DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 7(1), 1-25 (1981)

DEVELOPMENT OF BIODEGRADABLE AND INJECTABLE LATICES FOR CONTROLLED RELEASE OF POTENT DRUGS

R. Gurny*), N. A. Peppas**),
D. D. Harrington***, and G. S. Banker*)

*Department of Industrial and Physical Pharmacy
**School of Chemical Engineering
***School of Veterinary Medicine
Purdue University
West Lafayette, Indiana 47907

ABSTRACT

A new dosage form designed to provide parenteral drug delivery over a prolonged period of time following a standard injection has been investigated using testosterone as the model drug. The drug carrier consists of a biodegradable, biocompatible polymer in which the drug is dispersed on a molecular level. The

*Address reprint requests to Industrial and Physical Pharmacy Department, School of Pharmacy, Purdue University, West Lafayette, IN 47907.

polymer itself is formulated as a pseudolatex with high solid content (40.0%). The formulation has a low viscosity (97 cps) which can be injected easily through a hypodermic needle. The histopathology study showed good tissue compatibility of the pseudolatices and in-vivo tests on rats confirmed a prolonged release of drug over a 14 day period. The stability of the biodegradable poly-d,ℓ-lactic acid latex was found not to be significantly changed over 120 days of storage at room temperature (25°). A six month study showed a slight increase (10%) in the viscosity of the parenteral product when stored at room temperature. The increase was attributed to partial coalescence of the polymer particles which had an initial diameter of 0.45 μm.

INTRODUCTION

Injectable systems intended to provide continuous controlled drug release over periods of days, weeks or months have been investigated for the past three decades. Synthetic materials used in the early investigations included wax[1], silicone networks[2-6], and various types of hydrogels[7], which were formulated so as to be applicable in sustained drug delivery systems. A common problem of these systems was the sequence of histopathological reactions occuring while the pro-

ducts were in contact with the natural tissue. Only relatively recently has the use of biodegradable polymers as drug carrying tissue implants been considered as an approach to overcoming the major shortcoming of inadequate biocompatibility of earlier systems. Biodegradable polymers investigated for this use have included polylactic acid (PLA)[8-16] and polylactic glycolic acid (PLGA)[17-19], glycerides[20], polyamino acids, polyvinyl alcohol derivatives, polysaccharide derivatives and protein derivatives. The majority of implants currently in use in agriculture are compressed pellets of essentially pure drug, which are placed in an area of meat producing animals not consumed by man, such as the ear. Such sites may also have the advantage of producing relatively slow drug absorption. The direct contact of crystalline drug and localized lesions of high drug solute concentrations from such implants causes them to be less than ideal, especially for applications in the human body. However, the majority of these drugs have been utilized in implants.

A new system for sustained drug delivery by the parenteral route has been investigated in our laboratories; this system is based on the principal of drug inclusion or entrapment in a latex or pseudolatex dispersion, the latter being used as the drug carrier[21].

Biodegradable polymers are used to fabricate the dispersion in this case. The resultant drug carrying biodegradable colloidal or near colloidal formulations have low viscosities and can readily be injected with a conventional hypodermic needle to form a drug depot. Another unique feature of the biodegradable pseudolatex dispersions described here is that drug may be molecularly dispersed in the latex particles; in this way no crystalline drug is in contact with the implanted tissue, which further enhances the biocompatibility of these systems. The molecularly entrapped drug(s) are designed, so as to be released by slow diffusion from the matrix as the biodegradable polymeric structure breaks down.

PLA is known to undergo slow degradation[23-26] when introduced into animal tissues and to break down to lactic acid, a metabolite of low toxicity.

