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Injectable PLGA Adefovir microspheres; the way for long term therapy of chronic hepatitis-B

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ABSTRACT

For patient convenience, sustained release Adefovir Poly-d,l-lactic-*co*-glycolic acid (PLGA) microspheres were formulated to relieve the daily use of the drug which is a problem for patients treated from chronic hepatitis-B. PLGA microspheres were prepared and characterized by entrapment efficiency, particle size distribution and scanning electron microscopy (SEM). *In-vitro* release and *in-vivo* studies were carried out. Factors such as drug: polymer ratio, polymer viscosity and polymer lactide content were found to be important variables for the preparation of PLGA Adefovir microspheres. Fourier transform infrared (FTIR) analysis and differential scanning calorimetry (DSC) were performed to determine any drug-polymer interactions. One way analysis of variance (ANOVA) was employed to analyze the pharmacokinetic parameters after intramuscular injection of the pure drug and the selected PLGA microspheres into rats. FTIR and DSC revealed a significant interaction between the drug and the polymer. Reports of SEM before and after 1 and 24 h release showed that the microspheres had nonporous smooth surface even after 24 h release. The entrapment efficiency ranged between 55.83 and 86.95% and *in-vitro* release studies were continued for 16, 31 and 90 days. The pharmacokinetic parameters and statistical analysis showed a significant increase in the $T_{max,}$ AUC_{0-t} and MRT, and a significant decrease in the C_{max} of the tested formulation ($p < 0.05$). Results demonstrated that PLGA Adefovir microspheres could be used for long-term treatment of chronic hepatitis-B instead of the daily dose used by the patient.

1. Introduction

Chronic Hepatitis B virus infection is a global public health problem, so suppression of the replication of hepatitis B virus is the main effective mechanism of the antiviral drugs used in the treatment of chronic hepatitis B which cause liver cirrhosis and hepatocellular carcinoma (Liver, 2017; D'souza and Foster, 2004; Marcellin et al., 2003).

Adefovir dipivoxil is a prodrug of Adefovir, which makes inhibition and termination of the replication of virus B (Izzedine et al., 2004). Oral administration of a daily dose (10 mg) of Adefovir dipivoxil may continue for several years or lifelong (Sokal et al., 2008). So, injectable sustained release formulations like biodegradable polymeric microspheres were developed to prevent progression of the disease, particularly to cirrhosis, liver failure, and hepatocellular carcinoma and also the sustained virological response is also increased by extending treatment duration (Tang et al., 2014).

Poly (d,l-lactic-*co*-glycolic acid) (PLGA) is one of the most interesting polymers in the field of controlled drug delivery systems, which were approved by the FDA, as it is biodegradable, undergoes erosion after long time and achieves sustained drug release (Danhier et al., 2012; Makadia and Siegel, 2011).

LUPRON DEPOT® is one of the marketed drugs loaded PLGA microspheres. Leuprolide acetate microspheres are commercial products used for the treatment of prostate cancer (Soloway et al., 2002). Also, RISPERDAL CONSTA® (risperidone loaded PLGA microspheres) is indicated for the maintenance treatment of schizophrenia (Eerdekens et al., 2004). Such products can be intramuscularly or subcutaneously administered at 1 month or even 6 month intervals.

Adefovir is available in the market as tablets but not as parenteral sustained formulation (Baker, 2005). So, the aim of this study was to formulate PLGA loaded Adefovir microspheres to attain a prolonged period of release as possible (more than one month), also to reduce the side effects, drug toxicity, the frequency of administration, improve the patient compliance and also the bioavailability of the drug (Han et al., 2016). Some variable parameters were performed to evaluate the formulated microspheres such as entrapment efficiency determination, particle size distribution, scanning electron microscopy, *in-vitro* and *in-vivo* drug release profile. Fourier transform infrared (FTIR) analysis and

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differential scanning calorimetry (DSC) were also performed. Selected formulation from the *in-vitro* studies was used to determine the pharmacokinetics of the drug in rats.

2. Materials and Methods

2.1. Materials

Adefovir was kindly supplied by Eva Pharma., Egypt. PLGA of different viscosity grades and different ratios of lactide and glycolide were purchased from LACTEL International Absorbable Polymers, 2200 River-chase Center, Suite 501 Birmingham, USA. Polyvinyl alcohol (PVA) (molecular weight of 70,000:100,000 Da), dibasic potassium hydrogen phosphate, hydrochloric acid, monobasic sodium hydrogen phosphate and methylene chloride were purchased from El-Nasr Pharmaceuticals Chemicals Co., Egypt. Methanol and acetonitrile (Fisher chemical® HPLC gradient grade) were also purchased. All other chemicals were of analytical grade and used as received. Preparation of buffer and its dilutions were done with Millie-Q demineralized double-distilled water.

2.2. Preparation of Adefovir loaded PLGA microspheres

PLGA microspheres loaded with Adefovir were prepared by emulsion solvent evaporation method using distilled water as continuous phase containing PVA as an emulsifier. PVA solution (0.5%) was prepared in distilled water by heating to facilitate the solubility of PVA, and then allowed to cool at room temperature. The drug and polymer were weighed and dissolved in 20 ml methylene chloride at room temperature. The above organic phase was slowly added to 100 ml of 0.5% PVA solution at room temperature and emulsified by Heidolph PZP stirrer at 1600 rpm for 5 h. Microspheres formed were filtered, washed with water and dried overnight at room temperature (Goyal et al., 2011; Han et al., 2016; Nila et al., 2014). Formulations were prepared as shown in Table 1.

