



LIPOPHILIC AND HYDROPHILIC DRUG LOADED PLA/PLGA IN SITU IMPLANTS: STUDIES ON THERMAL BEHAVIOR OF DRUG & POLYMER AND OBSERVATION OF PARAMETERS INFLUENCING DRUG BURST RELEASE WITH CORRESPONDING EFFECTS ON LOADING EFFICIENCY & MORPHOLOGY OF IMPLANTS

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ABSTRACT

Initial burst effect leading to poor drug loading is a major drawback associated with *in situ* implants. In order to address a solution to this problem the influencing factors need to be identified. The aim of this work was to study whether some important formulation variables, namely, lipophilicity/ hydrophilicity of drugs, different polymer types, polymer molecular weights and drug/ polymer ratios could influence initial drug burst with corresponding loading efficiency of *in situ* implants. Dexamethasone and dexamethasone phosphate were used as lipophilic and hydrophilic model drug, respectively. As a part of material characterization the thermal behavior of Dexamethasone, DL-PLA (Mw 28.6 kDa) and PLGA was studied by DSC and TGA. Dexamethasone and dexamethasone phosphate loaded DL-PLA (Mw 28.6 kDa and 11.7 kDa) and PLGA (Mw 12.9 kDa) implants with different drug/ polymer ratios (0.5:9.5, 1:9, 1.5:8.5, 2:8 and 3:7) were prepared *in situ* in phosphate buffer, pH 7.4. Lipophilic dexamethasone was found to be loaded more efficiently than hydrophilic dexamethasone phosphate. Drug loading efficiency with DL-PLA was found to be more than that with PLGA. Loading efficiency was found to increase with decreasing drug/ polymer ratios in formulation. Polymer molecular weight did not show any influence on loading efficiency. Extent of burst during implant formation, as measured by drug content analysis of aqueous buffer media, complemented drug loading efficiency of corresponding implant. Morphology of implant matrices, as studied by SEM, supported the experimental results. The types of drug, polymer and drug/ polymer ratios have profound influence on drug burst release and loadings in implants. Therefore, these factors can be manipulated to reduce the burst and improve loading efficiency of *in situ* implants.

Keywords: Biodegradable polymer, Dexamethasone, Dexamethasone phosphate, Drug/polymer ratio, Biocompatible water miscible solvent, DMSO, SEM, DSC, TGA, Patient compliance

INTRODUCTION

Injectable *in situ* forming implant systems have been developed in recent years in order to avoid the surgical intervention as associated with administration and withdrawal of conventional implants and thus improve patient compliance¹. These biodegradable delivery systems are generally liquid formulations that form semi-solid or solid depot after injection. The drug is dissolved or dispersed in a concentrated solution of water insoluble biodegradable polymer in a water miscible biocompatible solvent. Upon injection into aqueous body fluids, the solvent diffuses into the surrounding aqueous environment while water diffuses into the polymer matrix leading to the formation of solid *in situ* implant, which retards the drug release^{2,3}. A number of researchers have extensively reported drug delivery using this method⁴⁻¹².

General advantages of parenteral *in situ* implant systems are localized or systemic prolonged drug delivery periods, drug dosage reduction along with reduction of adverse effects and reduced frequency of administration¹³. The manufacturing conditions are mild and uncomplicated especially for sensitive drug molecules¹⁴. A major problem associated with this system, however, is the possibility of initial burst effect during the first few hours after injection into the body leading to poor loading of drug in implant. Since the injectable implant system is administered as a liquid, the reason for the burst effect may be attributed to a lag between the injection and formation of the solid implant. During this lag period, as no encapsulating matrix exists, the initial burst of drug may lead to local or systemic side effects^{15,16}.

Thermal analysis methods involve a series of techniques that measure material properties as a function of externally applied temperature. Measurements of thermal analysis are conducted for the purpose of evaluating the physical and chemical changes that may take place in a heated sample. Thermal methods can be extremely useful during the course of preformulation studies. During the course of research and drug development, thermal methods can be used to evaluate compound purity, polymorphism, solvation, degradation, drug-excipient compatibility, and a wide variety of

other desirable characteristics¹⁷. Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA) are among the most widely used thermal methods of analysis of materials¹⁸.

The aim of this research was to thoroughly investigate the thermal properties of materials constituting the *in situ* implants, namely the drug dexamethasone and the biodegradable polymers DL-PLA and PLGA by DSC and TGA. In order to reduce the burst release and increase corresponding loading efficiency of implants a basic understanding of the factors influencing the process is needed. With this view on mind another objective of this work was to investigate the effects of a number of parameters on drug loading efficiency of *in-situ* implants namely, different drug (lipophilic dexamethasone and hydrophilic dexamethasone phosphate) and polymer types (DL-PLA and PLGA), different polymer molecular weights (DL-PLA Mw 28.6 kDa, DL-PLA Mw 11.7 kDa and PLGA Mw 12.9 kDa) and different drug/ polymer ratios (0.5:9.5, 1:9, 1.5:8.5, 2:8 and 3:7). Thorough morphological investigation of *in situ* implant matrices so formed by SEM was also an objective of the research.