Histopathological examination of implanted poly-d,ℓ-lactic acid samples shows that these materials disappear from the implant site at a rate of 5 to 10% per month with only the mildest and most transient inflammatory response[27]. PLA in the d,ℓ-form was thus chosen as the polymer carrier for pseudolatex preparation and drug entrapment in this work. Poly-d,ℓ-lactic acid is reported to have good stability and relatively slow degradation rate in comparison to the ℓ

type and to the copolymer of the PLA with glycolic acid. Histopathological and in vitro chemical kinetic studies for PLA have been reported in the recent literature[23,25,28] The product of PLA biodegradation is lactic acid, a normal intermediate in carbohydrate metabolism and the end product of the anaerobic metabolism of glucose or glycogen[29]. Lactic acid can be converted to glucose or oxidized through pyruvic acid to carbon dioxide and water. This latter pathway to carbon dioxide and products is suggested by radioactive implant studies which show that the major fraction of radioactivity is lost via respiration. The biodegradable formulations of PLA are normally films or cylinders which must be injected subcutaneously by a trochar or must be surgically implanted. Earlier work in the field of molecular entrapment of drugs in latices[30-34] coupled with more recent research on the preparation of pseudolatices[35-37] directed the investigators toward this new type of biodegradable, drug carrying, injectable controlled release system.

MATERIALS AND METHODS

Latex Preparation - The polymer latices for the dispersion studies were prepared by conventional methods described earlier[35-37]. Poly-d,ℓ-lactic acid (average molecular weight 62,400, Sandoz Pharmaceuticals, East

Hanover, NJ) was used as a drug carrier and [4-^{14}C] - testosterone (> 50 mCi/mmol, Amersham Corp., Arlington Heights, IL) was employed as a drug marker and was molecularly dispersed in the latex system. The drug tracer was added to the polymer solution during the emulsification step as reported earlier[21,35,36] Pluronic F68 [PLU] (BASF Wyandotte Corp., Wyandotte, Mich.), Tween 80 (Atlas Chemicals Industries Inc., Wilmington, Del.), or sodium lauryl sulfate USP [NaLS] (Fisher Scientific Corp., Fairlawn, NJ) were used as the surfactants in the latex preparation (Table 1). The latices were prepared to have a total solid content of 40.0%. The apparent viscosity (Brookfield Viscometer - RVT 7, Spindel No. 3) of the latices (A,B,C) was measured at 20^{O} and was found to be between 97 - 623 cps (Table 1). The apparent average particle size of a selected latex was determined by scanning electron microscopy, (SEM) (Jeolco Scanning Electron Microscope, JSM-U3, Tokyo, Japan), measuring 100 particles. The latex was diluted with an equal volume of a 10% w/v methylhydroxypropylcellulose (Methocel E5 Premium, Dow Chemical Company, Midland, (MN) solution in water to facilitate preparation of a sample for SEM. This sample was dried at 30^{O} for 24 hours and coated with gold and carbon in a vacuum evaporator equipped with a rotating stage (Edwards,

TABLE 1

Composition of the Investigated Formulations

Formulation	PLA	PLGA	PLU 68	Tween 80	NaLS	Water	Peanut Oil	Testosterone* [μCi/ml]	Viscosity [cps]
[A]	36**	--	4	--	--	60	--	100	97
[B]	36	--	--	4	--	60	--	--	183
[C]	36	--	--	--	4	60	--	--	623
[D]	36**	--	--	--	--	64	--	--	--
[E]	--	--	4	--	--	96	--	--	--
[F]	--	--	--	4	--	96	--	--	--
[G]	--	--	--	--	4	96	--	--	--
[H]	--	--	--	--	--	--	100	10	--
[I]	--	36**	--	--	--	96	--	--	--

*Calculated as μCi of final latex.
**All values are w/w of final latex.
*** > 100 mesh particles.

Model E12-E2, Manor Royal, Crawley, Sussex, England).

In-vitro Evaluation - The in-vitro release of drug from the latex system [A] (see Table 1) was determined by measuring the dissolution rates by a dialysis procedure. The rate of diffusion of neat drug from peanut oil, [H] (10 μCi per ml), was used as the reference system of these studies. Dialysis tubing, 3/8" diameter X 1" long, (Fisher Scientific Company, Pittsburgh, PA) was washed with redistilled water prior to use. Fifty ml of Krebs buffer* was warmed to $37^{o} \pm 1^{o}$ in a jacketed beaker (4" x 2") and stirred slowly with a magnetic stirrer (60 rpm). A 1 ml sample was pipetted into the dialysis bag, and suspended in the buffer. Samples of 0.5 ml were removed from the sink at predetermined time points. The samples were counted for 10 minutes with PCS (Amersham/Searle, Arlington, Heights, IL) as the scintillation fluid in a Beckman LS-250 (Beckman Instruments, Fullerton, CA) liquid scintillation counter. A quenching curve was constructured using a series of ^{14}C standards.

