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Physico-chemical characterization of a polymeric injectable implant delivery system

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Abstract

Physico-chemical properties of an injectable polymeric implant system were evaluated and utilized to predict and understand the in vivo release of a model drug. The injectable implant system is based on the principle that a water insoluble polymer, dissolved in a biocompatible solvent, will precipitate upon contact with water. The solubility parameter of poly(DL-lactide) and DL-lactide-co-glycolide copolymers were experimentally determined by evaluating the solubility of these polymers in hydrogen bonding solvents having solubility parameters ranging from 8.9 to 14.8 $(cal/cm^3)^{1/2}$. The appropriate Flory-Huggins interaction parameters were then calculated at 25 and 37°C. Analysis of ternary phase diagrams indicated that the quantity of water needed to initiate precipitation, as well as the precipitation threshold, increased with increasing temperature in agreement with theoretical calculations. Rats were subcutaneously administered formulations comprised of polymer concentrations above and below the precipitation threshold, i.e., 40% w/w polymer. Formulations with polymer concentrations below the precipitation threshold exhibited approximately twice the initial release compared to formulations having a polymer content above the precipitation threshold. A key factor affecting the initial release of a model drug from formulations was the polymer content of the formulation with respect to the precipitation threshold. The reported method of analysis may be utilized to screen polymers and biocompatible solvents for use in these injectable implant systems.

1. Introduction

The use of implants has been proposed for the sustained delivery of several therapeutic classes, including: contraceptive steroids [1], peptide hormones [2,3], prostaglandins [4], narcotic antagonists [5], anti-arrhythmics [6] and anti-cancer agents [7]. Although the therapeutic potential for implant systems is immense, the commercialization of these systems has been limited. One possible factor contributing to the lack of commercialization may be related to the difficulty of administration. For implants fabricated ex vivo, a surgical incision must be made or the implant administered via a trocar. In an attempt to provide a system that is more likely to have greater acceptance by patients, a novel implant system has been developed that may be injected as a liquid and subsequently solidify in situ [8]. This injectable implant system is comprised of a water insoluble biodegradable polymer dissolved in a water miscible biocompatible solvent. Upon intramuscular or subcutaneous injection into an aqueous environment, the biocompatible solvent diffuses out of the polymer while water diffuses into the polymer matrix. Due to the polymer's water insolubility, the polymer coagulates or precipitates upon contact with water thus resulting in a solid polymeric implant.

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This technology has been utilized for the delivery of model proteins [9], LHRH antagonists [10], narcotic antagonists [11], growth factors [12] and anti-inflammatory agents [13].

The release characteristics of compounds from these injectable implant systems is analogous to that reported for implant systems prepared ex vivo. More specifically, a relatively high initial release of the active agent is observed followed by a more sustained release profile that follows the square root of time relationship [14]. For conventional implants, the initial burst of drug has been linked to tissue irritation. Since injectable implants are administered as a liquid, it is reasonable to assume that there is a lag between the injection and the formation of the solid implant. During this lag time it is reasonable to anticipate that the initial burst of drug may be equivalent to or exceed that of conventional implant systems. This study was therefore initiated to understand the in situ implant formation process using polymer/solvent interaction theories [15,16] and ultimately control the observed initial burst of drug.

2. Materials and methods

2.1. Materials

Solvents: *N*-methyl-2-pyrrolidone (NMP, ISP Chemicals); dimethyl sulfoxide (DMSO, Cryoserv®, Research Industries); ethyl acetate (Fisher Scientific); acetone (Fisher Scientific); propylene glycol (Arco Chemical Company) and absolute ethanol (Pharmco). Polymers were either standard materials, e.g., Resomer® R 104 (DL-PLA, Boehringer Ingelheim) or custom synthesized, e.g., poly(DL-lactide-co-glycolides) or PLGs (Birmingham Polymers Incorporated). Solvents and polymers were used as supplied. Phosphate buffered saline (PBS, pH 7.4) was prepared with reagent grade materials and de-ionized water followed by sterile filtration.