MATERIALS AND METHODS

Materials

Dexamethasone BP2003/USP27 (Batch No: DX-010505) and Dexamethasone phosphate BP2003/USP27 (Batch No: DSP-020505) were from Savan Group UK. Poly (DL-lactide) designated by DL-PLA-30 in the present research (Trade name: Resomer® R203; Average molecular weight (Mw) 28.6 kDa; Inherent Viscosity 0.25-0.35 dl/g, 0.1% in chloroform), Poly (DL-lactide) designated by DL-PLA-10 (Trade name: Resomer® R202, Average molecular weight 11.7 kDa; Inherent Viscosity 0.16-0.24 dl/g, 0.1% in chloroform) and Poly (DL-lactide-co-glycolide), 50:50 molar ratio DL-lactide: glycolide designated by 50/50 PLGA-12 (Trade name: Resomer® RG502; Average molecular weight 12.9 kDa; Inherent Viscosity 0.16-0.24 dl/g, 0.1% in chloroform) were from Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany. Acetonitrile (HPLC grade) and Methanol (HPLC grade) were from Fisher Scientific, UK and Dimethyl sulfoxide (DMSO) from Gaylord Chemical Corporation, USA.

Differential scanning calorimetry (DSC) of drug and polymers

Thermal analysis of dexamethasone and polymers (DL-PLA 28.6 kDa and PLGA) was carried out by Differential scanning calorimetry (DSC) using TA Differential scanning calorimeter (DSC Q10 V9.4 Build 287). Approximately 5-10 mg of the sample for DSC analysis was weighed and spread uniformly in a hermetic aluminum pan to ensure proper thermal contact. The Universal Analysis software of DSC was used to determine the drug melting endotherm (T_m , determined at the onset of the endothermic peak) and polymer glass transition temperature (T_g , determined at the onset of heat capacity change) from the thermal profile.

Thermogravimetric analysis (TGA) of drug and polymers

Thermal analysis of dexamethasone and polymers (DL-PLA Mw 28.6 kDa and PLGA) was carried out by Thermogravimetric analysis (TGA) using TA Thermogravimetric analyzer (TGA Q50 V6.4 Build

193). The sample (approx. 5 to 10 mg) was placed in sample pans for TGA. The weight loss was measured on a TA Thermogravimetric Analyzer (TGA Q50 V6.4 Build 193), during heating from 0°C to 300°C for pure drug and 0°C to 100°C for polymer at a heating rate of 10°C/min. The weight loss was the difference between the initial and the final sample weight. The percentage of weight loss was calculated as the ratio of final sample weight to the initial sample weight multiplied by 100.

Preparation of Implants

The method employed to prepare Dexamethasone and Dexamethasone phosphate loaded *in situ* polymeric implants was similar to those adopted by Shah et al.⁴ and Shively et al.⁵ with some modification. Formulations varied with respect to types of drug and polymer, different polymer molecular weights and different drug/polymer ratios (Table 1).

Table 1: Composition of *in situ* implant formulations

Batch no	Drug*	Polymer	Polymer Mw (kDa)	Solvent [†]	Amount (% w/w)	
					Drug	Polymer
DX5-PLA30	DX	DL-PLA	28.6	DMSO	0.5	9.5
DX10-PLA30	DX	DL-PLA	28.6	DMSO	1	9
DX15-PLA30	DX	DL-PLA	28.6	DMSO	1.5	8.5
DX20-PLA30	DX	DL-PLA	28.6	DMSO	2	8
DX30-PLA30	DX	DL-PLA	28.6	DMSO	3	7
DX10-PLA10	DX	DL-PLA	11.7	DMSO	1	9
DX20-PLA10	DX	DL-PLA	11.7	DMSO	2	8
DX30-PLA10	DX	DL-PLA	11.7	DMSO	3	7
DX10-PLGA	DX	PLGA (50:50)	12.9	DMSO	1	9
DX20-PLGA	DX	PLGA (50:50)	12.9	DMSO	2	8
DXP5	DXP	DL-PLA	28.6	DMSO	0.5	9.5
DXP10	DXP	DL-PLA	28.6	DMSO	1	9
DXP15	DXP	DL-PLA	28.6	DMSO	1.5	8.5
DXP20	DXP	DL-PLA	28.6	DMSO	2	8

*DX stands for dexamethasone and DXP for dexamethasone phosphate; [†]Amount of solvent in each formulation was double the weight of polymer in the respective formulation

Biocompatible solvent DMSO was accurately weighed in the desired amount by analytical balance (Electronic balance: AY-200, Shimadzu, Japan) into glass vial. Weighed amount of polymer was incrementally added to DMSO (polymer/solvent ratio was 1:2) and dissolved over a period of time with the help of hot plate stirrer (Heating Magnetic Stirrer with Timer: VELP Scientifica, Europe) and vortex mixer (VM-2000: Disisystem Lab Equipments Inc, Taiwan). Temperature was maintained at 50°C while mixing.

After complete dissolution of the polymer, the solution was cooled to room temperature and weighed amount of the drug was incrementally added. Lipophilic drug dexamethasone was dispersed and hydrophilic dexamethasone phosphate dissolved in the polymer solution and final weight of the formulation taken. Formulations were taken up into 1 ml syringes and injected into pH 7.4 phosphate buffer contained in glass vessels through 21 G hypodermic needles. The amount of drug in each injection was calculated from the injection volume and the concentration of drug in formulation. Vessels with injected formulations were kept in incubator (Binder, Germany) for 1 hour maintaining the temperature at 37°C. After then implants were recovered, blotted dry with lint-free cotton and preserved in desiccators until necessary experiments.

Scanning electron microscopy (SEM)

The implants were analyzed for their surface morphology using scanning electron microscope (Philips XL 30, The Netherlands). The implants were initially spread on a carbon tape glued to an aluminum stub and coated with Au using a Sputter Coater under vacuum in a closed chamber.

