomposition of the active ingredient). This antiplasticizing effect of 20-OH on the carrier is demonstrated in Fig. 1 by a new peak for the glass transition temperature T_g. In addition to the peak at 43°C for poly(DL-lactic acid), there appears a new peak at 54°C in the systems containing 5.4%, 15%, and 21.3% of 20-OH melted with poly(DL-lactic acid). This may be related to the limited miscibility of both substances. At temperatures above 200°C, decomposition of poly(DL-lactic) acid occurs.

Rapid cooling of the melt resulted in tearing of the block from the wall of the test tube. This block was very brittle and could be readily broken up by gentle milling or sieving. Analysis of the content of 20-OH by UV spectrophotometry revealed an average content of 15.0% in 6 samples, with a range ±0.87%.

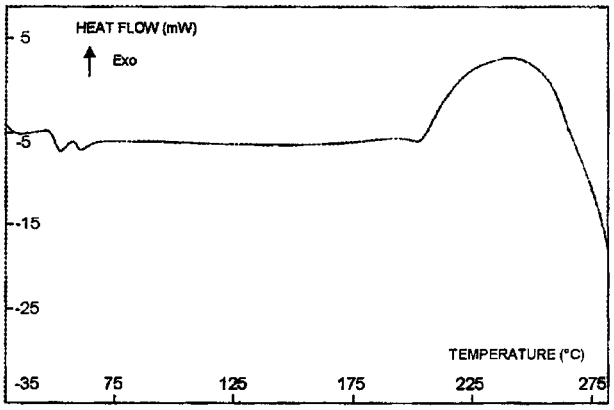

Figure 1. Thermogram of the melted mixture of poly(DL-lactic acid) and 20-OH (15%) (Calvet Calorimeter C 80 D, Setaram, heating rate 1°C/min).