2.2. Construction of ternary phase diagrams

A stock polymer solution (60.0% w/w) was prepared using NMP or DMSO. Serial dilutions, using the appropriate solvent, of the stock solutions were made to prepare 1.0 g vials containing 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60% w/w polymer. Vials containing polymeric solutions and a vial of PBS were placed in a thermostatted water bath at either 25 or 37°C. PBS was then added dropwise to vials in 1 to 10 μl aliquots until polymer precipitation was observed. This process was repeated until the precipitate no longer re-dissolved within 30 min at which time the total mass (calculated from volume) of buffer added to cause precipitation was recorded. The composition at precipitation was recorded using the known initial weights of solvent and polymer and the weight of PBS that was added. Precipitation compositions were then graphed on ternary plots.

2.3. Determination of polymer solubility

The solubility of a polymer within a solvent (ethyl acetate, acetone, NMP, DMSO, ethanol and propylene glycol) was determined gravimetrically. Excess polymer was added to solvent and allowed to equilibrate for 7 days. A known weight of sample, free of solids, was then added to a vial and the solvent removed under vacuum and heat if needed (e.g., NMP and DMSO). Polymers evaluated consisted of: 50/50 PLG having an inherent viscosity of 0.17 dl/g in chloroform at 45°C; 65/35 PLG (I.V. = 0.15 dl/g); 75/25 PLGs (I.V. values of 0.72, 0.20 and 0.11 dl/g); 85/15 PLG (I.V. = 0.09 dl/g) and resomer R104.

2.4. Calculation of Flory-Huggins parameters

As with solubility parameters, maximum solubility (polymer/solvent interaction) is achieved when the Flory-Huggins parameter approaches zero. The Flory-Huggins interaction parameter is the result of enthalpic (χ_H) and entropic (χ_s) contributions. The enthalpic contribution of the Flory-Huggins interaction parameters (χ_H) were calculated using the following equation [15].

$$\chi_H = \frac{V_1}{RT}(\delta_1 - \delta_2)^2 \quad (1)$$

where χ_H = enthalpic Flory-Huggins interaction parameter: (1) solvent; (2) polymer, V_1 = molar volume of solvent (cm^3/mol), R = gas constant, T = absolute temperature, and δ_i = solubility parameter, $(cal/cm^3)^{1/2}$.

Hildebrand solubility parameters for the various solvents utilized are listed in Table 1. The solubility par-

Table 1
Molar volumes and relevant solubility parameters of solvents[a]

Liquid	V (cm^3/mol)	Solubility parameter (cal/cm^3)$^{1/2}$			
		δ_D^b	δ_P^b	δ_H^b	$\delta_{(total)}^c$
Ethyl acetate	98.5	7.7	2.6	3.5	8.9
Acetone	74.0	7.6	5.1	3.4	9.8
NMP	96.6	8.8	6.0	3.5	11.2
DMSO	71.3	9.0	8.0	5.0	12.0
Ethanol	58.5	7.7	4.3	9.5	12.7
Propylene glycol	73.6	8.2	4.6	11.4	14.8
Water	18.0	7.6	7.8	20.7	23.4

[a]Reference [16]
[b]Hansen partial solubility parameters at 25°C: (D) dispersion; (P) polar; (H) hydrogen bonding interaction contributions where $\delta_D^2 + \delta_P^2 + \delta_H^2 = \delta_t^2$.
[c]Hildebrand solubility parameter at 25°C.

ameters for the various polymers were experimentally determined (Fig. 1).

The entropic contribution to the Flory-Huggins interaction parameter was calculated, assuming that the (χ_s) was negligible, using the method of Bristow and Watson [15,16] using the following relationship:

$$\left[\frac{\delta_1^2}{(RT)} - \frac{\chi}{V_1}\right] = \left[2\frac{\delta_2}{(RT)}\right]\delta_1 - \frac{\delta_2^2}{(RT)} - \frac{\chi_s}{V_1} \quad (2)$$

The left side of Eq. (2) was plotted versus the solubility parameter of the solvent. The y-intercept is then equal to $[-(\delta_2^2/(RT) - \chi_s/V_1]$. Using the y-intercept, δ_2,V_1, the entropic contribution (χ_s) may be calculated.

