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Thixotropically Based Biodegradable Intradermal Liquid Depot System Bearing Diltiazem Hydrochloride

M. K. CHOURASIA, M. S. ASHAWAT, A. K. JAIN, NEETI JAIN[1], A. JAIN, NITIN K. JAIN AND S. K. JAIN*
Pharmaceutics Research Projects Laboratory, Department of Pharmaceutical Sciences,
Dr. Hari Singh Gour Vishwavidyalaya, Sagar-470003.
[1]N. D. M. V. P. Samaj's College of Pharmacy, Nashik-422003.

Conventional treatment requires frequent administration of drugs to maintain effective concentration at the site of action. In the present work an implantable delivery system has been developed which when comes in contact with biological fluid, solidifies immediately and release the drug in a controlled manner for prolonged period of time. Polylactic acid, polyglycolic acid and copolymers of polylactic acid and polyglycolic acid were synthesized and characterized for various physicochemical attributes. IR spectra of the synthesized polymers was determined and it was found to be identical to that reported in the literature. Molecular weight of the polymers was determined with the help of viscosity measurement. Solubility and hydrolysis of the polymers was determined. Drug delivery systems were fabricated using synthesized polymers and characterized *in vitro* for various parameters. Drug content was estimated by direct measurement of absorbance whilst viscosity was determined using Ostwald viscometer. *In vitro* release for the formulations was determined for 10 d using dialysis tube diffusion technique, which revealed slow release of drug from formulation. On the basis of *in vitro* characterization studies, selected formulations were subjected to *in vivo* performance where drug plasma level was monitored after intradermal administration. *In vivo* studies revealed sustained release of the entrapped drug from formulations, which is reflected from the persistence of drug in blood for longer period of time.

For many decades, treatment of an acute disease or chronic illness has been accomplished by delivering drugs to patients, via various dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories. However, one concern that is applicable even today is that these drug delivery systems do not ensure maximum therapeutic responses. It is often necessary to take this type of delivery system several times a day to achieve and maintain effective concentration of the drug at the site of action. This results in fluctuating drug levels, premature biodegradation of the drug, undue toxicity, inability to attain effective concentration, and patient compliance. Current research is aimed at the development of drug delivery systems with maximum therapeutic benefits for safe and effective management of diseases.

*For correspondence
E-mail: drskjainin@yahoo.com

Polymeric drug delivery systems especially biodegradable polymers have been proves to be successful for controlled and site specific delivery of bioactive agents[1,2]. Polylactic acid and its copolymers with glycolic acid have been known for the past 20 years for its considerable chemical, biological and mechanical characteristics. These characteristics include structural simplicity, comparatively easy synthesis and simple degradation mechanism with no complex product formation. One of the reasons for the popularity of the lactide/glycolide material in drug delivery system is their relative ease of fabrication into various types of delivery systems. Polylactic acid, polyglycolic acid and their copolymers have been used extensively to form drug deliv-

ery systems like microcapsules[3,4], microparticles[5], pseudolattices[1] and nanoparticles[6].

Diltiazem is rapidly and completely absorbed through gastrointestinal tract after oral administration but it undergoes extensive first pass hepatic metabolism with a bioavailability of only 40%. Apart from that it is 80% bound to plasma protein, approximately 2-4% drug is excreted unchanged in urine and hence, it should be given two-three times a day for prolonged period of time to maintain the blood pressure[7,8]. Considering all these constrains of orally administered diltiazem and severity of the disease, it was felt necessary to design and develop a controlled release formulation that would deliver drugs at a constant rate for prolonged period of time and protect drugs from hepatic first pass metabolism.

Therefore in the present project an implantable prolonged controlled drug delivery system was developed that was implanted with the help of a hypodermic needle without any incision. This parenteral depot system of diltiazem is based on negative thixotropy, which solidified immediately after injecting and releases drug at a predetermined rate for prolonged periods of time.

MATERIALS AND METHODS

Diltiazem was generously supplied by M/s Plethico Pharmaceuticals, Indore. Polylactic acid, polyglycolic acid, hexafluoro-propan-2-ol, N-methyl-2-pyrrolidone and stannous octoate were procured from Sigma, St. Louis, Mo. Lauryl alcohol and acetonitrile were procured from CDH Chemicals, Mumbai. All other reagents were of analytical grade and water was double distilled.