The Au layer was coated to make the implant surface conductive to electrons in the SEM. The implants were then observed under SEM in varying magnifications and micrographs recorded.

The amount of drug that was actually loaded in implants during the fabrication process was determined by spectrophotometric analysis (UV-VIS Spectrophotometer: UV-1601, Shimadzu, Japan). Implant was crushed, weighed portion taken, and dissolved in 1 ml Acetonitrile by vigorous ultrasonication. For precipitating the polymer and extracting the drug, 9 ml of methanol or phosphate buffer, pH 7.4 was added for dexamethasone and dexamethasone phosphate, respectively. Centrifugation at 3000 rpm for 15 minutes separated the solid material. 1 ml supernatant was withdrawn into 100 ml volumetric flask. Volume was made with 20:80 methanol: water for dexamethasone or phosphate buffer, pH 7.4 for dexamethasone phosphate. The solution was analyzed spectrophotometrically for the drug content against the appropriate blank at 240 nm (λ_{max} of dexamethasone in methanol: water) and 239 nm (λ_{max} of dexamethasone phosphate in phosphate buffer, pH 7.4) for dexamethasone and dexamethasone phosphate, respectively.

Determination of extent of drug burst release

Extent of drug that had escaped (lost) in phosphate buffer, pH 7.4 without being loaded in the implant was determined. 5 ml buffer solution was withdrawn from the vessel 1 hour after the injection of implant formulation had been made and analyzed spectrophotometrically at a wavelength of 241.5 nm for dexamethasone and 242 nm for dexamethasone phosphate against the appropriate blank (Shimadzu UV-1601 Spectrophotometer, Japan). An identical volume of fresh buffer solution was replaced. Drug concentration was determined from the corresponding standard curves developed in phosphate buffer, pH 7.4.

Statistical analysis

Results were expressed as mean \pm S.D. Statistical analysis was performed by linear regression analysis. Coefficients of

determination (R^2) were utilized for comparison. The dependence, each of loading efficiency and drug loss during implant formation, was independently evaluated on variation of theoretical drug loads. Posthoc Tukey's tests were used to examine interrelationship between pairs of drug loading and drug burst for each theoretical drug loads. A P value of ≤ 0.05 was considered significant. Statistical analysis was performed using SPSS software.

RESULTS

The thermal behavior of the dexamethasone powder (as obtained from the source) and polymers DL-PLA-30 and 50/50 PLGA-12 was investigated by DSC (TA Instruments DSC Q10 V9.4 Build 287) and TGA (TA Instruments TGA Q50 V6.4 Build 193). A number of parameters could be measured from the various thermal events detected by DSC. From the melting endotherm of pure dexamethasone powder (Fig. 1) the onset and peak temperatures could be derived. The onset temperature was obtained by

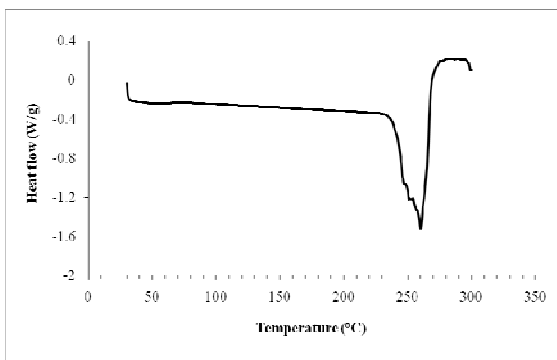


Fig. 1: DSC thermogram of pure dexamethasone powder

Fig. 3 and 4 graphically displays the thermogravimetric analysis of dexamethasone and the polymers (DL-PLA-30 and 50/50 PLGA-12), respectively. The weight loss vs. temperature profile of dexamethasone shows the weight of the drug to remain unchanged until the temperature of analysis reaches 235°C. Beyond this point the weight of the drug drastically fell reaching almost half its original weight by 300°C. For the polymers, as seen in Fig. 4, the

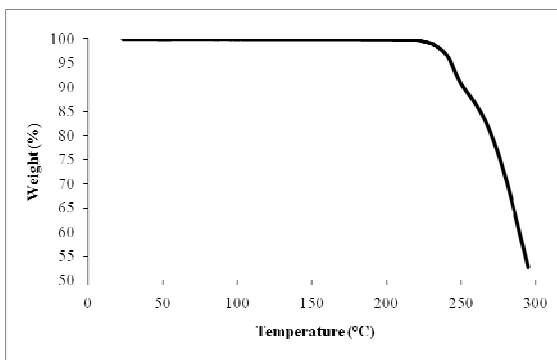


Fig. 3: Thermogravimetric analysis of dexamethasone (Temperature 0-300°C)

In situ biodegradable polymeric implants were analyzed for actual dexamethasone and dexamethasone phosphate content. Based on the experimentally determined drug load, the percentage of loading efficiency (% LE) of implants was determined with the formula

$$\% \text{ LE} = (\text{LD}/\text{AD}) \times 100$$

where LD is the amount of drug that was actually loaded in the implant and AD is the amount of drug originally added in the

extrapolation from the leading edge of the endotherm to the baseline. The peak temperature was the temperature corresponding to the maximum of the endotherm. It is the accepted custom that the extrapolated onset temperature is taken as the melting point; however, some users report the peak temperature in this respect¹⁸.