2.3. Estimation of Entrapment Efficiency of Adefovir Microspheres

Microspheres equivalent to 10 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by dissolving the microspheres in methylene chloride and then extracting with aliquots of 0.1 N hydrochloric acid by agitation in mechanical stirrer. Methylene chloride was evaporated then the solution was filtered through Whatman filter paper in 100 ml volumetric flask and the volume was adjusted to 100 ml using 0.1 N hydrochloric acid. The solution was diluted suitably and analyzed for drug content spectrophotometrically at λ_{max} (260 nm) after construction a calibration curve of Adefovir in 0.1 N hydrochloric acid with linearity range (5–40 µg/ml) by using GENESYS 10S spectrophotometer, USA. 0.1 N hydrochloric acid was used as a blank. The percent entrapment efficiency is calculated using the following equation (Sabry, 2013).

% Entrapment Efficiency = (Actual content/Theoretical content) × 100.

2.4. Particle Size Determination

Microspheres (50 mg) were suspended in distilled water (5 ml) containing 2% w/v of tween 80 to prevent microsphere aggregation. The above suspension was sonicated in a water bath and the particle size was expressed as volume mean diameter in micrometer using laser diffraction technique (Mastersizer 2000 Ver. 5.6).

2.5. Scanning Electron Microscopy Study (SEM)

Morphology was characterized by scanning electron microscopy using JEOL-T330A scanning microscope (Japan). Dry samples were placed on an aluminium plate and coated with gold. Pictures of microparticles were randomly taken. Other two samples were prepared by keeping them into 100 ml of Sörensen phosphate buffer and kept in a shaker at 37 ± 0.5 °C; one sample for 1 h and the other for 24 h. The microparticles were separated from the media and frozen at −80 °C for 4 h. The frozen samples were then lyophilized for 24 h in a freeze drier. The dried samples were sputter coated with gold and then SEM micrographs were obtained.

2.6. In-vitro Release Study

The dissolution of Adefovir pure powder and its release from the prepared microparticles were performed using the dialysis bag method. Microspheres equivalent to 10 mg of Adefovir were placed into cellulose dialysis bags (Molecular weight of 12,000:14000 Da) and then suspended in 100 ml of Sörensen phosphate buffer pH 7.4 in a closed bottle (Liu and Lv, 2014). The bottles were shaken at 50 rpm and 37 ± 0.5 °C. Each sample was run in triplicate. Aliquots of 3 ml were taken from each bottle at 0.5, 1, 2, 3, 4, 5, 6, 8 and 24 h then at 2, 3, 5, 7, 10, 13, 16, 21, 31, 41, 45, 55, 62, 70, 80 and 90 days. Samples were analyzed for the drug content spectrophotometrically at 260 nm against Sörensen phosphate buffer pH 7.4 as a blank. The dissolution medium was replaced with fresh medium to maintain a sink condition. Then, the average of the standard error of mean values was calculated (Ahmed et al., 2012).

2.7. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer using KBr disk method. The scanning range was 400–4000 cm^{-1} and the resolution was 1 cm^{-1}.

Table 1
Composition and entrapment parameters of Adefovir-loaded PLGA microspheres.

Batch no.	Formulation variables			Entrapment parameters		
	Polymer type	Drug: polymer ratio	Polymer inherent viscosity dL/g	Actual content (mg)	Theoretical content (mg)	Entrapment efficiency (%)
1	PLGA (50:50)	1:4	1.05	5.66	10	58.33
2	PLGA (50:50)	1:7	1.05	7.22	10	72.20
3	PLGA (50:50)	1:10	1.05	8.45	10	84.58
4	PLGA (50:50)	1:13	1.05	8.69	10	86.95
5	PLGA (50:50)	1:10	0.55–0.75	5.58	10	55.83
6	PLGA (65:35)	1:10	0.55–0.75	5.70	10	57.00
7	PLGA (75:25)	1:10	0.55–0.75	6.04	10	60.40
8	PLGA (85:15)	1:10	0.55–0.75	6.55	10	65.50

SDs did not exceed 1.5% of the reported value.