Synthesis of polymers:

The method of synthesis of polylactic acid (PLA), polyglycolic acid (PGA) and copolymers of polylactic acid and polyglycolic acid (PLGA) was similar to that described by Gilding and Reed[9] with slight modifications. Commercial lactide or glycolide was purified three times by dissolving repeatedly in the minimum quantity of ethlylacetate at 60° and crystallization was induced by cooling to room temperature. The purified dimer was dried at 40° in a vacuum oven for a minimum of 24 h prior to use.

Synthesis of PLA:

Homopolymers from lactide was prepared by placing purified lactide (4.5 g) and 0.1 ml of 8% stannous octoate solution in hexane into a pre dried polymerization tube (Quickfit type MF 24-3, fitted with vacuum release adaptor). The tube was immersed in a bath of silicone fluid at 160° and the contents heated for 6 h while being stirred. The hexane and liberated water were evaporated off as the tube was maintained under reduced pressure of 25 mm Hg using an attached vacuum pump with liquid nitrogen trap. After polymerization the tube was immersed in ice water and the solidified mass formed was dissolved in chloroform. The solution was filtered into excess methanol to precipitate out the polymer and after repeating the purification process the polymer obtained was vacuum dried at 80° for 12 h prior to use.

Synthesis of PGA:

Homopolymer from glycolide (3.3 g) was prepared similarly except that 0.01, 0.03 percent lauryl alcohol in hexane was employed as initiator in addition to the catalyst. It was impossible to form solutions of the resulting polymers for purification purposes as they failed to dissolve in all common organic solvents. The only poor solvent reported is hexafluoro-propan-2ol[10] (Gilding *et al.*, 1981), which is prohibitively expensive for routine use. The solid mass of crude polymer formed was used after grinding into suitable size fragments.

Synthesis of PLGA:

Copolymer from DL-lactide and glycolide was similarly prepared with 50 M each. lauryl alcohol in hexane (0.01%) was employed as an initiator in addition to the catalyst. After completion of the polymerization process the tube was immersed in an ice-bath, which resulted in the formation of amorphous mass that was dissolved in chloroform and precipitated with excess methanol. After repeating the purification process the copolymer obtained was vacuum dried at 80° for 12 h.

Polymer characterization:

IR spectra of PLGA were recorded using potassium bromide on an IR spectrophotometer (Shimadzu, Japan) whereas that of PLA and PGA were recorded on a Jasco model 810. The molecular weight of the polymer, either weight average or number average was determined using the Mark-Houwink equation. For determination of solubility, a weighed quantity (100 mg) of the polymer was mixed with 10 ml of solvent. The mixture was kept for a period of 24 h with intermittent shaking at room temperature and the extent to which the polymer dissolved in a particular solvent was determined. Hydrolysis of the polymers was determined on the basis of the fact that hydrolysis is the principal mode for the degradation of lactide, glycolide and co-polymers.

The hydrolysis is affected by the size and hydrophilicity of the particular polymers and the pH of environment. Hydrolysis studies were carried out for 15 d to study the effect of pH on PLA and co-polymer.

Design and development of biodegradable systems:

On the basis of pharmacokinetic parameters of diltiazem, the drug-input rate through the dermal layer requires to achieve effective plasma concentration was calculated[11,12] and using this the total drug concentration required in the formulation was calculated. The drug delivery system was prepared using the method reported by Dunni and Tipton[13]. Drug and synthesized polymer (10%, 15% and 20% w/w) were dissolved with stirring in N-methyl-2- pyrrolidone. This drug polymer solution was injected intradermally for achieving prolonged delivery of diltiazem as it would solidify as soon as it comes in contact with biological fluid or aqueous media.

Evaluation of the drug delivery system:

The drug content of the formulation was determined directly by measuring the absorbance against the solvent N-methyl-2-pyrollidone as a blank using spectrophotometer at 237 nm (Shimadzu 1601, Japan) while the viscosity was determined using an Ostwald viscometer.