Histopathology - A foreign body histopathologic evaluation of various latex systems and additives was carried out by injecting the test materials into the

---

*In [g/l]: NaCl: 6.9, KCl: 0.35, $CaCl_2$: 0.28, $MgSO_4$: $7H_2O$: 0.29, $NaHCO_3$: 2.10, KH_2PO_4: 0.16, Dextrose: 1.00.

anterior thigh muscles of white, adult, male sprague-Dawley rats (250-270g) using a sterile syringe equipped with a 21 gauge needle. Studies were carried out in 2 phases, the first of which evaluated the short term (3 to 4 day) acute tissue reaction to various surfacants, vehicles and control materials. In the second phase, tissue reaction to latex PLGA, latex PLA and PLU-F86, were evaluated in rats sacrificed at varying post injection (PI) time intervals. The samples evaluated, dosage and PI days of sacrifice are shown in Table 2. All rats were killed by CO_2 exposure over dry ice, necropsied immediately, and the thigh muscle with injection site, were removed and fixed in standard neutral phosphate-buffered formalin. Multiple transverse sections of the anterior thigh muscle group injection site were collected from each rat at approximately 2-3 mm intervals. Samples were processed using standard paraffin embedding techniques, sectioned and stained with hematoxylin and eosin[38].

Stability - The degradation of the polymer due to preparation of the latex, and the stability of the polymer in the latex system, were determined by molecular weight analysis. The average molecular weights of each polymer was determined by a gel permeation chromatograph (GPC) (Waters Associates, Inc., Milford, MA) equipped with a differential refractive index detector

TABLE 2

Materials Subjected to Histopathologic Examination

Sample	Injection Volume (ml)	Post Injection Days Evaluated
	Acute Studies	
I. Surfactant Group		
1. NaLS	0.25	3
2. Tween 85	0.25	4
II. Vehicle Group		
1. PLGA	0.25	3
2. PLA	0.25	4
III. Control Group		
1. Saline	0.25	3
	Long Term Studies	
I. Vehicle Group		
1. PLGA Latex	0.5	7,14,28,56,84
2. PLA Latex	0.5	7,14,28,56,84
II. Surfactant Group		
1. PLU - F68	0.5	7,28

and 5 columns (μ Styragel) (Waters Associates Inc., Milford, MA) having the following nominal exclusion limit designations: 10^6Å, 10^5Å, 10^4Å, 10^3Å, 10^2Å. Changes in molecular weight of the polymer were followed over a three month period. The solvent used was tetrahydrofuran (THF) (Waters Associates Inc., Milford,

MA) at a flow rate of 2 ml/min using a solvent delivery system type 6000 A (Waters Associates Inc., Milford, MA). The sample concentration was 0.1% and the injection volume was 500 µl using an injection loop U6K (Waters Associates Inc., Milford, MA). Samples of latex were dried under vacuum in order to remove the water with a minimal effect on the polymer stability; the polymer was redissolved in THF and filtered through a 0.45 µm Millipore filter (Millipore Corporation, Bedford, MA). This filtering process was carried out very slowly so as to avoid polymer degradation due to shear effects or breakage of the membrane. An indirect method of calibrating the GPC columns was successfully used to obtain average molecular weights averages for the PLA. Five polystyrene standards (Waters Associates Inc., Milford, MA, molecular weight average: 2,350 - 470,000) with a very narrow molecular weight distribution were analyzed to construct the calibration curve and to calculate the Q factor. Molecular size averages of an unknown molecular weight were converted to molecular weight by means of the Q factor method[39]. A Q factor of 41.4 was used for polystyrene[40].