2.5. Evaluation of formulations in rats

Injectable implant formulations comprised of 5% (w/w) naltrexone base, 35% (w/w) 75/25 PLG (I.V. = 0.11), 60% (w/w) NMP and 5% (w/w) naltrexone base, 57% (w/w) 75/25 PLG (I.V. = 0.11), 38% (w/w) NMP were prepared under aseptic conditions. Each formulation was subcutaneously injected into the midline dorsal area of 20 rats (250–300 g). Five animals from each group were terminated and implants retrieved at 0.5, 1, 2 and 4 hours post-administration. Implants were prepared for HPLC [17] analysis by lyophilization, reconstitution with 25:75 acetonitrile/methanol, sonication for 2 h and filtration.

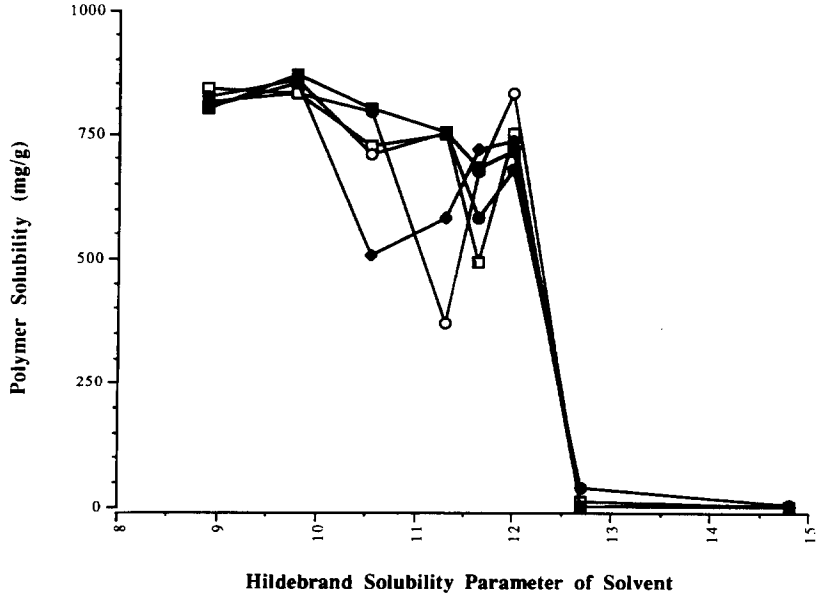

Fig. 1. Solubility of poly(DL-lactide-co-glycolide) polymers as a function of glycolide content (0, 50, 65, 75 and 85%) and Hildebrand solubility parameter in hydrogen bonding solvents at 25°C. Inherent viscosity of polymers ranged from 0.09 to 0.17 dl/g in chloroform at 45°C. Legend: Glycolide content: (●) 0%; (◆) 50%; (■)) 65%; (○) 75%; (□) 85%.

3. Results and discussion

Physical properties of the polymer that are likely to affect the coagulation properties of injected liquid polymers include the lactide to glycolide ratio and the polymer's molecular weight. To determine the effect of glycolide composition on the polymer solubility parameter, the solubility of polymers with different glycolide content were evaluated. The polymers included poly(DL-lactide), 85/15 poly(DL-lactide-co-glycolide), 75/25 poly(DL-lactide-co-glycolide), 65/35 poly(DL-lactide-co-glycolide) and 50/50 poly(DL-lactide-co-glycolide) of approximately the same molecular weight (inherent viscosities ranged from 0.09 to 0.15 dl/g). The solubility of the polymers was determined in ethyl acetate, acetone, NMP, ethanol, DMSO and propylene glycol and plotted according to the respective solvent's total solubility parameter (Table 1), Fig. 1. Analysis of Fig. 1 indicates that all polymers evaluated had two solubility maximas, one maxima at a solubility parameter of 9.8 $(cal/cm^3)^{1/2}$ and the other at approximately 13 $(cal/cm^3)^{1/2}$. Due to the polymer solubility at 9.8 $(cal/cm^3)^{1/2}$ being slightly greater than that at 13 $(cal/cm^3)^{1/2}$, the solubility parameter of the polymers evaluated was determined to be 9.8 $(cal/cm^3)^{1/2}$. Further analysis of Fig. 1 indicates that no consistent trend was observed as a function of the ratio of lactide to glycolide.