The DSC scan of dexamethasone exhibited the onset melting endotherm at 255°C and the peak at 260°C which complied with the findings of other researchers, such as those reported by Aiedeh et al.¹⁹ who obtained the endothermic melting peak at 260°C for pure dexamethasone. DSC scans of DL-PLA-30 and 50/50 PLGA-12 were also performed. Fig. 2 exhibits the onset temperatures of glass transition (T_g) at 57 and 45°C with the peaks appearing at 65 and 52°C for DL-PLA-30 and 50/50 PLGA-12, respectively which are at par reference which reports the glass transition temperatures (T_g) of DL-PLA to range between 55-60°C and 50/50 PLGA between 45-50°C²⁰ (Lactel® Absorbable Polymers).

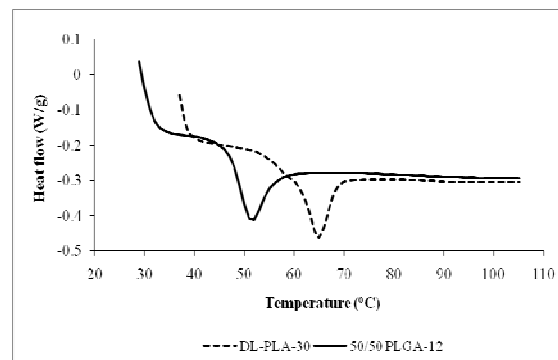


Fig. 2: DSC thermogram of DL-PLA-30 and 50/50 PLGA-12

thermogravimetric analysis was carried out in the temperature range of 0-100°C. The process of weight loss started at 35°C for DL-PLA 30 and 65°C for 50/50 PLGA-12. But insignificant weight loss (0.35 and 0.15% weight loss for DL-PLA 30 and 50/50 PLGA-12 only, respectively) was seen to occur by heating which could be due to the removal of loosely bound moisture in the polymer.

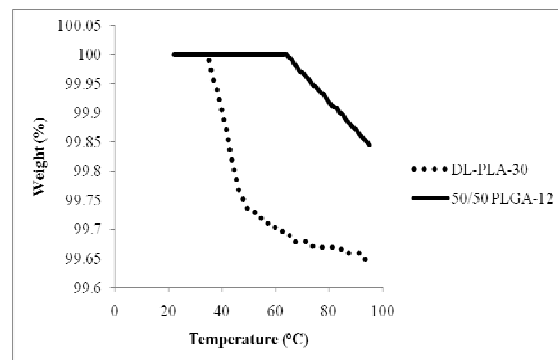


Fig. 4: Thermogravimetric weight loss vs. temperature profile of DL-PLA-30 and 50/50 PLGA-12

formulation. Drug burst in the surrounding media, i.e., drug not loaded while implants were formed, was measured. This experiment was performed by analysis of the aqueous buffer media 1 hour after the injection of implant formulation had been made.

The loading efficiency of all the batches was found to decrease with increasing drug loads in formulation and vice versa (Fig. 5). With DX-PLA30 series, the loading efficiency ranged between 59.25-

87.68% with decreasing theoretical drug loads from 30 > 5% (-ve correlation). The correlation was statistically significant ($p \leq 0.05$). The SEM micrographs of DX5-PLA30, DX10-PLA30 and DX30-PLA30 (5, 10 and 30% dexamethasone loaded DL-PLA-30 implant) surfaces (Fig. 6a, 6b and 6c) further supported this finding by being more and more rough, porous and nonuniform with increasing drug loads, thereby probably entrapping relatively lower amount of dexamethasone. The loading efficiency of DX-PLA10 batch was also found to follow the same pattern (52.89 - 76.25% for 30>10%

theoretical drug loads). Fig. 7a and 7b exhibit the SEM micrographs of DX10-PLA10 and DX30-PLA10 where the surfaces are seen to be increasingly porous with increased drug loads. The same for DX10-PLGA and DX20-PLGA was 52.89 and 71.48%. But this finding was significant ($p \leq 0.1$). SEM micrographs of DX10-PLGA and D X20-PLGA implant surfaces (Fig. 8a and 8b) were in accordance with this observation by being more and more porous with increasing drug loads.

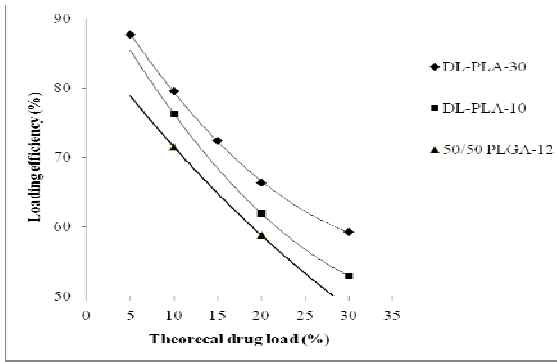


Fig. 5: Effects of drug load, types of polymer and polymer molecular weight variation on dexamethasone loading efficiency of implants

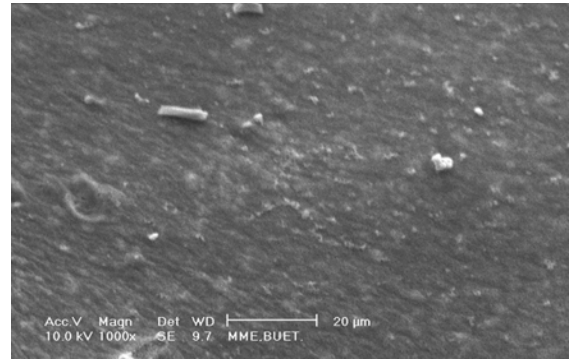


Fig. 6 (a)