In-Vivo Evaluation - The in-vivo release profiles of the drug entrapment products were determined employing rats as described previously in the histopathology section. Again a

0.2 ml sample of latex was injected in the rear thigh of the animal using a 21 gauge hypodermic needle. Blood samples of 200 µl were taken from the orbital sinus plexus and were digested in a solution of 200 µl of perchloric acid, 70% (Mallinchrodt Inc.), 200 µl of hydrochloric acid (1N), (Fisher Scientific Corp.), and 30 µl of hydrogen peroxide, 30% (Mallinchrodt Inc.), for at least 4 hours at 60°. After digestion, Aquasol-2 (New England Nuclear, Boston, MA) was added as the scintillation cocktail and the samples were scintillation counted for 10 minutes as described earlier.

RESULTS AND DISCUSSION

The goals of this ongoing research project were (i) to determine the feasibility of preparing a biodegradable polymer in latex form, (ii) to determine the histopathological compatibility of such a system, (iii) to determine the stability of the polymer in such aqueous dispersion form, (iv) to evaluate the release profile and rate of an entrapped drug in-vitro from the new system and (v) to preliminarily evaluate the release profile of a model drug from this new type of dosage form in a test animal.

To achieve these objectives a preparation method of a latex dispersion, of the polymer in a colloidal or near colloidal form, with adequate physical stability,

(freedom from settling and caking, dispersibility), and low viscosity to permit injection, was required. It was also necessary to developed a latex-drug formulation in which the drug could be incorporated without producing coagulation of the latex.

Figure 1 presents an electron micrograph of the particles of the typical PLA latex, as prepared, and/or

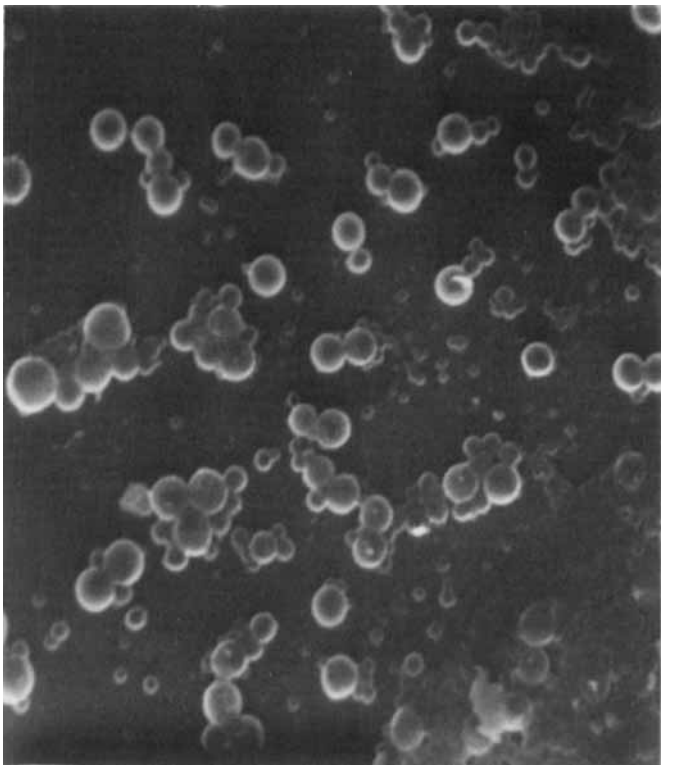

FIGURE 1

Electron Micrograph of the PLA Latex. 10,000X. Average Particle Size 0.45µm.

following loading with the test drug (testosterone). The average particle size of the latex shown [formulation A] is 0.45 μm ± S.D. 0.16. The viscosity of the latex shown, containing 40% solid, was 96 cps at 20°, immediately following manufacture, and increased about 10% following 6 months storage at room temperature.

Table 3 presents average molecular weight data for the polymer, and the polymer in latex form over a 120 day storage period. The weight average molecular weight of this polymer, $\bar{M}_w$ decreased 9 to 10% as a result of the latex manufacturing process. This molecular weight reduction was attributed primarily to a shear depolymerization rather than a chemical effect.

TABLE 3

Weight Average Molecular Weight of the PLA Polymer and of the Polymer in Latex Form as Measured over a Storage Period.

