Effect of Hydrophobic Excipients on the Release Behavior of Dexamethasone and Betamethasone from Biodegradable Poly (DL-lactide) Polymeric Implants

Fariba Khan, Tasnuva Mahmud, M. Saiful Islam, Reza-ul Jalil

Abstract

This study was conducted with the aim of formulating injectable in-situ forming implants of biodegradable poly (D,L-poly lactic acid) (DL-PLA) with steroidal drugs. Two steroidal drugs betamethasone sodium phosphate and dexamethasone sodium phosphate were selected as model drug. Different hydrophobic excipients such as sorbitan esters of lauric acid (Span 20), sorbitan esters of oleic acid (Span 80), cetyl alcohol, glyceryl monostearate, glyceryl dibehenate (Compritol®), glyceryl palmito-stearic ester (Precirol®) were used. In-vitro dissolution of 7 days was performed to investigate the effect of these excipients on the release of betamethasone and dexamethasone from in-situ forming DL-PLA implants. Drug release data were fitted in different models to characterize release mechanism. Both betamethasone and dexamethasone release were found to follow Korsmeyer model. Glyceryl palmitostearate was found to reduce the release of both betamethasone and dexamethasone most. Time for 25% release (t25), 50% release (t50), and 75% release (t75) of the drugs were calculated. Mean dissolution time (MDT) values were also calculated from the dissolution data. MDT (day) was 2.48±0.2 and 2.31±0.3 for betamethasone and dexamethasone respectively for implants containing no excipient. Incorporation of all the excipients (except span 20) increased these MDT values for both of the drugs indicating sustained release nature of the excipients.

Key words: in-situ implants, Poly (D,L-lactic acid), Betamethasone, Dexamethasone, Hydrophobic excipients, Sustained release

Introduction

In the past decades, several parenteral depot formulations such as implants based on biodegradable polymers have become commercially available to improve the efficacy and prolong the activity of pharmaceutical agents of a wide variety of types including, antibiotics, anti-cancer drugs, steroids, peptides, proteins and many other therapeutic agents. Biodegradable systems which are hydrolytically or enzymatically cleaved in vivo and non-degradable polymers. Biodegradation for controlling drug delivery has the advantage that the drug delivery device may not have to be removed from the site of action after drug delivery is completed and the accumulation of polymers in the body during prolonged applications is avoided. 

Poly(lactic acid) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) are the most frequently used biodegradable polymers (Anderson and Shive, 1997). Poly (lactic acid) (PLA) has been developed as a non-toxic biodegradable material which degrades in vivo to lactic acid and subsequently metabolizes to CO₂ and H₂O (Kulkarni et al., 1966). Yolles et al. (1971) has reported the use of this polymer for long-acting injectable parenteral drug delivery systems and also obtained a patent for implants containing cyclazosin.

Glucocorticoids are used to prevent or suppress inflammation in response to multiple inciting events, including radiant, mechanical, chemical, infectious, and immunological stimuli (Hardman et al., 1996). They inhibit the production of factors that are critical in generating the inflammatory response, including vasoactive and chemoattractive factors, and lipolytic and proteolytic enzymes. In addition, they decrease the extravasion of leukocytes to the injury site (Schleimer, 1997). Betamethasone sodium phosphate and dexamethasone sodium phosphate are two glucocorticoids having high potency. They are effective on multiple organ systems also. However, they have serious systemic side effects (Liapi and Chrousos, 1992; Donald et al., 1997).
Consequently, the controlled, continuous local delivery of these drugs at the implantation site via an *in situ* forming implant system has been considered as a means to avoid these side effects and achieve the goal of suppressing the local inflammatory response.

**Materials and Methods**

Betamethasone sodium phosphate and dexamethasone sodium phosphate were generous gift from Aristopharma Ltd. Bangladesh, D,L-poly lactic acid (DL-PLA, Boehringer Ingelheim, Ingelheim, Germany), dimethyl sulfoxide (DMSO) (Merck Limited, Mumbai, India), cetyl alcohol (BDH Chemicals Ltd., England), glyceryl monostearate (e.g. Danisco, UK), glyceryl dibehenate (e.g. Compritol AT088®, Gattefosse France), glyceryl palmitostearate (e.g. Precirol®, Gattefosse France), sorbitan monolaurate (BDH Chemicals Ltd., England), sorbitan monooleate (Loba Chemie, India), zero sized capsule shell (Globe Pharmaceuticals, Bangladesh) were also used as gift.