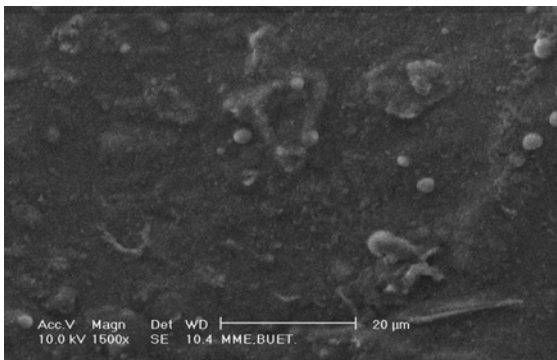


Fig. 6 (b)

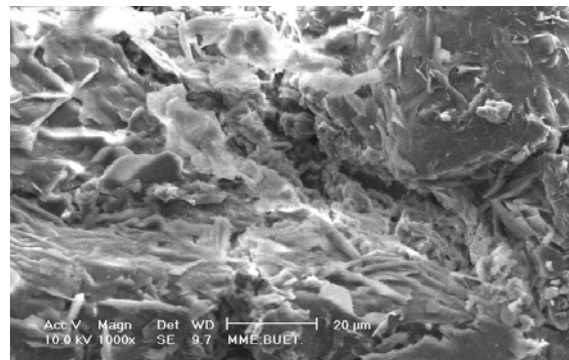


Fig. 6 (c)

Fig. 6: SEM micrographs of dexamethasone loaded DL-PLA-30 implant surfaces after formation: (a) DX5-PLA30 (b) DX10-PLA30 and (c) DX30-PLA30

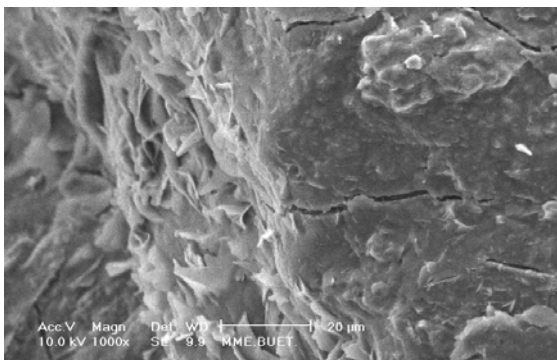


Fig. 7(a)

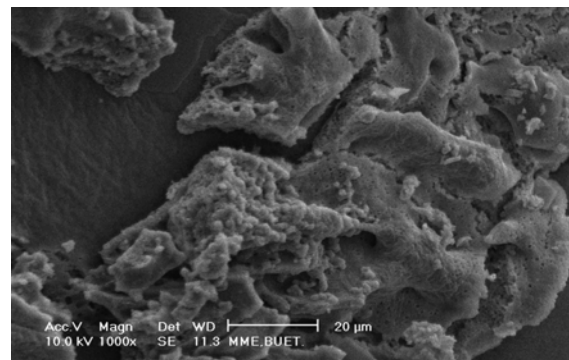


Fig. 7(b)

Fig. 7: SEM micrographs of dexamethasone loaded DL-PLA-10 implant surfaces after formation: (a) DX10-PLA10 and (b) DX30-PLA10

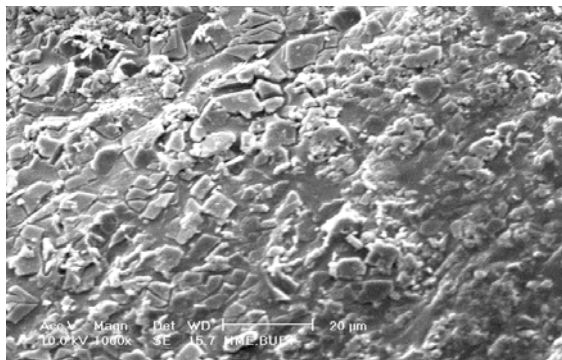


Fig. 8(a)

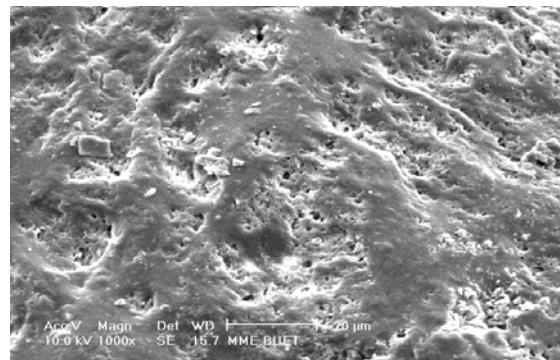


Fig. 8(b)

Fig. 8: SEM micrographs of dexamethasone loaded 50/50 PLGA-12 implant surfaces after formation: (a) DX10-PLGA and (b) DX20-PLGA

The loading efficiency of dexamethasone phosphate loaded DL-PLA-30 implants ranged between 38.15 – 68.8% for 20>5% theoretical drug loaded formulations (Fig. 9). The high correlation coefficient ($R^2 = 0.9986$) indicated the effect to be linear. The effect was found

to be statistically significant ($p \leq 0.05$). Interestingly, the SEM micrographs showed high concentration of drug on the implant surfaces (Fig. 10a, 10b and 10c), but the assay results exhibited otherwise probably indicating negligible drug presence inside.