***In vitro* evaluation:**

In vitro release of diltiazem from different formulations was performed using the dialysis tube diffusion technique. The formulation (2 ml) was placed in hermetically tied dialysis assembly. The entire system was kept immersed in 50 ml of PBS (pH 7.4) contained in a beaker at 37°. The contents of the beaker were stirred continuously with the help of a magnetic stirrer. Samples were withdrawn periodically for 10 d and drug content was assayed.

***In vivo* performance studies:**

In vivo evaluation of the designed drug delivery system was performed in a lower animal by measuring drug plasma concentrations. The following drug delivery systems, DILPLG-1, DILPLG-4 and DILPLG-7, which exhibited drug release nearer to the drug input rate required to maintain effective plasma concentration (calculated on the pharmacokinetic parameters of the drug) were selected for *in vivo* studies.

Wistar rats were used for the *in vivo* study, which was approved by animal ethical committee. Rats (18) were taken, weighed individually and divided into three groups of six animals each. Group one animals were administered DILPLG-1 intradermally with the help of 24 gauge needle while animals of second and third group were given DILPLG-4 and DILPLG-7, respectively. Following intradermal administration of drug delivery system, blood sample were collected from marginal eye vein of all animals using special heparinized curved cannula at scheduled time intervals for 15 d. The frozen serum was thawed at 25±10° and 100 µl of serum was pipetted into a clean dry Eppendroff tube. To this 200 µl of dilute hydrochloric acid was added vortexed for 5 min followed by addition of 100 µl of 0.1 M sodium hydroxide solution. Volume was made up to 500 µl with deionized water and vortexed. Acetonitrile was added to make the volume up to 1.0 ml, vortexed for 5 min and centrifuged at 10 000 rpm for 10 min at 25±1°. The supernatant was filtered through Whatman membrane filter (0.45 µm) into another Eppendroff tube and 20 µl volume of this solution was injected into the HPLC system (Shimadzu RF 350, Japan).

RESULTS AND DISCUSSION

Polylactide was synthesized by simple and convenient method reported by Gilding and Reed[9]. Lactide and glycolide were polymerized indigenously to obtain poly (dl-lactide and glycolide) of desired quality. Copolymer of equal ratio of 50 M of each dimer was obtained at 200°. Time taken for polymerization was 4.5 h and reaction was initiated by adding 0.01% lauryl alcohol in the presence of 0.03% stannous octoate as catalyst. Table 1 presents the reaction conditions for the synthesis of different polymers along with their appearance characteristics. IR spectroscopy data of the synthesized polymers were recorded using an IR spectropho-

TABLE 1: DETAIL OF SYNTHESIS AND APPEARANCE OF VARIOUS POLYMERS

Polymer code	Temperature	Reaction time (h)	Lauryl alcohol concentration	Stannous Octoate Concentration	Polymer colour	Texture
PLA	160°	6	-	0.2%	Amber	Amorphous
PGA	200°	5	0.03%	0.09%	Cream	Hard
50:50 PLGA	200°	4.5	0.01%	0.03%	Cream	Amorphous

tometer, which were found to be much identical to those of the polymers reported in the literature (Table 2). Polymers were found to be hydrophobic in nature, which is reflected from their solubility in various aqueous and non-aqueous solvents. Polymers were found to be soluble in organic solvents while PGA exhibited solubility only in hexafluroisopropanol and hence it was not selected for further studies (Table 2). Viscosity and average molecular weight of the synthesized polymers were found to be 10 250±375 and 12 050±292 with PLA and PLGA, respectively. Hydrolysis studies were carried out for 15 d to study the effect of pH on PLA and copolymer, PLGA. It was observed that the hydrolysis was more under basic conditions than acidic (Table 2).

Formulation of drug delivery systems necessitates the determination of drug input rate that was calculated to be 100 ng/h for 150 g of rat. Therefore, the total drug required for 15 d delivery is 1.767 mg but considering drug-skin partitioning and polymer-drug partitioning, 2.5 mg drug was taken for the formulation of drug delivery system. Drug content of different formulations was determined by spectrophotometric method against the solvent N-methyl-2-pyrollidone as a blank at 237 nm and viscosity was determined using an Ostwald viscometer (Table 3). *In vitro* studies were performed to ascertain the behaviour of the formulation especially with respect to its drug release profile.