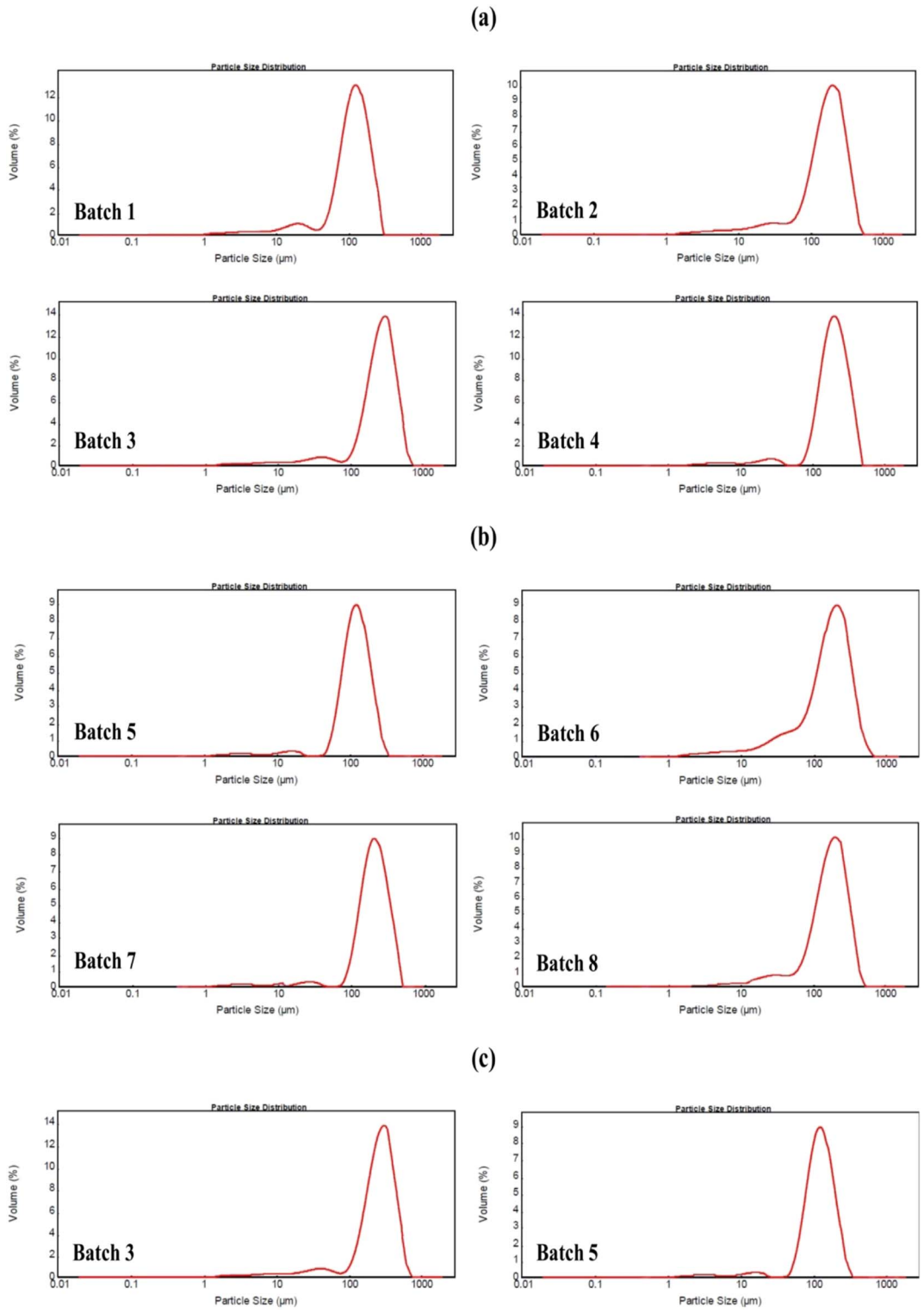


Fig. 1. Effect of (a) the drug: polymer ratio, (b) polymer lactide content and (c) polymer viscosities on the distribution of particle size of Adefovir-PLGA microspheres.

2.8. Differential Scanning Calorimetry (DSC)

The DSC thermograms were recorded on a Shimadzu-DSC 50. Samples (2.5 mg) were heated in hermetically sealed aluminium pans over a temperature of 30–300 °C at a constant rate of 10 °C/min under a nitrogen purge (30 ml/min). The physicochemical status of the Adefovir in the microspheres was measured by differential scanning calorimetry (DSC) analysis.

2.9. HPLC Analysis

High performance liquid chromatography (HPLC) (Agilent 1200 series, Germany) with Agilent Zorbax ODS® 5 µ C_{18} (250 × 4.6 mm I.D., 5 µm particle size) column was used for the chromatographic separation. Elution pumps ran modified isocratic mobile phase which consisted of methanol, acetonitrile and distilled water (70:10:20% v/v). The autosampler utilized acetonitrile as a rinse solution, injection volume was 20 µl and flow rate of mobile phase was 1.1 ml/min.

Table 2
Distribution of particle size of Adefovir-loaded PLGA microspheres (%).

Batch no.	200:350 µm	125:200 µm	60:125 µm	<60 µm
1	1.51	22.51	73.51	2.47
2	4.46	63.14	30.69	1.71
3	6.75	89.58	2.89	1.78
4	7.20	91.60	0.7	0.5
5	6.50	44.65	45.2	3.65
6	8.30	49.87	35.32	6.51
7	11.71	55.17	28.01	5.11
8	17.13	61.66	20	1.21

SDs did not exceed 1.5% of the measured value.

Photodiode array detector (DAD) was used and detection was performed at 260 nm. The column was operated at room temperature.