**Preparation of *in-situ* forming implants and *In vitro* dissolution study**

Poly (DL-lactide) (DL-PLA) solution with 400 mg dimethyl sulfoxide (DMSO) was prepared where rest of the ingredients (drug and excipient) were added according to Table I. Few ml of this solution was taken into hard gelatin capsule shell (zero sized) with the help of glass syringe (Microstat Tuberculin, USA)) and hypodermic needle (Becton Dickinson & Company, USA). The capsule shell was immersed instantly inside a glass vials containing dissolution media of 100 ml phosphate buffer (pH 7.4). Implants were formed *in-situ* in the dissolution media. Dissolution vessels (glass vials) containing the dissolution media were kept in a thermostatic water bath (Memmert GmbH & Co. KG, Germany) maintaining the rpm at 50 and temperature at 37±0.5°C. Dissolution samples were then withdrawn from each of the vials at predetermined intervals. This study was carried out for seven days. Dissolution samples were analyzed spectrophotometrically at 241 nm (betamethasone) and 241.5 nm (dexamethasone) (BP 2003). This whole procedure was performed in triplicate.

**Kinetic Analysis of Dissolution Data**

To study the mechanism of drug release from the implants, the release data were fitted to zero-order, first-order, and Higuchi equation (Higuchi, 1963). These models fail to explain drug release mechanism. Therefore, the dissolution data was also fitted to the well-known exponential equation (Korsmeyer equation), which is often used to describe the drug release behavior from polymeric systems (Korsmeyer et al., 1983).

\[
\log \left( \frac{M_t}{M_f} \right) = \log k + n \log t
\]

where, \(M_t\) is the amount of drug release at time \(t\); \(M_f\) is the amount of drug release after infinite time; \(k\) is a release rate constant incorporating structural and geometric characteristics of the implant; and \(n\) is the diffusional exponent indicative of the mechanism of drug release.

To clarify the release exponent for different batches of sustained release implants, the log value of percentage drug dissolved was plotted against log time for each batch according to the equation 1. A value of \(n < 0.45\) indicates Fickian (case I) release; \(> 0.45\) but < 0.89 for non-Fickian (anomalous) release; and \(> 0.89\) indicates super case II type of release. Case I generally refers to diffusion controlled drug release, Case II refers to the erosion of the polymeric chain and anomalous transport (Non-Fickian) refers to a combination

**Table I: Composition of Sustained release Biodegradable Implants of Betamethasone/ Dexamethasone sodium phosphate (20 mg)**

<table>
<thead>
<tr>
<th>Batch</th>
<th>DL-PLA (mg)</th>
<th>Cetyl Alcohol (mg)</th>
<th>Glyceryl Dibehenate (mg)</th>
<th>Glyceryl Palmitostearate (mg)</th>
<th>Glyceryl Monostearate (mg)</th>
<th>Span 20 (mg)</th>
<th>Span 80 (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>200</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>200</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>200</td>
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</tbody>
</table>
of both diffusion and erosion controlled drug release (Peppas, 1985).

Mean dissolution time (MDT) was calculated from dissolution data using the following equation (Mockel and Lippold, 1993):

$$\text{MDT} = \frac{(n/n+1)}{K^{-1/n}}$$

Where, \( n \) = release exponent and \( k \) = release rate constant.

**Results and Discussion**

DL-PLA implants of betamethasone and dexamethasone were formed in-situ (Fig. 1) in the dissolution media. Dissolution study was conducted for 7 days.

![Fig.1. Physical morphology of in-situ forming biodegradable DL-PLA implants of betamethasone (a) and dexamethasone (b)](image)

Figure 2 shows the *in vitro* release of betamethasone from DL-PLA implants. Implants containing no excipient (blank) released 76.12 ±2.01% betamethasone in pH 7.4 buffer media after 7-days. Cetyl alcohol (CA) (batch A) caused a little sustained release of the drug. It was 73.52±2.014% while CA was incorporated into the implant. CA is an emulsifying and stiffening agent. It occurs as waxy, white flake which is practically insoluble in water (Kibbe, 2000). This water insoluble nature of the CA might play a role in retarding the release of the betamethasone slightly. Moreover, CA is also used as sustained release excipient in extending the release of the drug (Nguyen et al., 2008; Castellanos et al., 2008). Batch B, C, D containing glyceryl dibehenate, glyceryl palmitostearate, and glyceryl monostearate respectively also reduced the release of betamethasone but in a greater extent (Fig. 2). Glyceryl dibehenate (GB) reduced the release of the drug to 72.14±3.124%. GB is a tablet and capsule lubricant (Abramovici et al., 1986). It is a mixture of glycerides of fatty acids, mainly behenic acid and is also practically insoluble in water (Kibbe, 2000). This lipophilic nature might hinder the release of the water soluble betamethasone. But most importantly, GB has a good release retarding capacity which played the major role in controlling the release of betamethasone from DL-PLA implants (Li et al., 2006; Obaidat and Obaidat, 2001; Perez et al., 1993). Similar but more pronounced reduced release was observed while glyceryl monostearate (GMS) was incorporated in the implant formulation (batch D). Percent release of betamethasone was 67.45±4.014% for this batch. GMS is a lipophilic emulsifying agent (HLB 3.8) which is practically insoluble in water (Kibbe 2000). Besides, it is a lubricant for tablet manufacturing and may be used to form sustained release matrices for solid dosage forms (Peh and Yuen, 1995). However, GMS retarded the release of betamethasone from in-situ forming implants in this experiment where implants were made using biodegradable DL-PLA polymer. This release retardant activity of GMS from biodegradable implantable dosage form was also evident previously (Peri et al., 1994). Amongst the excipients, glyceryl palmitostearate (GPS) was able to reduce the release of betamethasone most. After incorporation of this excipient, betamethasone release was 58.21±2.214% after 7 days while this figure was 76.12 ±2.01% for the blank (Fig. 2b). GPS is also a water insoluble excipient which is used as tablet and capsule lubricant. It is a mixture of mono-, di-, and tri-, glycerides of C16 and C18 fatty acids (Kibbe 2000). Due to this more lipophilic nature of the GPS, it has been used as a matrix former in sustained release dosage forms (Shaikh et al., 1991). Gao et al.
(1995) also showed that GPS could be a good release retardant for steroids while it was used in a biodegradable injectable dosage form.