	Neat Polymer	Latex Formulation				
Day		0	30	60	90	120
Average[a]	62,400	57,020	56,740	56,230	54,932	55,396
Standard Deviation	325	1,617	2,037	1,340	1,136	1,748

[a]Three measurements

A more critical finding was that the PLA did not significantly change in molecular weight in pseudolatex form when stored over a 4 month period (Figure 2). The Q factor used for the PLA molecular weight determination was 30.42. The in-vitro dissolution dialysis release of (100 μCi/ml) [4-^{14}C] testosterone product in PLA latex [A], together with the profiles of a conventional oil based formula is shown in Figure 3. The oil based formulation [H] (10 μCi/ml) released the active compound at a rate more than ten times faster than the latex system, where the compound is molecularly dispersed in the polymer matrix. The release of the drug from the latex system in the Krebs buffer

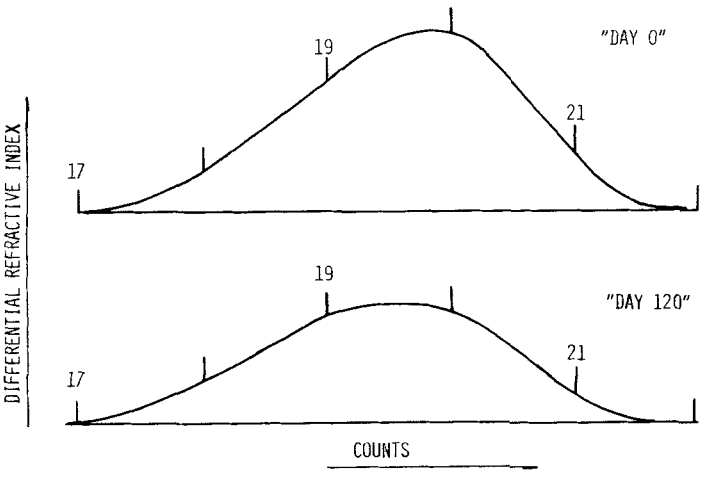

FIGURE 2

GPC Analysis of the Neat Polymer and the Polymer Latex [A] after 120 Days of Storage at Room Temperature.

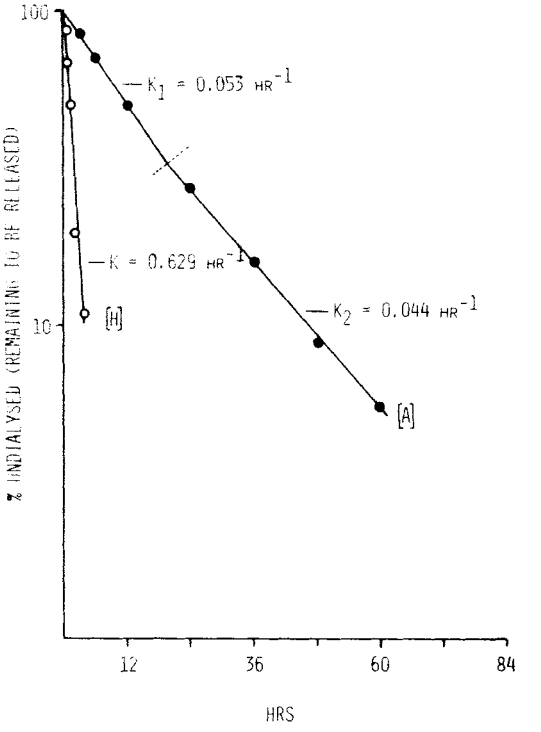

FIGURE 3

In Vitro Release from Latex [A] and Solution [H] Loaded with Testosterone.

(simulated tissue fluid), shows a somewhat more rapid release over the first 12 to 24 hours. This initial more rapid release may be the result of drug located on the particle surface, the second slower part of the release profile being due to drug diffusion out of the polymer particles and breakdown of polymeric material.