2.10. Quantification of Adefovir in Plasma

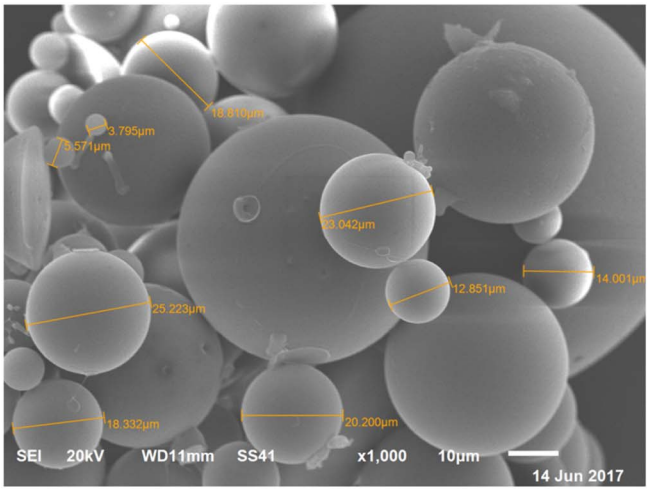
A stock solution of Adefovir was prepared at a concentration 600 µg/ml using methanol as a solvent. It was serially diluted with methanol to provide standard solutions at concentrations 400, 300, 200, 100, 20, 10 µg/ml of Adefovir. 500 µl of standard solutions were pipetted into 2 ml polypropylene microcentrifuge tube and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was mixed with 500 µl of plasma for 60 s followed by addition of 1 ml methanol. The concentrations in standard plasma samples were 200, 150, 100, 50, 10, 5 µg/ml. The mixture was mixed for another 60 s and centrifuged at 12,000 rpm for 5 min at 25 °C. 20 µl of the supernatant after its separation was automatically injected into the HPLC system for analysis. A calibration curve was constructed by plotting the area under the curve against Adefovir concentrations. Validation parameters such as linearity range, limit of detection and limit of quantification were included and calculated according to the requirements of the International Conference on Harmonization (ICH) guidelines (Guideline, 2005).

2.11. Pharmacokinetic Study

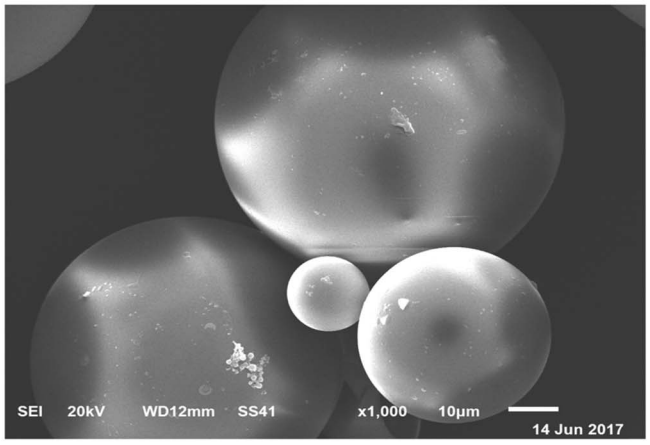
Adult albino male rats (weighing 220–260 g) were used for the bioavailability study. Animals were housed at the standardized conditions in the animal house of the Faculty of Pharmacy, Zagazig University, Egypt. All animal procedures were performed in accordance with the approved protocol for use of experimental animals set by the standing committee on the animal care of the Faculty of Pharmacy, Zagazig University (approval number: P1-10-2017).

Twelve rats were used and randomly divided into two groups. One group received pure Adefovir after dissolving in polyethylene glycol (PEG) 400, while the other group was given Adefovir microspheres (batch 3) after reconstitution in 0.5% carboxymethylcellulose w/v. Batch 3 was chosen for this assay as it showed higher entrapment efficiency (84.58%) with lower polymer content when compared with batch 4 which showed very close entrapment efficiency (86.95%) to batch 3 but with more polymer content. The formulations were administered intramuscularly by using gauge needles 18 and equivalent amount of 60 mg of Adefovir/kg, which was calculated according to (Reagan-Shaw et al., 2008). 12 times of therapeutic dose (10 mg) for Adefovir was considered to be safe when used in rats according to the specifications of Adefovir (De Clercq, 2003). For the first group, orbital blood samples (0.5 ml) were collected at 0.5, 1, 2, 3, 4, 6, 8 and 24 h. While for the second group, 0.5 ml blood samples were collected at 4, 8, 12 and 24 h then at 2, 3, 5, 9 and 15 days. The concentration of Adefovir in each plasma sample was calculated after analyzing by the HPLC method according to the prepared calibration curve of Adefovir in plasma which was mentioned previously.

The pharmacokinetic parameters including the maximum plasma concentration (C_{max}), the time required to reach maximum plasma concentration (T_{max}), area under the plasma concentration–time curve (AUC), mean residence time (MRT) and total clearance (Cl) were



(A)



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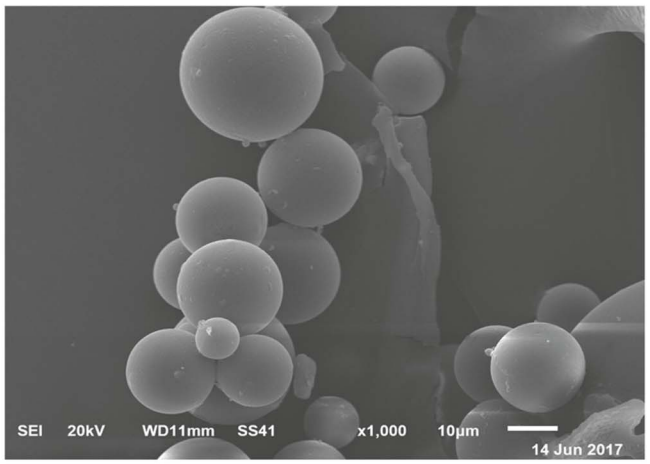

(C)

Fig. 2. SEM of Adefovir microspheres (batch 3)
(A): Before exposing to Sörensen phosphate buffer pH 7.4.
(B): After exposing to Sörensen phosphate buffer pH 7.4 for 1 h.
(C): After exposing to Sörensen phosphate buffer pH 7.4 for 24 h.