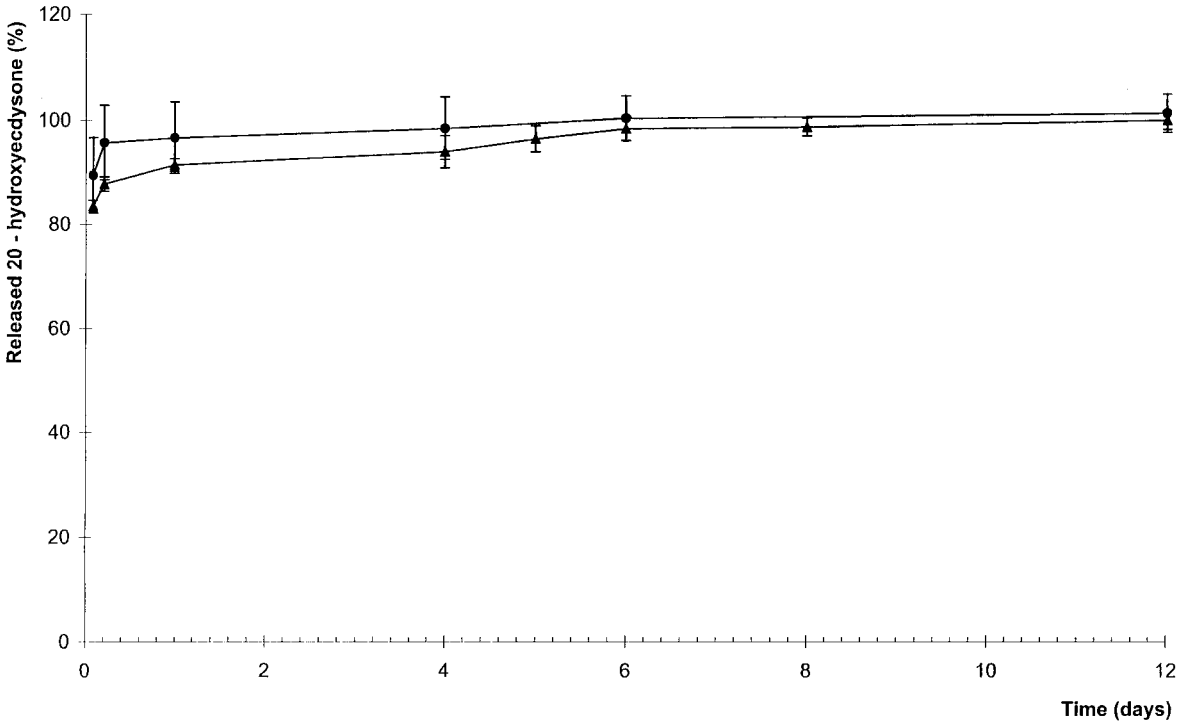

Figure 2. Release of 20-OH from microparticles into isotonic Tris buffer, pH 7.4, at 37°C (n = 4, SD bars): ● carrier, poly(DL-lactic) acid, 15% 20-OH, sieve fraction 25–100 μm; ▲ carrier, poly(L-lactic) acid, 30% 20-OH, sieve fraction 25–100 μm.