Sorbitan monolaurate (Span 20) and sorbitan monooleate (Span 80) were also used in the betamethasone implant formation (Table I). Batch E containing span 20 increased the release of the drug (82.14±2.214) which is shown in Figure 2. It might be due to less lipophilic nature (HLB 8.6) of span 20 (Kibbe 2000). On the other hand, span 80 (batch F) showed more release controlling capacity of betamethasone compared with span 20 (Fig. 2). Betamethasone release was 62.89±3.12% for this batch. This is attributable to the fact that presence of hydrophobic excipient, span 80, in the matrix impedes the rate of entrance of dissolution medium within the matrix and hence the dissolution of water soluble

Fig. 2. *In vitro* release profiles of betamethasone from biodegradable DL-PLA implants after 1 day (a) and 7-day (b) of dissolution where blank=no excipient, A=cetyl alcohol, B=glyceryl dibehenate, C=glyceryl palmitostearate, D=glyceryl monostearate, E=span 20 and F=span 80. Data are represented as mean ± SD (n=3)

Fig. 3. *In vitro* release profiles of dexamethasone from biodegradable DL-PLA implants after 1 day (a) and 7-day (b) of dissolution where blank=no excipient, A=cetyl alcohol, B=glyceryl dibehenate, C=glyceryl palmitostearate, D=glyceryl monostearate, E=span 20 and F=span 80. Data are represented as mean ± SD (n=3)
betamethasone was slowed considerably. The hydrophobic nature of surfactant, span 80 is due to its very low HLB value of 4.3 (Kibbe, 2000).

These excipients, except span 20, were also able to control the burst release of water soluble betamethasone sodium phosphate from DL-PLA implants as shown in Figure 2a. In the figure, release data of betamethasone after 1 (one) day is shown.

Biodegradable DL-PLA implants were also made with dexamethasone sodium phosphate, another potent glucocorticoids having a comparative glucocorticoid potency of 25-80 (Liapi and Chrousos, 1992; Donald et al., 1997). Similar release patterns, as were observed in case of betamethasone, were also observed for dexamethasone for individual excipient (Fig. 3a). Dexamethasone release after 7-days of dissolution was 83.25±2.01% from implants formulated without excipient (blank). After the incorporation of CA (batch A), GB (batch B), GPS (batch C), GMS (batch D), span 20 (batch E) and span 80 (batch F), dexamethasone release was 80.21±2.01%, 74.12±3.12%, 66.12±4.01%, 91.21±2.21%, and 71.21±3.12% respectively (Fig. 3b). Incorporation of these excipients also reduced dexamethasone release markedly after 1 (one) day of dissolution (Fig. 3a).

The release rate kinetic data for all the models is shown in Table II. Release data for betamethasone and dexamethasone of all the batches were fitted in Korsmeyer equation. Regression co-efficient ($r^2$) values ranged from 0.934 to 0.99 for betamethasone and 0.948 to 0.99 for dexamethasone and indicated diffusion controlled release mechanisms of the drugs. Value of release exponent "n" determined from the various formulations ranged from 0.24 to 0.272 for betamethasone and 0.185 to 0.247 for dexamethasone (Table II). The log k values are also shown in Table II where the values ranged from 0.241 to 0.396 for betamethasone and 0.21 to 0.383 for dexamethasone. The value of "n" and log k was found to vary with type of excipients in the DL-PLA implants.