The authors acknowledge that this type of evaluation is made difficult by the fact that the relative contribution of dispersion, polar and hydrogen bonding interactions is not constant from one solvent to another. Hansen partial solubility parameters are included in Table 1 to illustrate this fact. For example, according to Table 1, the hydrogen bonding Hansen partial solubility parameter is relatively constant (~ 3.4) for ethyl acetate, acetone and NMP but dramatically increases with subsequent solvents. Also note that the Hansen polar solubility parameters for ethanol and propylene glycol (4.3 and 4.6, respectively), two relatively polar solvents, are less than the respective parameters for less polar materials such as DMSO, NMP and acetone (8.0, 6.0 and 5.1, respectively). Although there are inherent difficulties in the analysis of this type of experiment, it is thought to be superior to theoretical treatments which do not incorporate hydrogen bonding or polar effects. Any inaccuracy in determining the solubility parameter of these polymers will be reflected in quantitative differences in the calculated Flory-Huggins parameters (to follow) but the trends will remain the same.

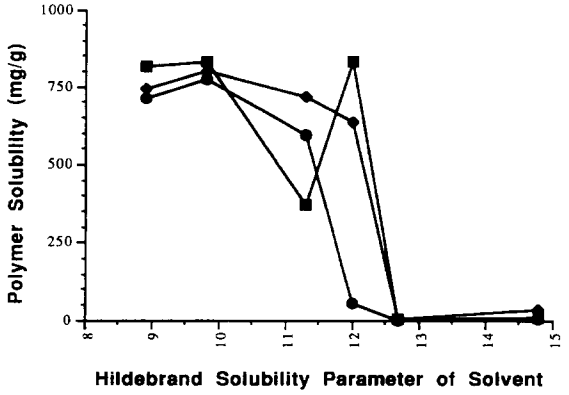

Fig. 2. Solubility of 75/25 poly(DL-lactide-co-glycolide) as a function of inherent viscosity and Hildebrand solubility parameter in hydrogen bonding solvents at 25°C. Inherent viscosity was determined in chloroform at 45°C. Legend: Inherent viscosity: (■)) 0.11 dl/g; (◆) 0.2 dl/g; (●) 0.72 dl/g.

The effect of the polymer's molecular weight was indirectly evaluated by determining the solubility of a 75/25 poly(DL-lactide-co-glycolide polymer) as a function of inherent viscosity with multiple solvents (Fig. 2). Analysis of Fig. 2 indicates that there is apparently no effect of molecular weight on the solubility parameter of this series of polymers. It did appear, however, that the polymer solubility was inversely proportional to the molecular weight of the polymer in higher total solubility parameter solvents (e.g., ~ 13 $(cal/cm^3)^{1/2}$) which have a greater hydrogen bonding component.

Using the experimentally determined solubility parameter of the polymer (9.8 $(cal/cm^3)^{1/2}$), the respective solubility parameters of the two solvents commonly utilized in this injectable implant system (e.g., NMP and DMSO) and Eqs. 1 and 2, the Flory-Huggins interaction parameter was calculated at 25° and 37°C (Table 2).

Analysis of Table 2 indicates that the Flory-Huggins interaction parameters for NMP, at a given temperature, were less than that for DMSO. Indicating, that NMP is a better solvent for these selected polymers compared to DMSO. If NMP is a better solvent than DMSO, it may be predicted that more non-solvent (e.g., water) is needed to desolvate the polymer. In addition, Table 2 predicts that as the temperature is increased from 25 to 37°C the interaction parameter decreases for both

Table 2
Flory-Huggins interaction parameter for selected polymers in NMP and DMSO as a function of temperature

Solvent	Temperature (°C)	χ_H^a	χ_S^b	χ^c
DMSO	25	0.48	0.29	0.77
	37	0.46	0.19	0.65
NMP	25	0.24	0.40	0.64
	37	0.23	0.26	0.49

[a]Calculated using Eq. (1).
[b]Calculated using Eq. (2). At 25°C, y-intercept = 0.173, $R^2 = 0.998$ and at 37°C, the y-intercept = 0.165, $R^2 = 0.998$.
[c]Flory-Huggins interaction parameter is the sum of enthalpic and entropic contributions.