The designed drug delivery system was studied for *in*

TABLE 2: CHARACTERIZATION OF SYNTHESIZED POLYMERS

Parameter		PLA	PGA	PLGA
Wavelength obtained in cm^{-1}	C=O ester	1750	1790	1759
	C-H stretch	3025	3060	2997.2
	C-H bend	1460	1460	1425.3
	C-O stretch	1200	1228	1132
	C-C stretch	910	910	956
	O-H stretch of acid	-	-	3517
Solubility	Chloroform	+++	-	+++
	Carbon tetrachloride	++	-	+++
	Ethyl acetate	++	-	+++
	NMP	++	-	+++
	DMSO	+++	-	++
	Hexafluoroiso-propanol	++	++	++
	Acetone	++	-	-
	Benzene	-	-	-
	Ether	-	-	-
Percent Polymer Hydrolyzed	pH 6.8	21.42±0.96	-	23.43±0.87
	pH 7.4	84.75±2.12	-	78.26±2.28
	pH 12.7	94.16±2.97	-	86.74±2.58
Viscosity average molecular weight		10250±140	-	12050±200

PLA: Polylactic acid; PGA:Polyglycolic acid; PLGA: Polylactide co glycolide; NMP: N-methyl-2- pyrrolidone; DMSO: Dimethyl sulfoxide.

TABLE 3: COMPOSITION AND EVALUATION OF VARIOUS FORMULATIONS

Formulations			Viscosity (poise)	% Drug content
Formulation code	D/P ratio	Quantity of NMP (ml)		
DILPLA-1	15:85	2	1.37±0.21	98.6±3.6
DILPLGA-1	10:90	1	1.51±0.26	99.9±3.8
DILPLGA-2	10:90	2	1.50±0.27	99.5±3.6
DILPLGA-3	10:90	3	1.42±0.21	99.4±3.2
DILPLGA-4	15:85	1	1.39±0.19	98.6±3.4
DILPLGA-5	15:85	2	1.21±0.11	98.5±3.0
DILPLGA-6	15:85	3	1.03±0.10	98.3±2.8
DILPLGA-7	20:80	1	1.02±0.08	99.7±3.4
DILPLGA-8	20:80	2	1.02±0.09	99.5±3.1
DILPLGA-9	20:80	3	1.01±0.03	99.2±3.2

D/P: Drug/Polymer, NMP: N-methyl-2- pyrrolidone.

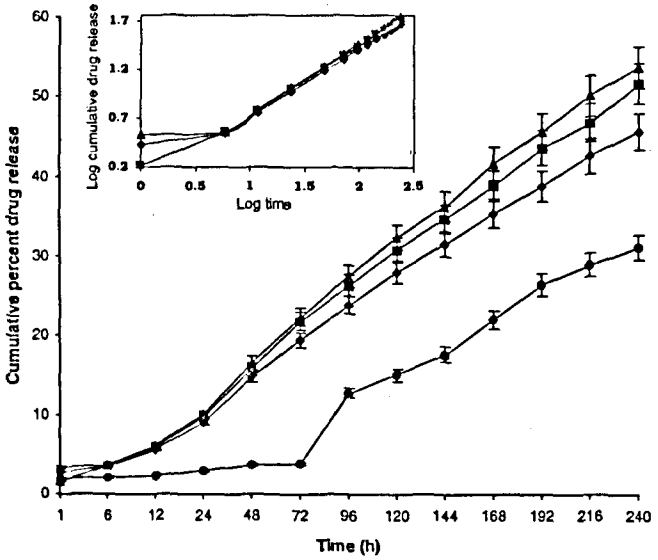

Fig. 1: *In vitro* drug release profile of DILPLA, DILPLGA 1, 2 and 3.

In vitro drug release from DILPLA (-●-), DILPLGA-1 (-♦-), DILPLGA-2 (-■-) and DILPLGA-3 (-▲-) was performed using the dialysis tube diffusion technique employing cellophane membrane. Inset figure shows the log of cumulative drug release Vs log time plot for DILPLGA 1, 2 and 3.