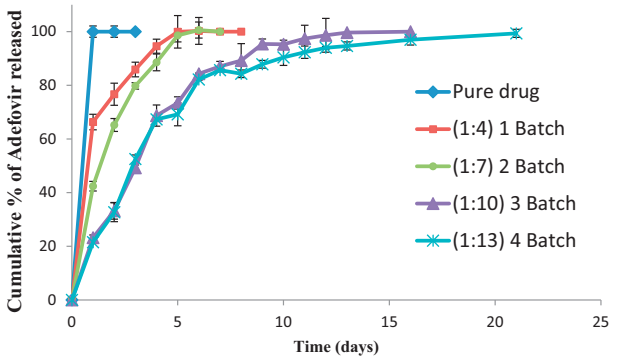

Fig. 3. Effect of the drug: polymer ratio on the *in-vitro* release of Adefovir loaded PLGA microspheres (n = 3).

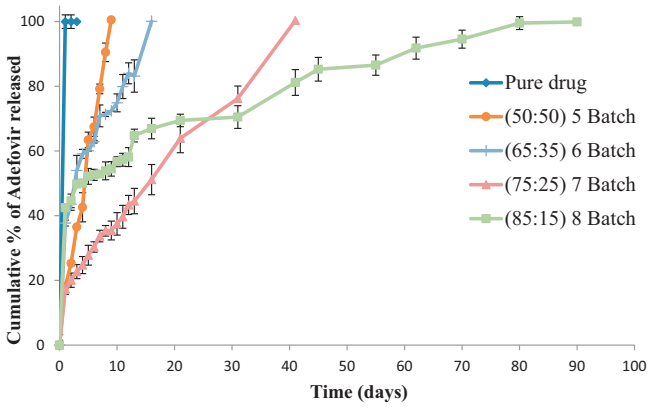

Fig. 4. Effect of lactide content of the polymer on the *in-vitro* release of Adefovir loaded PLGA microspheres (n = 3).

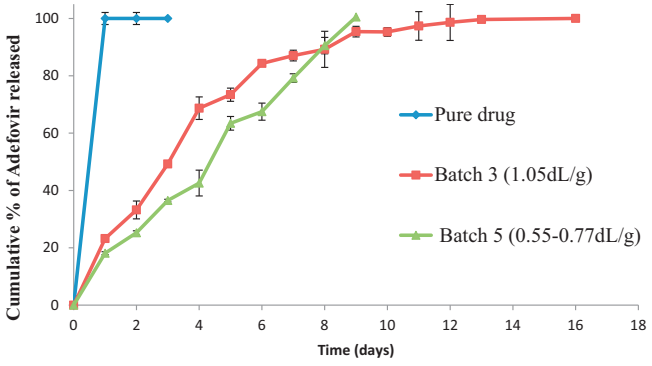

Fig. 5. Effect of inherent viscosity of the polymer on the *in-vitro* release of Adefovir loaded PLGA microspheres (n = 3).

calculated for Adefovir using the pharmacokinetic software WinNonlin Standard Edition Version1.1 (Pharsight, Mountain View, California) using non compartmental method.

2.12. Statistical Analysis

One way analysis of variance (ANOVA) was employed to analyze the pharmacokinetic parameters obtained from the two groups by using GraphPad Prism version 5.04.

3. Results and Discussion

3.1. Estimation of Entrapment Efficiency of Adefovir Microspheres

The entrapment efficiency and the actual values of Adefovir loading within the formulated PLGA microspheres are shown in Table 1. Results from Table 1 show that the entrapment efficiency of Adefovir was increased by increasing drug to polymer ratio, the inherent viscosity and lactide to glycolide ratio but with different degrees. The entrapment efficiency was increased from 58.33 to 86.95% for PLGA microspheres respectively when the ratio of drug: polymer was increased from 1:4 (batch 1) to 1:13 (batch 4). Batch 3 and batch 4 showed very close entrapment efficiency (84.58 and 86.95%). Also, the entrapment efficiency was increased from 55.83 to 84.58% when the viscosity of PLGA was 0.55–0.75 (batch 5) and 1.05 dl/g (batch 3), respectively. These results are in agreement with previous publications (Ramya Shivani and Krishna Sailaja, 2014; Rathod et al., 2012) who stated that the entrapment efficiency of microspheres increased with increasing the polymer concentration due to increasing the viscosity of the internal phase and the availability of the polymer for encapsulating the drug which may result in reduction of drug loss during evaporation.