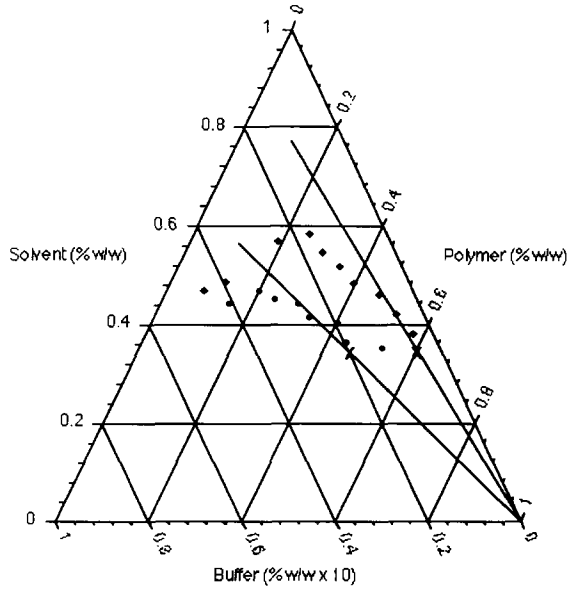

Fig. 3. Ternary phase diagram of polymer precipitation as a function of 75/25 poly(DL-lactide-co-glycolide), *N*-methylpyrrolidone, and phosphate-buffered saline, pH 7.4. Composition at 25°C (♦) and 37°C (●). The precipitation threshold, i.e., the tangent drawn from the 100% polymer point to the precipitation profile, is marked by (X).

solvents, suggesting that more water is needed to desolvate the polymer as the temperature is increased from 25 to 37°C.

Having characterized the solubility of the polymer and solvent, the interactions between polymer, solvent and non-solvent (water) were evaluated through the construction of ternary phase diagrams. Due to the fact that titration of distilled water (without buffering components) results in the same visual properties, the observed precipitation reported herein is the result of polymer precipitation and not precipitation of buffering components. Ternary phase diagrams were constructed using 75/25 PLG (I.V. = 0.11) as a function of solvent (e.g. NMP or DMSO) and temperature (e.g. 25 or 37°C) (Figs. 3 and 4, respectively). Those compositions above the precipitation curve are single phase systems while compositions below the curve are two phase systems, i.e., precipitated polymer and dissolved polymer. Analysis of Figs. 3 and 4 indicates that the quantity of water needed to coagulate or precipitate the polymer at 37°C was approximately twice that at 25°C. For example, 40% polymer in NMP requires 2% water at 37°C compared to 1% water at 25°C (Fig. 3). Comparison of Figs. 3 and 4 indicates that NMP solvent systems require almost twice as much water to initiate implant formation compared to DMSO systems. These results are in complete agreement with that predicted by Table 2.

Figs. 3 and 4 also indicate that the quantity of water required for precipitation or implant formation decreases as the polymer concentration is increased. Practically, this has important implications with regard to the design of appropriate in vitro methods and ultimately, in vivo release characteristics. The subcutane-

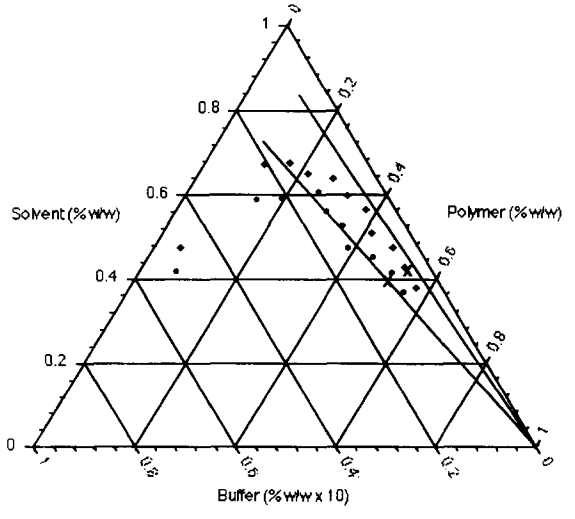

Fig. 4. Ternary phase diagram of polymer precipitation as a function of 75/25 poly(DL-lactide-co-glycolide), dimethyl sulfoxide, and phosphate buffered saline, pH 7.4 composition at 25°C (♦) and 37°C (●). The precipitation threshold, i.e., the tangent drawn from the 100% polymer point to the precipitation profile, is marked by (X).