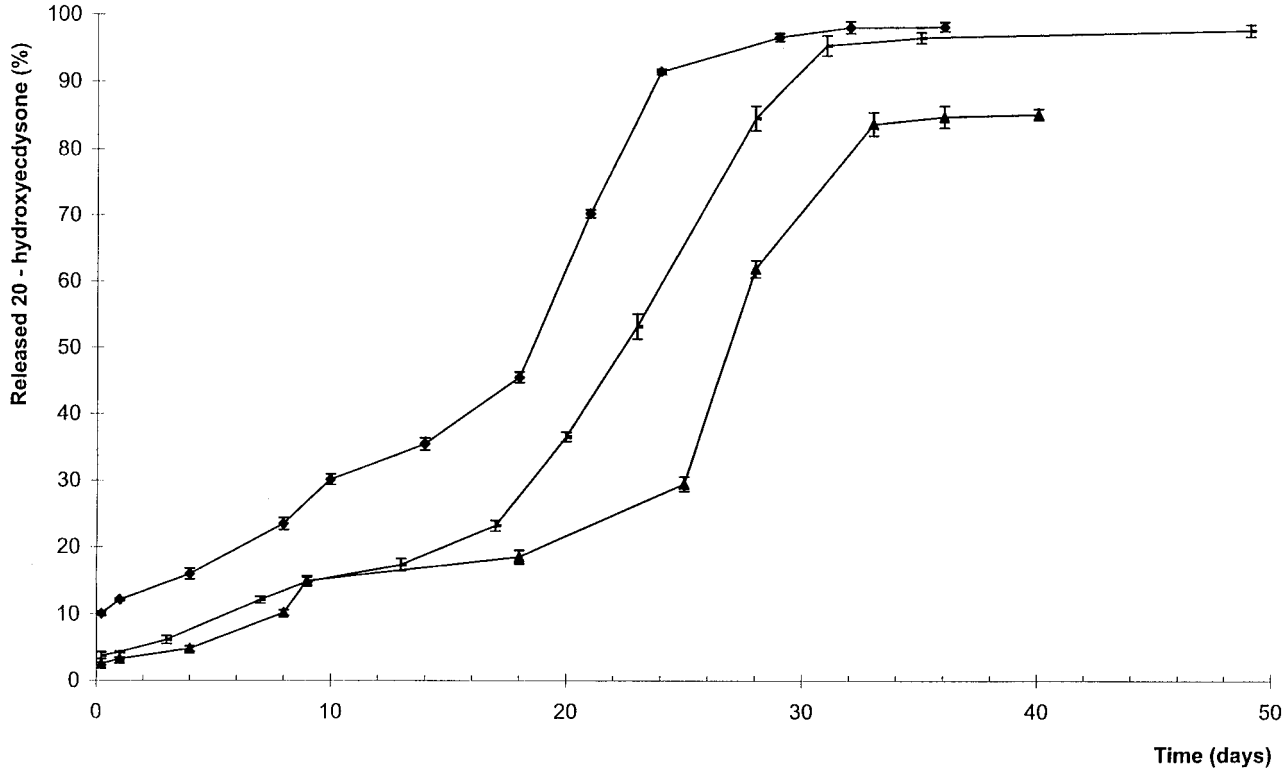

Figure 3. The effect of size on the release of 20-OH from full PDLLA cylinders, diameter 2 mm, into isotonic Tris buffer, pH 7.4, at 37°C ($n = 4$, SD bars): ◆ length of device 2 mm; ● length of device 5 mm; ▲ length of device 10 mm.

Cylindrical devices with a mean diameter of 2.02 mm showed deviations of ±0.05 mm from the diameter, and 10-mm cylinders had weight variation of ±4.0%.

In Vitro Release of 20-Hydroxyecdysone

Figure 2 shows the course of release of 20-OH from the microparticles. The initial burst effect, expressed as the share of the drug released during the first day, was higher than 90%; the remaining amount of the drug was released in 10 days. The generally rapid rate of release of 20-OH is due to both a short diffusion path in microparticles and a much larger surface area for diffusion/dissolution.

The cylindrical devices, however, gave a considerably lower rate of release of 20-OH. Figure 3 shows the course of release of the active ingredient from the full-cylinder devices, which differed in length. Burst effect decreased with increasing length of cylinders. It is in accordance with the theoretical assumptions relating to the ''edge effects'' of cylinders. After the burst stage, there was a stage of slow continuous release. The duration of this stage increased with increasing length of the cylinders. Similar dependence on the size has already been reported in a previous work (8). In the cylinders 10 mm in length, the duration of this stage was about 25 days. After this period, more rapid release related to degradation of the polylactic acid carrier took place that lasted up to 10 days. This contrasts to a sigmoidal-shape release profile of drug from microparticles (17,18). This type of release profile would clearly be unacceptable for in vivo 20-0H delivery.

A more favorable release profile, sigmoidal but nearer to zero-order kinetics, is presented in Fig. 4 for the hollow cylinders. After a relatively small burst effect, the release rate was faster than for full devices of the same outer dimensions. Continuous release was observed in the devices with an outer diameter of 2 mm and a length of 5 mm. Hollow cylinders with a length of 10 mm had the same initial velocity during the first 10 days with a subsequent period of slow release and pulse from progressively swelled matrices after 20 days of release. Slower release occurred in the devices with a length of