Also, the entrapment efficiency increased from 55.83 to 65.50% when the lactide content was 50% (batch 5) and 85% (batch 8), respectively, while the change in lactide concentration from 50% (batch 5) to 65% (batch 6) and to 75% (batch 7) did not significantly affect the entrapment efficiency of microspheres.(Sharma et al., 2016)reported that the entrapment efficiency for formulation with PLGA 75:25 and PLGA 50:50 were almost the same, despite the difference in lactide concentration. This may be due to that the change in lactide concentration from 50 to 75% does not significantly influence the characters of nanoparticles. Also, he found that in case of the polylactic acid (PLA), when the lactide concentration is increased from 50 to 100%, the hydrophobic interaction between the lipophilic drug and PLGA polymer is increased, which result in higher entrapment efficiency.

3.2. Particle Size Determination

Fig. 1 and Table 2 demonstrate the distribution of particle size of Adefovir microspheres. The particle size measurements demonstrated in the table ranges between < 60 and 350 µm. It is clear that increases in the polymer concentration, polymer lactide content and polymer viscosities showed increases in the percentage of the prepared microspheres that show higher particle sizes. These results were observed for PLGA polymers by (Chorny et al., 2002; Patel and Patel, 2014) who explained that increasing polymer concentration, polymer viscosities and lactide concentration cause increasing the viscosity of the polymer solution, so coarse emulsions are obtained which led to the formation of large particles during the diffusion process (Quintanar-Guerrero et al., 1996).

3.3. Scanning Electron Microscopy Study(SEM)

The formulated microspheres were well formed, and Adefovir loading did not affect the morphology of the formulated microspheres when compared with the blank microspheres. Fig. 2 shows the scanning electron microscopy of Adefovir microspheres (batch 3) before and after exposing to Sörensen phosphate buffer pH 7.4 for 1 h and 24 h. The microspheres showed a spherical, nonporous smooth surface even after exposing to the buffer for 24 h. A partial barrier was created by the oily phase around the polymer, leading to a slow release of the organic solvent to the aqueous phase and resulting in the formulation of microspheres with nonporous smooth surface (Ahmed et al., 2012).

3.4. In-vitro Release Study

Figs. 3, 4 and 5 show the release of Adefovir from microspheres according to different factors that found to play a significant effect on the drug release. For instance, Fig. 3 demonstrates the effect of drug: polymer ratio on the release mechanism. It is obvious that the release of pure drug occurred after 24 h. In contrast, the release from batches 1 and 2 reached 100% after 7 days. On the other hand, batches 3 and 4

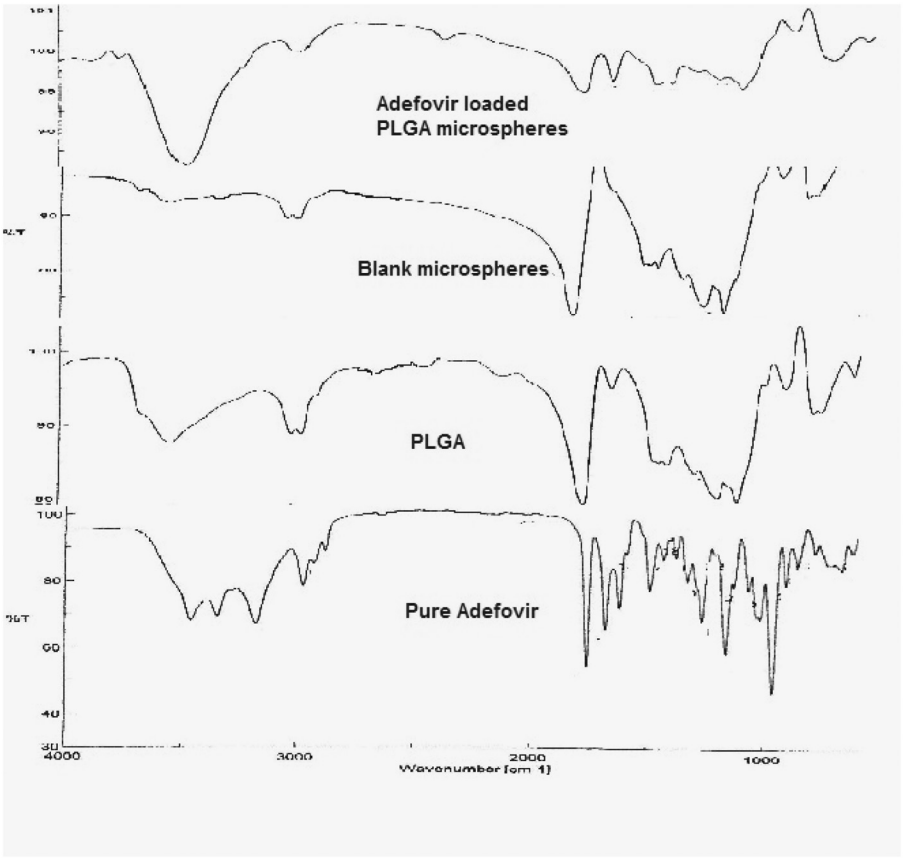

Fig. 6. FTIR spectra of pure Adefovir, PLGA, blank microspheres and Adefovir-loaded PLGA microspheres (batch 3).