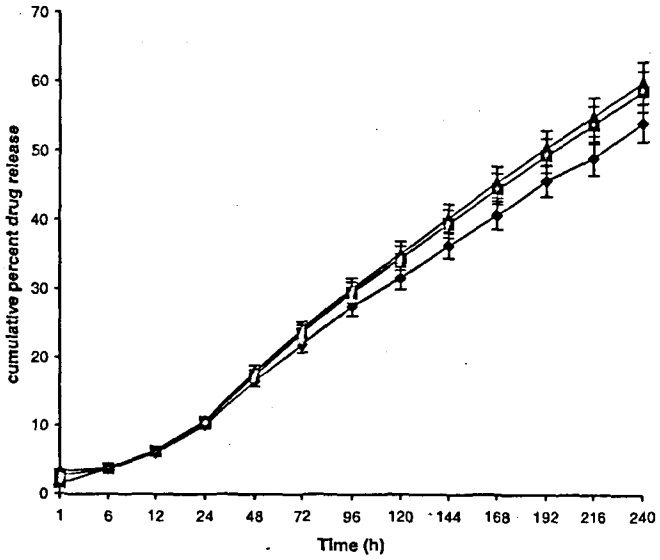

Fig. 2: *In vitro* drug release profile of DILPLGA 4, 5 and 6.

In vitro drug release from DILPLGA-4 (-●-), DILPLGA-5 (-■-) and DILPLGA-6 (-▲-) was performed using the dialysis tube diffusion technique employing cellophane membrane. Inset figure shows the log of cumulative drug release Vs log time plot for DILPLGA 4, 5 and 6.

vitro drug release in PBS of pH 7.4. A linear relationship was obtained between cumulative percent drug release versus time. The *in vitro* drug release profile exhibited initial fast drug release from 3, 6, 9 formulation on d 4, after day 5, a decline in release was observed, which could be due to fast release of superficial drug from systems. The release of diltiazem was found to be 31.3±1.21 and 53.72±2.15% from DILPLA and DILPLG-3, respectively after 240 h while it was 58.56±2.68 and 59.89±1.46% from DILPLG-5 and DILPLG-6, respectively (figs. 1 and 2). After 24 h, 10.71±0.36 and 11.2±0.28% drug release was recorded from DILPLG-7 and DILPLG-9, respectively while after 240 h it was 61.55±2.89 and 64.59±3.12% (fig. 3). The release kinetics from the system was established by determining the diffusional release exponent from a curve of logarithm of cumulative drug release versus logarithm time (inset fig. 1-3). The slopes of the straight line of these curves were recorded as the value of diffusional release exponent (η), which were found in between 0.72 to 0.88, which is an indication of non-fickian release kinetics.

The *in vivo* performance of both drug delivery systems was evaluated by periodic measuring the drug plasma concentration and pharmacokinetic parameters. The drug delivery systems (DILPLGA-2, DILPLGA-5 and DILPLGA-8) which exhibited the drug release nearer to the drug input (2.5 mg) required maintaining effective plasma concentration (calculated on the pharmacokinetic parameter of the drug) was selected for the *in vivo* performance studies. All drug delivery systems were injected intradermally to Wistar rats into the middle dorsal area without making any incision.