The time taken to release 25% ($t_{25}$), 50% ($t_{50}$), and 75% ($t_{75}$) of betamethasone and dexamethasone from DL-PLA implants formulated with different excipients was determined from the dissolution data (Table III). DL-PLA implants of betamethasone containing no excipient (blank) required 0.04±0.01 and 3.9±0.2 days to release 25% and 75% of drug respectively. All the excipients increased these values for betamethasone except span 20 (batch E). Amongst the excipients, glyceryl palmitostearate caused maximum time to release 25% (0.15±0.02 day) and 75% (12.14±0.2 day) of betamethasone. These excipients also increased $t_{25}$, $t_{50}$, and $t_{75}$ of dexamethasone from DL-PLA implants (see Table III). $t_{25}$, $t_{50}$, and $t_{75}$ values for dexamethasone were 0.11±0.01 and 13.4±0.5 day respectively as glyceryl palmitostearate was incorporated in the implants. Where as these

Table II: Mathematical Modeling and Drug Release Kinetics of Betamethasone and Dexamethasone Implants

<table>
<thead>
<tr>
<th>Batch</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi</th>
<th>Korsmeyer Model</th>
<th>LogK</th>
<th>n</th>
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<tr>
<td>Betamethasone Sodium Phosphate</td>
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<td></td>
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<tr>
<td>A</td>
<td>0.625</td>
<td>0.767</td>
<td>0.819</td>
<td>0.95</td>
<td>0.295</td>
<td>0.264</td>
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<tr>
<td>B</td>
<td>0.62</td>
<td>0.764</td>
<td>0.812</td>
<td>0.94</td>
<td>0.302</td>
<td>0.267</td>
</tr>
<tr>
<td>C</td>
<td>0.643</td>
<td>0.755</td>
<td>0.823</td>
<td>0.934</td>
<td>0.396</td>
<td>0.25</td>
</tr>
<tr>
<td>D</td>
<td>0.629</td>
<td>0.753</td>
<td>0.821</td>
<td>0.959</td>
<td>0.335</td>
<td>0.268</td>
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<tr>
<td>E</td>
<td>0.61</td>
<td>0.792</td>
<td>0.807</td>
<td>0.936</td>
<td>0.241</td>
<td>0.272</td>
</tr>
<tr>
<td>F</td>
<td>0.604</td>
<td>0.723</td>
<td>0.797</td>
<td>0.938</td>
<td>0.351</td>
<td>0.25</td>
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<tr>
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<td>0.603</td>
<td>0.76</td>
<td>0.799</td>
<td>0.948</td>
<td>0.266</td>
<td>0.24</td>
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<tr>
<td>Dexamethasone Sodium Phosphate</td>
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<tr>
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<td>0.881</td>
<td>0.989</td>
<td>0.275</td>
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<td>B</td>
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<td>0.863</td>
<td>0.975</td>
<td>0.3</td>
<td>0.24</td>
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<tr>
<td>C</td>
<td>0.615</td>
<td>0.725</td>
<td>0.802</td>
<td>0.948</td>
<td>0.383</td>
<td>0.229</td>
</tr>
<tr>
<td>D</td>
<td>0.662</td>
<td>0.776</td>
<td>0.848</td>
<td>0.968</td>
<td>0.332</td>
<td>0.24</td>
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<td>E</td>
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<td>F</td>
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<td>0.866</td>
<td>0.98</td>
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<td>0.821</td>
<td>0.804</td>
<td>0.986</td>
<td>0.21</td>
<td>0.185</td>
</tr>
</tbody>
</table>
values were only 0.07±0.01 and 2.9±0.1 day for implants containing no excipient (blank).

Mean dissolution time (MDT) value is used to characterize drug release rate from a dosage form and indicates the drug release retarding efficiency of polymer. MDT values for betamethasone and dexamethasone were also calculated from the dissolution data (Table III). As shown in the table, MDT values of the implants formulated without excipient were 2.48±0.2 and 2.13±0.3 day for betamethasone and dexamethasone respectively. Except span 20, all the excipients increased this MDT values. But implants prepared with glyceryl palmitostearate showed the highest MDT values. It was 7.68±0.5 day for betamethasone and 8.76±0.4 day for dexamethasone.

Conclusion

Results of the present study demonstrated that different types of lipophilic surfactants and lubricants/diluents could be successfully employed for formulating sustained release biodegradable DL-PLA implants for betamethasone and dexamethasone. Incorporation of the excipients resulted differences in release behavior, especially the burst effect, which is a very crucial point in efficacy and safety of in-situ forming injectable implant. This can be expected to reduce the frequency of administration and decrease the dose-dependent side effects associated with repeated administration of conventional betamethasone and dexamethasone tablets.

Acknowledgments

Authors are grateful to Aristopharma Ltd., Bangladesh for providing betamethasone sodium phosphate and dexamethasone sodium phosphate as gift sample.

References


*Received : May 13, 2009;
Accepted : January 06, 2010*