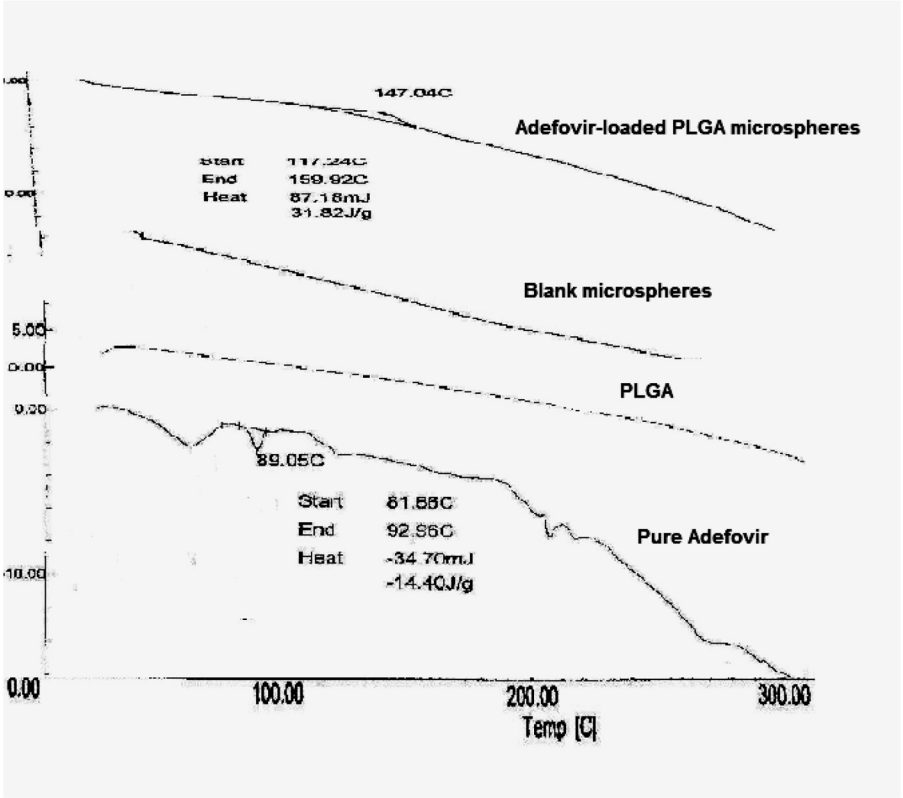

Fig. 7. DSC thermograms of pure Adefovir, PLGA, blank microspheres and Adefovir-loaded PLGA microspheres (batch 3).

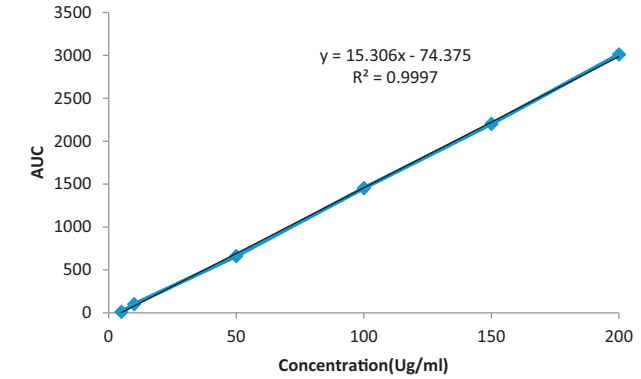

Fig. 8. Standard calibration curve of Adefovir in rat plasma.

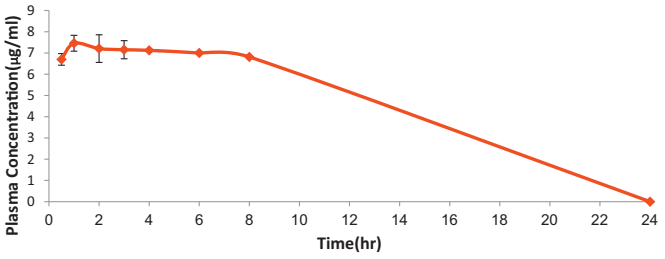

Fig. 9. Plasma concentration *versus* time of pure Adefovir after intramuscular injection in rats (n = 6).

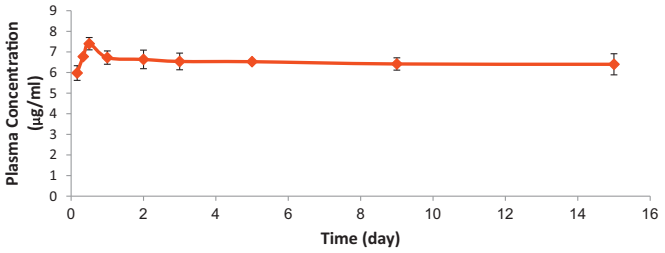

Fig. 10. Plasma concentration *versus* time of Adefovir loaded PLGA microspheres (batch 3) after intramuscular injection in rats (n = 6).