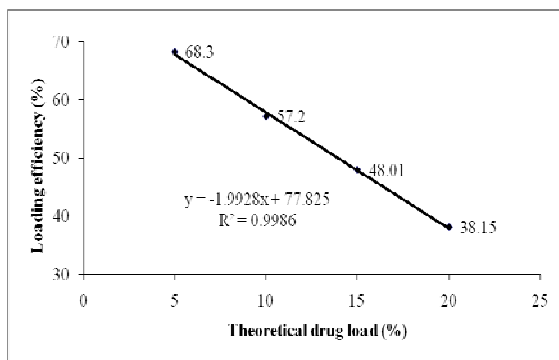


Fig. 9: Effect of dexamethasone phosphate load on the loading efficiency of DL-PLA-30 implants

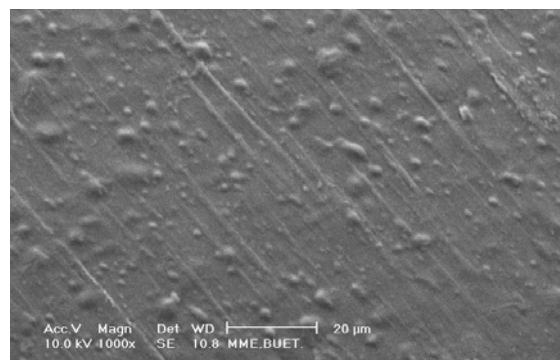


Fig. 10(a)

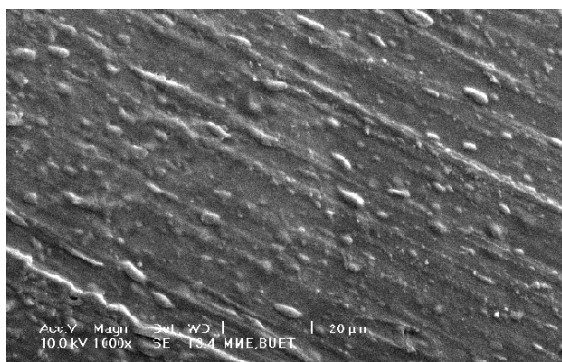


Fig. 10(b)

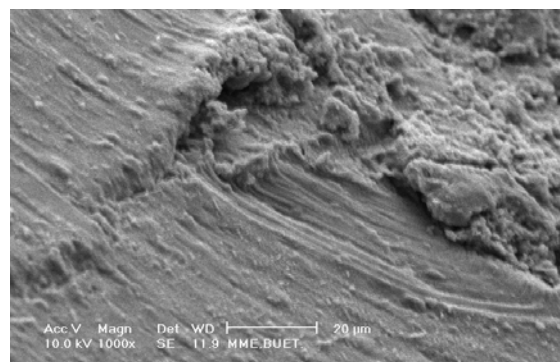


Fig. 10(c)

Fig. 10: SEM micrograph of dexamethasone phosphate loaded DL-PLA-30 implant surface after formation: (a) DXP5 (b) DXP10 and (c) DXP20

Drug burst release experiments showed the extent of burst to increase with increasing drug loads in formulation and vice-versa (Table 2). Fig. 11 and 12 graphically show the interrelationship

between (%) drug loading efficiency and (%) drug burst during implant formation. Correlation coefficient between the two data sets was statistically found out for all the batches. Increased drug escape

in the surroundings was found to complement with decreased loading efficiency and vice versa, indicating good correlation of both the experiments. The correlation coefficient between dexamethasone loading efficiency and dexamethasone burst for DL-PLA-30 and DL-PLA-10 implants was found to be -0.9 and -0.96, respectively. With DL-PLA-30 implants the finding was statistically significant ($p \leq 0.05$). But for DL-PLA-10 implants the correlation was insignificant ($p = 0.093$). For dexamethasone phosphate loaded DL-PLA-30 implants drug loss increased with increasing drug loads ($R^2 = 0.999$; $p = 0.001$). With the increase in theoretical drug loads the

extent of dexamethasone phosphate lost increased which correlated with the already found decreasing actual drug loading data.

The correlation coefficient of interrelationship was found to be -0.997 and it was significant ($p = 0$). The possible reasons for deviation from the ideal value 1 could be attributed to loss of drug due to the adherence of viscous polymeric formulation in syringe, experimental errors, etc. Correlation coefficient calculation for 50/50 PLGA-12 implants was not attempted due to the presence of only two data points (two drug loads).

Table 2: Effects of drug load, types of polymer and polymer molecular weight variation on extent (%) of burst and (%) drug loading efficiency of *in situ* implants

Batch no*	Actual drug content (% w/w) Mean \pm S.D.	% drug burst Mean \pm S.D.	Loading efficiency (%)
DX5-PLA30	4.38 \pm 0.25	0.25 \pm 1.55	87.68
DX10-PLA30	7.95 \pm 0.65	0.95 \pm 1.69	79.54
DX15-PLA30	10.86 \pm 0.70	1.47 \pm 1.80	72.39
DX20-PLA30	13.29 \pm 0.85	2.65 \pm 1.83	66.35
DX30-PLA30	17.76 \pm 1.95	6.40 \pm 2.05	59.25
DX10-PLA10	7.63 \pm 0.45	1.25 \pm 1.74	76.25
DX20-PLA10	12.38 \pm 0.80	3.70 \pm 2.08	61.90
DX30-PLA10	15.88 \pm 2.25	7.82 \pm 2.95	52.89
DX10-PLGA	7.15 \pm 0.55	1.55 \pm 2.15	71.48
DX20-PLGA	11.75 \pm 0.90	4.60 \pm 2.67	58.75
DXP5	3.44 \pm 0.90	1.45 \pm 1.26	68.8
DXP10	5.72 \pm 0.87	3.75 \pm 1.85	57.2
DXP15	7.20 \pm 1.58	6.50 \pm 2.37	48.01
DXP20	7.63 \pm 1.76	8.95 \pm 2.75	38.15