Histopathology

Acute Studies - Histopathologic examination of muscle injection sites disclosed that all agents evaluated in the acute studies, with the exception of saline, caused muscle necrosis and inflammatory reactions. However, the degree of necrosis as well as nature of the inflammatory response to various agents differed. Using the amount of muscle degeneration and necrosis seen, in conjunction with the character of the inflammatory reaction (elecited as criteria), agents were placed in one of 3 groups.

Group I agent, Tween 85, appeared to be least irritating. Muscle degeneration and necrosis occurred. However, lesions consisting of a large focus muscle degeneration and myofiber lysis or liquefaction similar to that observed with agents in groups II and III were not evident. The inflammatory cell infiltrate consisted largely of microphage although some neutrophils were present. The finding of basophilic muscle fibers with multiple centrally placed nuclei (rowing) was observed and interpreted as early attempts at regeneration. A mild steatitis was sometimes observed.

Group II agent produced lesions of intermediate severity; agent placed in this category was sodium

lauryl sulfate. The injection site lesions commonly appeared as a large, irregularly shaped focus of coagulative muscle necrosis. In some lesions, there was separation of myocytes within muscle bundles due to edema and fibrin deposition. Small foci of extravasation of erythrocytes were occasionally seen. Mineralization of myocytes at the periphery of lesions was also common in this group. The inflammatory cell infiltrate, as in group I, was primarily mononuclear. Evidence of attempts at muscle regeneration was also found. Some inflammation of adipose tissue adjacent to foci of muscle necrosis was usual.

Group III agents included latex PLGA and PLA. Morphologically, lesions produced by these agents differed from those seen with group II agents primarily in that there was both a necrotizing myositis as well as a steatitis with liquefaction. Liquefaction was accompanied by marked infiltration by neutrophils. Other features of the lesions were similar to those seen in group II rats.

Long Term Studies

PLU-F68: Histologically, muscle necrosis in rats injected with PLU-F68 was minimal. Lesions in animals killed on PI day 7 appeared as small foci composed of a few multinucleated giant cells and macrophages with

finely vacuolated cytoplasm. Lesions in rats killed on PI day 28 contained only macrophages.

PLGA Latex: Lesions in muscle at PI day 7 consisted of myofiber necrosis with a dense infiltration by macrophages, accumulation of multinucleated giant cells and mild steatitis. Liquefaction of necrotic muscle and adipose time was pronounced in the PI day 14 muscle sections. Multinucleated giant cells and macrophages with foamy cytoplasm were numerous in the tissue surrounding the foci of liquefaction. Small fatty cysts were present in adipose tissue adjacent to muscle lesions. Necrotic muscle debris in sections collected on PI day 28 had been largely removed and replaced by macrophages and multinucleated giant cells. Adipose tissue in these sections also contained small cysts similar to those seen in PI day 14 sections. Lesions in muscles sections from rats killed on PI days 56 and 84 were similar except for size. PI day 84 lesions were smaller. Both were composed of dense accumulations of macrophages and a few multinucleated giant cells.

PLA Latex: Examination of PI day 7 sections revealed a large irregular focus of liquefactive necrosis involving muscle and adipose tissue with neutrophil infiltration. The periphery of the lesion was infiltrated by macrophages. Multinucleated giant

cells and myofibers showing evidence of attempted regeneration were also present. Lesions in sections of muscle collected on PI day 14 were composed of a dense admixture of macrophage, multinucleated giant cells and some lymphocytes. Variable size vacuoles, some containing small amounts of unstained granular material, were found in some portions of the lesions. PI day 28 lesions were smaller than those found in PI day 14 sections but contained a similar inflammatory cell population. However, many of the macrophages and giant cells had a vacuolated cytoplasm. Variable size fatty cysts like those seen in PI day 14 sections were also present. Except for a reduction in size, lesions in PI day 56 sections were identical to those seen in PI day 28 muscle. No lesions were found in PI day 84 muscle sections examined.