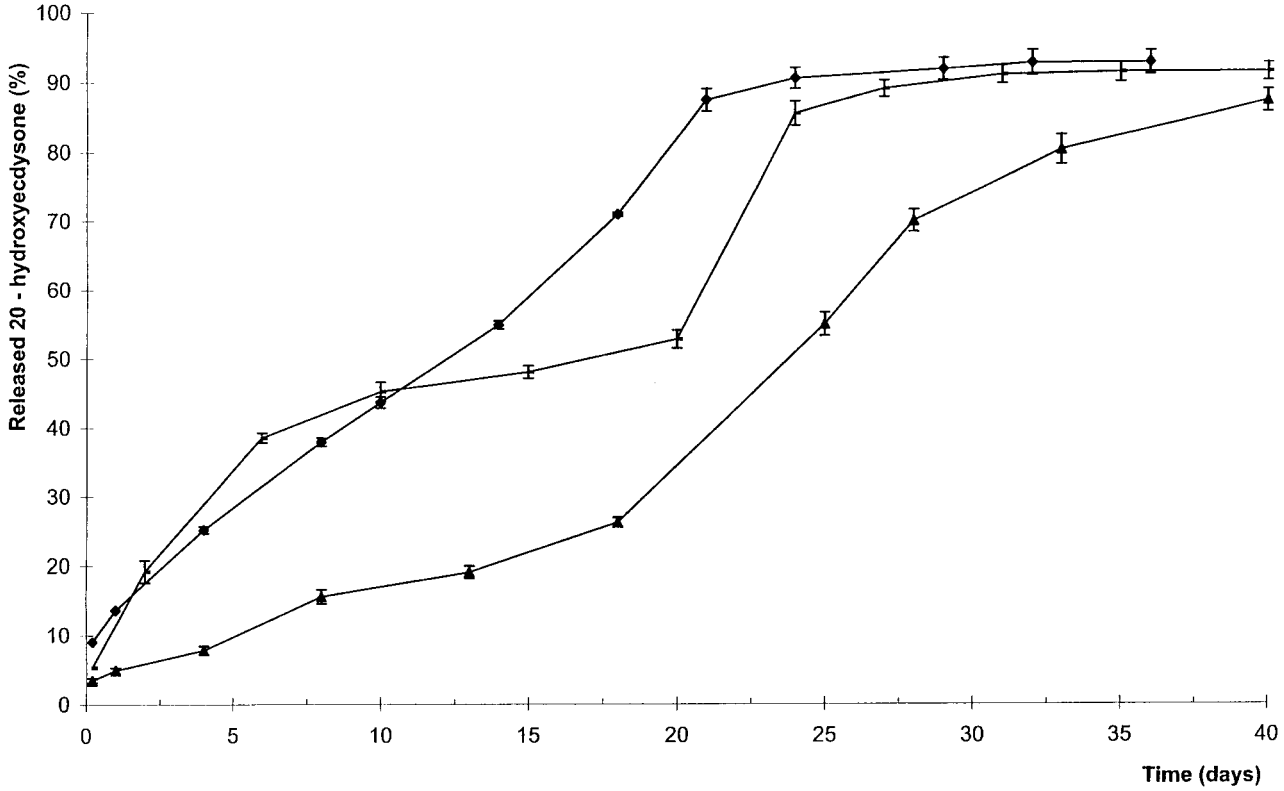

Figure 4. The effect of size on the release of 20-OH from PDLLA hollow cylinders into isotonic Tris buffer, pH 7.4, at 37°C (n = 4, SD bars): ○ outer diameter 2 mm, length of device 5 mm; □ outer diameter 2 mm, length 10 mm; △ outer diameter 4 mm, length of device 10 mm.

10 mm and an outer diameter of 4 mm. The difference may be related to the thickness of the wall of the hollow cylinder. In the devices with an outer diameter of 2 mm, the thickness of the wall was 42 ± 6 μm; in the devices with an outer diameter of 4 mm, the thickness of the wall was 55 ± 9 μm. The values of the dissolution half-lives presented in Table 2 confirmed the higher rate of dissolution for hollow cylinders.

Table 2

20-OH Dissolution Half-life ($t_{1/2}$) of PDLLA Cylindrical Implants

Type of Device	Diameter/Length	$t_{1/2}$ (days)
Full cylinders	2/2	19
	2/5	22
	2/10	26
Hollow cylinders	2/5	12
	2/10	18
	4/10	24

CONCLUSIONS

It is possible to formulate a biodegradable delivery system that will allow prolonged delivery of 20-OH over a period of 40 days in vitro. The shape and size of the system had a marked effect on drug release, with the most favorable release profile being obtained with hollow cylindrical devices. This device can now be taken forward for in vivo studies.

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REFERENCES

1. R. Lafont and D. H. S. Horn, in *Ecdysone. From Chemistry to Mode of Action* (J. Koolman, Ed.), Georg Thieme, Stuttgart, 1989, pp. 39–64.