*The batch numbers indicate theoretical drug loads in respective polymeric implant. For e.g., DX5-PLA30 stands for theoretically 5% dexamethasone loaded DL-PLA-30 implant; DXP20 for theoretically 20% dexamethasone phosphate loaded DL-PLA-30 implant

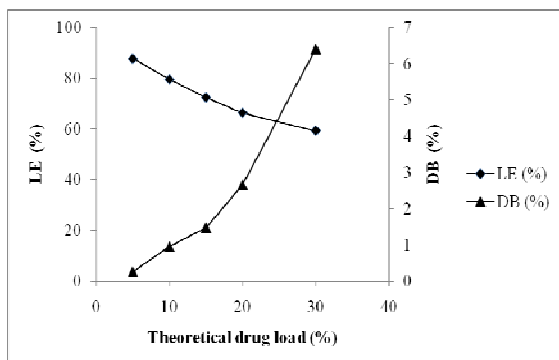


Fig. 11(a)

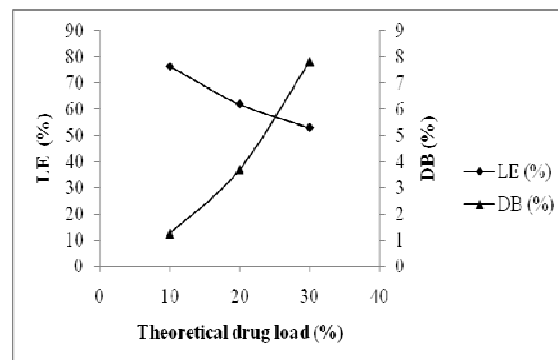


Fig. 11(b)

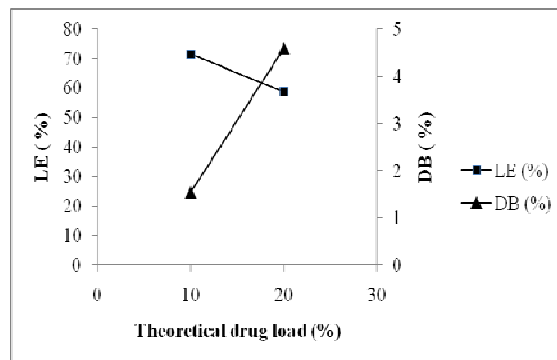


Fig. 11(c)

Fig. 11: Interrelationship between dexamethasone loading efficiency (LE) and dexamethasone burst (DB) for (a) DL-PLA-30 (b) DL-PLA-10 and (c) 50/50 PLGA-12 implants

The effect of lipophilicity and hydrophilicity of drug on corresponding burst effect and loading efficiency of implants was also observed (Fig. 13). The loading efficiency of lipophilic dexamethasone was compared with that of hydrophilic dexamethasone phosphate for 5, 10, 15 and 20% theoretically drug loaded DL-PLA-30 implants. It could be clearly seen from the graph that the loading efficiency of the lipophilic drug was much higher

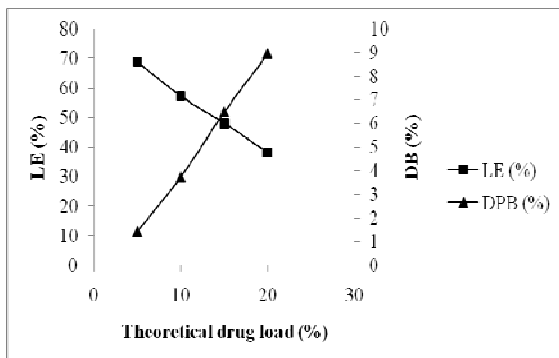


Fig. 12: Interrelationship between dexamethasone phosphate loading efficiency (LE) and dexamethasone phosphate burst (DPB) for DL-PLA-30 implants

DISCUSSION

Thermogravimetric analysis represented a powerful adjunct to DSC analysis in the present research. The T_g of DL-PLA-30 was found to be 55°C in the DSC scan of present research (Fig. 2) but around 55°C, no change in polymer weight was seen to occur in the TG thermogram (Fig. 4). These findings are at par the phenomenon that solid-liquid or solid-solid phase transformations (as observed in DSC) are not accompanied by any loss of sample mass and would not register in a TG thermogram¹⁷. The melting endotherm of dexamethasone as seen in DSC thermogram of the drug given in fig. 1, on the other hand, was seen to appear at 237°C which correlated with the weight loss vs. temperature profile of dexamethasone (Fig. 3) showing the weight of the drug to drastically fall beyond 235°C reaching almost half its original weight by 300°C. These findings suggest decomposition reactions (as identified by the melting endotherm in DSC) to be accompanied by weight changes and they can be thus identified by a TG weight loss over the same temperature range.