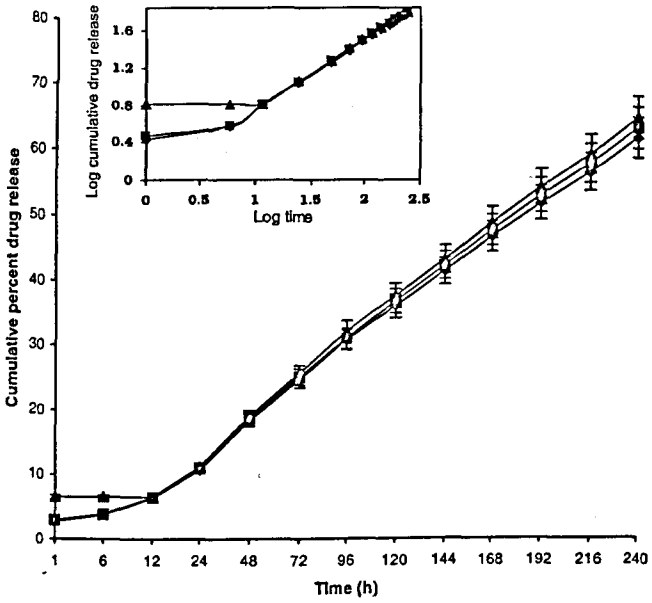

Fig. 3: *In vitro* drug release profile of DILPLGA 7, 8 and 9.

***In vitro* drug release from DILPLGA-7 (-●-), DILPLGA-8 (-■-) and DILPLGA-9 (-▲-) was performed using the dialysis tube diffusion technique employing cellophane membrane. Inset figure shows the log of cumulative drug release Vs log time plot for DILPLGA 7, 8 and 9.**

Fig. 4 exhibited initial peak drug plasma concentration 180.3±6.1 and 270.6±9.3 ng/ml with DILPLGA-2 and DILPLGA-8 respectively, which declined to 105.4±3.2 and 210.6±6.5 ng/ml. The initial high drug serum concentration could be due to burst release of the drug from the formulation. The formulation PLGA-2 exhibited initial peak drug serum concentration 180.3±6.1 ng/ml that is comparatively less than the other formulations (DILPLGA-5, 223.7±7.5 ng/ml and DILPLGA-8, 270.6±9.3 ng/ml). This could be due to high percentage of polymer ratio, which promptly formed implant; hence drug available for diffusion to plasma was less. Further the formulation DILPLGA-5 exhibited maximum drug serum concentration up to last day of the studies (15 d), sustained action was maintained and was very much in the therapeutic range. DILPLGA-8 showed maximum $AUC_{0\text{-}240\ h}$ (52 377±910 ng.h/ml) compared to DILPLGA-2 (39 471±830 ng.h/ml) and DILPLGA-5 (50 402±945 ng.h/ml) as shown in

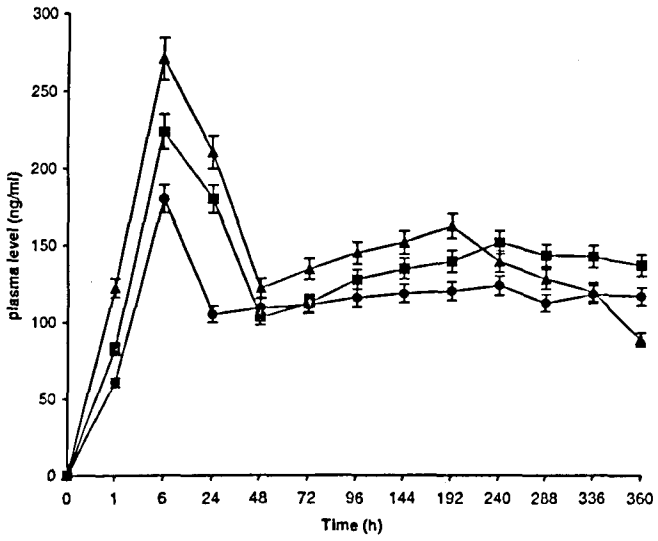

Fig. 4: Drug serum concentration Vs time plot after implanting intradermally polymeric systems.

Plasma level of DILPLGA-2 (-●-), DILPLGA-5 (-■-) and DILPLGA-8 (-▲-) following intradermal administration in albino rats.

TABLE 4: PHARMACOKINETIC PARAMETERS OF VARIOUS FORMULATIONS

Formulation	Pharmacokinetic parameters		
	Cp (ng/ml)	Tp (h)	AUC (ng.h/ml)
DILPLGA-2	180.3±6.1	6	39471±830
DILPLGA-5	223.5±7.8	6	50402±945
DILPLGA-8	270.6±9.3	6	52377±910

Table 4. However, formulation DILPLGA-5 showed better sustained drug release as compared to other formulations, because a constant drug serum level could be achieved till last day of the study.

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