Figure 4 shows the results of the drug release studies carried out on an average of 6 rats for each formulation tested. The rapid release of the drug from the oil solution seen in-vitro obviously occured in-vivo. The much slower release of the entrapped drug from the PLA latex is seen in the in-vivo study. While the drug containing PLA latex A product does not produce an ideal release profile in-vivo (showing a peak before the plateau), it does demonstrate the ability to

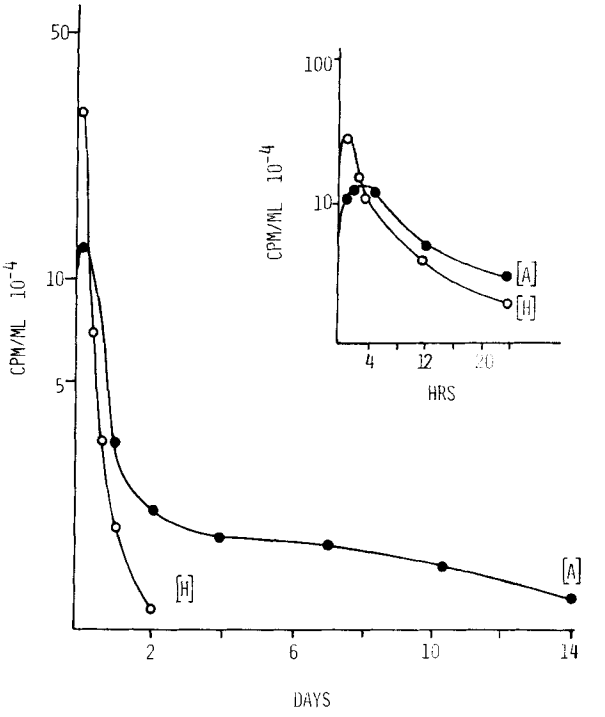

FIGURE 4

In Vivo Release from Latex [A] and Solution [H] Loaded with Testosterone Tested on Six Animals Over 14 Days.

produce a protracted, nearly steady state blood level from day 4 through day 14.

CONCLUSION

In conclusion, this study has demonstrated that a biodegradable polymer may be prepared as a colloidal or near colloidal latex, which appears to have good molecular weight stability at room temperature storage.

Drug entrapment at a molecular level is possible in such latices and the resultant products may readily be injected through a standard hypodermic syringe. The formulation [A] had fairly good tissue compatibility and the system appears to have a potential to produce prolonged blood drug levels in-vivo. The drug containing latex systems did provide an initial (and usually undesirable) burst of drug release, and released more rapidly than do implants. Nevertheless, this new dosage form for parenteral drug delivery appears to show promise for controlled prolonged drug release of an injectable product with good tissue biocompatibility. This new approach to using biodegradable polymer as a drug carriers is thought to be a promising step toward the development of clinically useful new parenteral products. Research is continuing to produce more uniform total drug release profiles through formula modifications, by better controlling the drug entrapment within the latex particles and the compactness of the injected depots.

ACKNOWLEDGMENT

The authors acknowledge the technical assistance of Miss I. Kri nitzsky and D. Taylor.

REFERENCES

1) F.J. Saunders and A.L. Raymond, U.S. Patent 2.413.419 (1946)

2) D.R. Mishell and M.E. Lunekin, Fertil. Steril., 21, 99 (1970)

3) T.J. Roseman and W.I. Higuchi, J. Pharm. Sci., 59, 353 (1970)

4) G. Beuagiano, M. Ermini, C.C. Chang, K. Sundaram, and F.A. Kincl, Acta Endocrinol., 29, 63 (1970)

5) E.M. Coutinho, C.E.R. Nattos, A.R.S. Sautanna, J.A. Filho, M.C. Silva, and J.H. Tatum, Contraception 2, 313 (1970)

6) E.M. Coutinho, and A.R.da Silva, Fertil and Steril, 25, 170 (197r)

7) B.D. Ratner and Hoffman in "Hydrogels for Biomedical Application", J. Andrade, editor, American Chemical Society, Symposium Series No. 31, Washington, D.C., 1976, p. 1

8) S. Yolles, J. Eldridge, T. Leafe, J.H.R. Woodland, D.R. Blanke and F. Meyers in "Advances in Experimentor Medicine and Biology", Vol. 47, Plenum Press, 1974, p. 177