The effect of four independent variables could be considered to affect drug burst effect and loading efficiency of implants - drug and polymer load, polymer type, polymer molecular weight (Fig. 1) and physicochemical nature of the drug itself, namely, lipophilicity/hydrophilicity (Fig. 13). The drug and polymer load was seen to affect loading efficiency significantly. The possible reason for relatively lower burst and higher loading efficiency with lower drug loads could be the greater availability of the polymer for trapping the drug in implant and vice versa. In actual drug load assays of implants, 10-20% theoretical drug loaded implants exhibited low coefficients of variation (SD/Average) indicating homogeneous distribution of dexamethasone. The greater coefficient of variation, as found with 30% drug loaded implants, could be attributed to increased drug loss resulting in higher percentage of unloaded drug. Similar results were also obtained by Sampath et al.²¹ while preparing and characterizing poly (L-lactic acid) gentamicin delivery systems. The SEM micrographs of different drug loaded implants were in support of these findings by exhibiting the lower drug loaded implants to have stronger matrices with lower porosity and vice versa. According to the experimental findings of Mathew et al.²², drug/ polymer ratio was also the most significant factor influencing entrapment efficiency of ketorolac tromethamine-loaded albumin microspheres. Similar effects were also observed by Madan et al.¹ who strongly inferred initial burst effect of rosiglitazone to substantially reduce from PLGA implant systems by increasing

than the hydrophilic one for the same theoretical loads and the variation in loading efficiency became more and more pronounced with increasing drug loadings.

The difference between the actual drug loading of dexamethasone and dexamethasone phosphate for each corresponding therapeutic drug loads was statistically significant ($p \leq 0$).

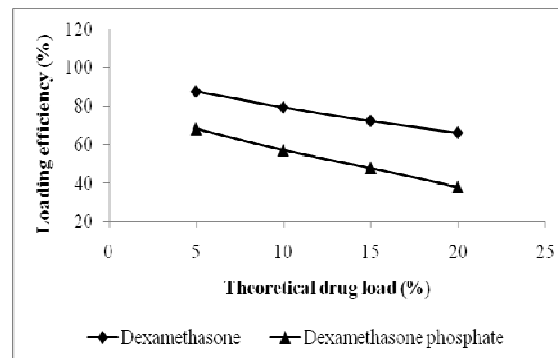


Fig. 13: Comparison of dexamethasone and dexamethasone phosphate loading efficiency of in situ implants

polymer concentration in formulation. According to them the slow release mechanism for higher polymer concentration can be explained by a reduction in permeability due to changes in the morphology of the polymer. Increased polymer concentration may provide the matrix with lower tortuosity and poor porosity for diffusion of drug. Moreover, higher amount of polymer may result in viscous microenvironment of the system inhibiting the movement of water into the matrix for easy diffusion of drugs into the surroundings leading to lower burst effect.

As for the effect of polymer variation, the loading efficiency varied by more than 8 ($p = 0.002$) and 7% ($p = 0.0003$) between DL-PLA-30 and 50/50 PLGA-12 for 10 and 20% theoretical drug loaded implants, respectively. The same between DL-PLA-10 and 50/50 PLGA-12 was found to be more than 3 ($p = 0.0096$) and 4% ($p = 0.031$) for 10 and 20% theoretical drug loading, respectively. The better loading efficiency of DL-PLA over PLGA can be attributed to the higher hydrophobicity of the homopolymer than the copolymer favoring better entrapment of hydrophobic drug dexamethasone²³.

Interestingly, the amount of dexamethasone actually loaded did not seem to depend much on polymer molecular weight alone. As can be seen in fig. 5, for 10% theoretical loading, the descending loading efficiency order was DL-PLA-30 (mol. wt 28.6 kDa) > DL-PLA-10 (mol. wt 11.7 kDa) > 50/50 PLGA-12 (mol. wt 12.9 kDa). So, no obvious correlation could be inferred. Perugini *et al.*²⁴ also found Clodronate encapsulation in biodegradable microspheres not to depend on polymer molecular weight. But then again, in the present study, the data was not sufficient enough to draw any rigid conclusion in this regard.

Lipophilicity/hydrophilicity of the implantable drug was seen to significantly affect drug burst and implant loading. Being a highly water soluble drug, dexamethasone phosphate probably partitioned more in the outer aqueous media than its lipophilic counterpart dexamethasone when the formulation was injected, leading to relatively more burst and less efficient loading. Moreover, the physical state of the drug in the formulation may have also played a role here. Dexamethasone phosphate, probably being in dissolved state in polymeric solution gave rise to a greater burst than the dispersed dexamethasone in formulation.

CONCLUSION

Loss of drug due to burst effect from *in situ* implant leading to inefficient loading is a disadvantage with serious consequences

associated with this otherwise advantageous dosage form. In order to approach elimination or at least minimization of the problem the contributing factors were studied. Dexamethasone and Dexamethasone phosphate loaded PLA and PLGA *in situ* implants were prepared in Phosphate buffer, pH 7.4. Formulation variables were different polymer types, polymer molecular weights, drug/polymer ratios and lipophilicity/ hydrophilicity of drug. Being more hydrophobic, PLA retarded burst release more efficiently and improve loading as compared to PLGA. Implants with more drug/polymer ratios gave rise to more burst and less efficient loading because of lesser availability of the polymer for holding the drug, thereby generating relatively weaker matrices. SEM micrographs also conformed to the observations. Polymer molecular weight did not have any effect on drug burst and loading. But data for such comment was not sufficient. Because of relatively more partitioning of hydrophilic drug dexamethasone phosphate in aqueous buffer during implant formation, it was found to undergo more burst effect and poor loading compared to lipophilic dexamethasone. Another contributing factor for this effect could be the physical state of the drug in formulation. Hydrophilic dexamethasone phosphate was in dissolved state in formulation whereas lipophilic dexamethasone was dispersed. Drug burst effect correlated significantly with implant loading efficiency in the present research.

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