ous and intramuscular environment has been shown to have limited amounts of water, e.g., 8.9% ($N=23$, w/w) [18]. The reported quantity of water represents total water, i.e., intracellular and extracellular. It must also be remembered that not all extracellular water is in contact with the injected liquid polymer. With this understanding, one could predict for formulations comprised of lower polymer concentrations ($<35\%$ w/w), that the formation of an implant may be dependent on the rate of fluid flow into the injection site. Since fluid flow in human adipose tissue may range from 7 to 53 ml/100 g per min. [19], implant formation may be erratic. If fluid flow into the injection site is relied upon to form the implant, the observed initial release may also be erratic. If implant formation is not rapid, drug dissolved in the biocompatible solvent is more likely to diffuse out of the polymer and into the surrounding tissue, resulting in a high initial release that causes local or systemic toxicity. If the available water is limited, the initial in vivo release of drug should be inversely proportional to the concentration of polymer. More specifically, formulations utilizing higher polymer concentrations should, based on Figs. 3 and 4, initially release less drug compared to formulations fabricated with lower polymer concentrations.

Conventional in vitro testing methods usually contain a significant excess of buffer. For example a 50 mg drop of formulation may be added to 5 ml of water, i.e., 1% w/w polymer in buffer. This is in dramatic contrast to the in vivo environment in which the injected formulation may be present in significant excess, compared to the aqueous phase.

The initial release of a model lipophilic drug (e.g., naltrexone) from injectable implants as a function of polymer concentration was therefore evaluated in rats, Fig. 5. Analysis of Fig. 5 indicates that the percent of naltrexone released from formulations (5% w/w naltrexone base) comprised of 57% 75/25 PLG (I.V. = 0.09) and 38% NMP was significantly lower than that observed for formulations comprised of 5% naltrexone base, 35% 75/25 PLG (I.V. = 0.09) and 60% NMP. The initial variability observed in Fig. 5 may be due to the observed incomplete implant formation in the first 2 h post-administration, resulting in a loss of implant contents upon the removal of implants. As predicted by comparison of Figs. 3 and 4, indicating that less water is required to initiate the precipitation of the equivalent amount of polymer in DMSO systems,

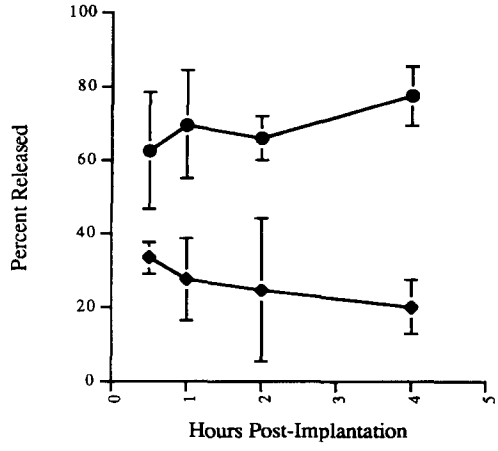

Fig. 5. Percent of naltrexone released from recovered implants in rats within the first 4 h of administration. Injectable implant formulations were comprised of 5% naltrexone as a function of 75/25 poly(DL-lactide-co-glycolide) concentration. Inherent viscosity of polymer was 0.11 dl/g. Data represent means of 5 animals (± s.d.). Legend: (●) 35% polymer, 5% naltrexone, 60% *N*-methyl pyrrolidone; (◆) 57% polymer, 5% naltrexone, 38% *N*-methyl pyrrolidone.

the initial release of naltrexone from DMSO injectable implant formulations was less than that observed for NMP based systems (data not shown). Comparable results have also been reported by Radomsky et al. [10].

In summary, injectable implants represent a novel biodegradable implant system for the delivery of drugs. Conventional Flory-Huggins and cohesive energy density theories can be utilized to understand these systems. This report has described powerful experimental techniques that can be utilized to predict the effect of different solvents, polymers and temperature on the properties of injectable implants.