2. L. Opletal, J. Dvoøák, M. Sovová, F. Krátký, J. Èeøovský, J. Hofbauer, M. Dittrich, and P. Solich, Phytotherapeutic aspects of diseases of the circulatory system. 6. *Leuzea carthamoides* (Willd.) DC. The present state of research and possible use of the taxon, Èesk. Slov. Farm., 46, 247–255 (1997).
3. S. Okui, T. Otaka, M. Uchiyama, T. Takemoto, H. Hikino, S. Ogawa, and N. Nishimoto, Stimulation of protein synthesis in mouse liver by insect-moulting steroids, Chem. Pharm. Bull., 16, 384–387(1968).
4. T. Otaka, M. Uchiyama, S. Okui, T. Takemoto, H. Hikino, S. Ogawa, and N. Nishimoto, Stimulatory effect of insect metamorphosing steroids from *Achyranthes* and *Cyathula* on protein synthesis in mouse liver, Chem. Pharm. Bull., 16, 2426–2429 (1968).
5. S. Ogawa, N. Nishimoto, and H. Matsuda, Pharmacology of ecdysones in vertebrates, in *Invertebrate Endocrinology and Hormonal Heterophylly* (W. J. Burdette, Ed.), Springer, New York, 1974, pp. 341–344.
6. R. Wada, S.-H. Hyon, and Y. Ikada, Kinetics of diffusion-mediated drug release enhanced matrix by degradation, J. Controlled Release, 37, 151–160 (1995).
7. O. Ike, Y. Shimizu, R. Wada, S.-H. Hyon, and Y. Ikada, Controlled cisplatin delivery system using poly(DL-lactic acid), J. Controlled Release, 13, 230–234 (1992).
8. X. Zhang, U. P. Wyss, D. Pichora, and M. F. A. Goosen, A mechanistic study of antibiotic release from biodegradable poly(DL-lactide) cylinders, J. Controlled Release, 31, 129–144 (1994).
9. X. Zhang, U. P. Wyss, D. Pichora, and M. F. A. Goosen, Biodegradable controlled antibiotic release devices for osteomyelitis: optimization of release properties, J. Pharm. Pharmacol., 46, 718–724 (1994).
10. S.-Y. Lin, Controlled release of adriamycin HCl from polymeric needle devices, Biomat. Artif. Cells Artif. Organs, 16, 801–814 (1988).
11. B. Fritz and B. Feliciano, Does the rain forest of Brazil hold the secret to muscle growth? Muscle Fitness, 12, 88–90, 166–168 (1994).
12. J. Píš , M. Budìšínský, K. Vokáè, V. Laudová, and J. Harmatha, Ecdysteroids from the roots of *Leuzea carthamoides*, Phytochemistry, 37, 707–711 (1994).
13. M. Dittrich and L. Melichar, Influence of processing conditions on the release of sodium fluoride from oligomeric L-lactic acid matrices, Biomaterials, 17, 1591–1597 (1996).
14. M. Asano, H. Fukuzaki, M. Yoshida, M. Kumakura, T. Mashimo, H. Yuasa, K. Imai, H. Yamanaka, U. Kawaharada, and K. Suzuki, In vivo controlled release of luteinizing hormone–releasing hormone agonist from poly(DL-lactic acid) formulations of varying degradation pattern, Int. J. Pharm., 67, 67–77 (1991).
15. E. M. Filachione and C. H. Fisher, Lactic acid condensation polymers. Preparation by batch and continuous methods, Ind. Eng. Chem., 36, 223–228 (1944).
16. X. Zhang, U. P. Wyss, D. Pichora, and M. F. A. Goosen, An investigation of the synthesis and thermal stability of poly(DL-lactide), Polym. Bull., 27, 623–629 (1992).
17. R. Bodmeier, K. H. Oh, and H. Chen, The effect of addition of low molecular weight poly(DL-lactide) on drug release from biodegradable poly(DL-lactide) drug delivery systems, Int. J. Pharm., 51, 1–8 (1989).
18. A. Kishida, J. B. Dressman, S. Yoshioka, Y. Aso, and Y. Takeda, Some determinants of morphology and release rate from poly(L)lactic acid microspheres, J. Controlled Release, 13, 83–89 (1990).