9) S. Yolles, U.S. Patent 3.887.699 (1975)

10) T.R. Jackanicz, H.A. Nash, D.L. Wise and J.B. Gregory, Contraception, 8, 227 (1973)

11) S. Yolles, T.D. Leafe and F.J. Meyer, J. Pharm. Sci., 64, 115 (1975)

12) S. Yolles, T.D. Leafe, J.H.R. Woodland and F.J. Meyer, J. Pharm. Sci., 64, 348 (1975)

13) R.H. Renning, L. Malspeis, S. Frank and R.E. Notari, Natl. Inst. Drug Abuse Res. Mongr. Ser., (4) 43 (1976)

14) C. Thies, Natl. Inst. Drug Abuse Res. Mongr. Ser. (4), 19 (1976)

15) N. Mason, C. Thies, and T.J. Cicero, J. Pharm. Sci., 65, 847 (1976)

16) J.H.R. Woodland, S. Yolles, D.A. Blake, Martin Helrick, and F.J. Meyers, J. Med. Chem. 16, 897 (1973)

17) R.G. Sinclair, Environ. Sci. Technol., I, 955 (1973)

18) A.D. Schwope, D.L. Wise, and J.F. Howes, Natl. Inst. Drug Abuse Res. Mongr. Ser., (4), 13 (1976)

19) D.L. Wise, Acta Pharm. Fuecica, 13, 34 (1976)

20) M.F. Sullivan, and D.R.Kalkwarf, Natl. Inst. Drug Abuse Res. Mongr. Ser., (4), 27 (1976)

21) R. Gurny, M.A. Gonzalez, D. Kildsig, and G.S. Banker, Drug Dev. Ind. Pharm. (submitted for publication)

22) Kronenthal T.L. in "Polymers in Medicine and Surgery" Plenum Press, New York, 1975, p. 119

23) A.F. Hegyeli, J. Biomed. Mater Res., 1, 205 (1973)

24) R.J. Ruderman, E. Bernstein, E. Kairinen, and A.F. Hegyeli, J. Biomed. Mater Res., 1, 215 (1973)

25) R.K. Kulkarni, E.G. Moore, A.F. Hegyeli, and F. Leonard, J. Biomed. Mater. Res., 5, 169 (1971)

26) R.A. Miller, J.M. Brody, and D.E. Cutright, J. Biomed. Mater. Res., 77, 711 (1977)

27) R.K. Kulkarni, K.C. Pani, C. Neuman, and F. Leonard, Arch. Surg., 93, 839 (1966)

23) J.M. Brody, D.E. Cutright, R.A. Miller, and G.C. Battistone, J. Biomed. Mater. Res., 1, 155 (1973)

29) J.M. Anderson and D.F. Gibbons, Biomat., Med. Dev., Art. Org., 2, 235 (1974)

30) H. Goodman, and G.S. Banker, J. Pharm. Sci., 59, 1131 (1970)

31) C.T. Rhodes, K. Wai, and G.S. Banker, J. Pharm. Sci., 59, 1578 (1970)

32) C.T. Rhodes, K. Wai, and G.S. Banker, J. Pharm. Sci., 59, 1581 (1970)

33) J.C. Boylan and G.S. Banker, J. Pharm. Sci., 62, 1177 (1973)

34) A.B. Larson and G.S. Banker, J. Pharm. Sci., 65, 838 (1976)

35) A.M. Ortega, 1977 Ph.D. Thesis, School of Pharmacy, Purdue University

36) J. Vanderhoff, M.S. El-Asser, and J. Ungerstad, U.S. Patent application number 867,037 (1977)

37) M.S. El-Asser in "Advances in Emulsion Polymerization and Latex Technology," G. Poehlein editor, Lehigh University Press, Bethlehem, PA 1975

38) G.L. Luna, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathologic, 3rd edition, McGraw-Hill Book Company, New York (1968).

39) K.H. Altelt, Theory and Mechanics of Gel Permeation Chromatographic in "Advances in Chromatographic, Vol. 7, M. Dekker, New York, p. 3, 1968.

40) Waters Associates Inc., personal communication, May 1978.