References

[1] T.J. Roseman. Silicone rubber: a drug-delivery system for contraceptive steroids, in: A.C. Tanquary and R.E. Lacey (Eds.), Controlled Release of Biologically Active Agents, Plenum Press, New York, 1974, pp. 99–115.

[2] R. Burns, G. McRae and L. Sanders. A one year controlled release implant for the luteinizing hormone releasing hormone superagonist RS-49947. II Clinical performance results, J. Controlled Rel., (1990) 233–241.

[3] A.K. Kwong, S. Chou, A.M. Sun, M.V. Sefton and M.F.A. Goosen. In vitro and in vivo release of insulin from poly(lactic acid) microbeads and pellets, J. Controlled Rel., 4 (1986) 47–62.

[4] E.S. Nuwayser and D.L. Williams. Development of a delivery system for prostaglandins, in A.C. Tanquary and R.E. Lacey (Eds.), Controlled Release of Biologically Active Agents, Plenum Press, New York, 1974, pp. 145–164.

[5] B.C. Yoburn, A.H. Cohen and C.E. Inturrisi. Pharmacokinetics and pharmacodynamics of subcutaneous naltrexone pellets in the rat, J. Pharm. Exp. Therapeutics, 237 (1986) 126–130.

[6] A. Sintov, W. Scott, M. Dick and R.J. Levy. Cardiac controlled release for arrhythmia therapy: lidocaine-polyurethane matrix studies, J. Controlled Rel., 8 (1988) 157-165.

[7] Y.F. Maa and J. Heller. Controlled release of 5-fluorouracil from linear poly(ortho esters), J. Controlled Rel., 13 (1990) 11–19.

[8] R.L. Dunn, J.P. English, D.R. Cowsar, D.P. Vanderbilt. Biodegradable in situ forming implants and methods of producing the same. US Patent No. 4938763, July 3, 1990.

[9] W.J. Lambert and K.D. Peck. An in situ forming biodegradable system for the controlled release of proteins, Pharm. Res., 10 (1993) S-92.

[10] M.L. Radomsky, G. Brouwer, B.J. Floy, D.J. Loury, F. Chu, A.J. Tipton and L.M. Sanders. The controlled release of ganirelix from the Atrigel™ injectable implant system, Proc. Int. Symp. Control. Rel. Bioact. Mater., 20 (1993) 458–459.

[11] B.A. Coonts, B.K. Lowe, R.L. Norton, A.J. Tipton, G.L. Yewey and R.L. Dunn. Plasma concentrations of naltrexone following subcutaneous and intramuscular injections of Atrigel™ formulations in dogs, Pharm. Res., 10 (1993) S-192.

[12] E.G. Duysen, G.L. Yewey, J.L. Southard, R.L. Dunn and W. Huffer. Release of bioactive growth factors from the Atrigel delivery system in tibial defect and dermal wound models, Pharm. Res., 10 (1993) S-83.

[13] A.J. Tipton, S.M. Fujita, K.R. Frank and R.L. Dunn. A biodegradable injectable delivery system for nonsteroidal anti-inflammatory drugs, Pharm. Res., 8 (1991) S-196.

[14] T. Higuchi. Mechanism of sustained-action medication, J. Pharm. Sci., 52 (1963) 1145–1149.

[15] G.M. Bristow and W.F. Watson. Cohesive energy densities of polymers: part 1. Cohesive energy densities of rubbers by swelling measurements, Trans. Faraday Soc., 54 (1958) 1731–1741.

[16] A.F.M. Barton. Handbook of Solubility Parameters and Other Cohesion Parameters, 2 ed., CRC Press, Boca Raton, 1991, pp. 385–390.

[17] A. El-Koussi and E.R. Garrett. Electrochemical chromatographic determinations of morphine antagonists in biological fluids, with applications, J. Pharm. Sci., 73 (1984) 621–624.

[18] A. Martinsson. On the composition of human adipose tissue, Acta Med. Scand., 182 (1967) 795–803.

[19] S.L. Nielsen. Adipose tissue blood flow determined by the washout of locally injected $^{133}\times$enon, Scand. J. Clin. Lab. Invest., 29 (1972) 31–36.