Table 3
Pharmacokinetic parameters after intramuscular injection of pure Adefovir (in hours) and Adefovir loaded PLGA microspheres batch 3 (in days), (mean ± SD, $n = 6$).*

Parameter	Pure Adefovir	Adefovir loaded PLGA microspheres (batch 3)
C_{max}	7.45 + 1.98 (µg/ml)	7.4* ± 1.88 (µg/ml)
T_{max}	1.00 ± 0.00 (hr)	0.5* ± 0.00 (d)
K_{el}	0.011 ± 0.003 (hr^{-1})	0.0019* ± 0.055 (d^{-1})
$t_{1/2}$	62.16 ± 3.52 (hr)	359.10* ± 11.66 (d)
AUC_{0-t}	56.47 ± 3.89 (µg/ml·h)	97.43* ± 6.58(µg/ml·d)
$AUC_{0-\infty}$	667.55 ± 10.54 (µg/ml·h)	3413.14* ± 8.64 (µg/ml·d)
$AUMC_{0-\infty}$	3.53 ± 0.55 (µg/ml·hr^2)	3.45* ± 0.78 (µg/ml·d (Baker, 2005))
MRT	96.55 ± 4.36 (hr)	503.62* ± 7.89 (d)

* Significant at $P < 0.05$ level.

showed a gradual release that reached 100% after 16 and 21 days respectively that show extended release. From the preceding result, it clearly demonstrates that increasing the concentration of the polymer is accompanied by a reduction in the drug released, so extended action can be obtained (Freiberg and Zhu, 2004). Another factor that found to affect the drug release is the polymer lactide content. Fig. 4 results indicate that increasing the lactide percentage from 50 to 85 produced a significant prolongation time of the drug release, that enhances the sustaining effect. Batches 5, 6, 7 and 8 showed a sustained release of the drug that continued for 9, 16, 41 and 90 days respectively. The slower release of the drug from batches with higher lactide content can be attributed to slow drug diffusion from the microspheres since the hydrophobicity is expected to be increased by increasing the lactide content. This is in accordance with Budhian et al. (2005) and Okada et al. (1994) who mentioned that increasing the lactide content cause a reduction in hydration and swelling of the polymer that leads to slower diffusion of the drug from the polymer matrix.

Moreover, the effect of inherent viscosity of the polymer on the drug release is shown in Fig. 5. It can be observed that batch 5 of polymer with lower viscosity (0.55–0.77 dL/g) showed a complete release of the drug after 9 days only. On the contrary, batch 3 which poses the same polymer concentration and the lactide content like batch 5 but with a higher inherent viscosity (1.05 dL/g) gave a prolonged time for releasing the drug where it reached 100% after 16 days compared to batch 5 (9 days). This assumption is expected because increasing the viscosity causes reduction of the permeability of the polymer followed by reduction of the drug release (Zidan et al., 2006).

3.5. Fourier Transform Infrared Spectroscopy

FTIR was performed to determine the possible type of interaction between Adefovir and PLGA. Fig. 6 showed that the characteristic shoulders of Adefovir were traced at 3456 cm^{-1} (N—H stretching), 3175 cm^{-1} (C—H aromatic), 2969 cm^{-1} and 2923 cm^{-1} (C—H aliphatic stretching), 1579 cm^{-1} (C=C aromatic) and 1258 cm^{-1}(C—O stretching). In case of Adefovir PLGA loaded microsphere, the characteristic N—H peak of the drug became broader and showed higher intensity than that of the pure drug and also the disappearance of the carbonyl group band of PLGA. This may be attributed to the intermolecular hydrogen bonding between the drug and the polymer, which reflect a significant interaction between the drug and the polymer.

3.6. Differential Scanning Calorimetry

Fig. 7 illustrates the DSC thermograms for the same samples which were used for FTIR analysis. The DSC trace of Adefovir showed a small endothermic peak at 89.05 °C, which is corresponding to its melting point.

It is clear that the incorporation of Adefovir within PLGA showed a new broad endothermic peak at 147.04 °C which is higher than that of the pure drug. This result indicated the interaction of Adefovir with carboxyl terminal ends of PLGA thus proving formation of an amorphous solid solution in the polymeric matrix (Guo et al., 2015).

3.7. Quantification of Adefovir in Plasma

Fig. 8 reflected the calibration curve of Adefovir in the plasma. A good linearity was obtained within the concentration range (5–200 µg/ml) with a good correlation coefficient (R (Baker, 2005)) of 0.999. The limit of detection was calculated to be 1.426 µg/ml while the limit of quantification was 4.756 µg/ml.

3.8. Pharmacokinetic Study

Plasma concentration-time profiles of Adefovir after intramuscular administration of Adefovir solution and Adefovir loaded PLGA microspheres were shown in Fig. 9 and Fig. 10.

After intramuscular injection of the pure drug solution, the peak plasma concentration of Adefovir C_{max} (7.45 µg/ml) reached after the first hour, it maintained for 8 h then gradually disappeared from the plasma after 24 h.

In contrast, after injection the Adefovir loaded PLGA microspheres, the peak plasma concentration C_{max} (7.4 µg/ml) reached after 6 h, then a relatively steady state concentration of 6.5 µg/ml was observed and continued for 15 days. The half-life of the drug showed a very

significant difference between pure drug and loaded microspheres as shown in Table 3. In the first case, it was 62.16 h while in case of PLGA loaded microspheres it was 359.10 days. A result of more attractive is that $AUC_{0-\infty}$ was 667.55 μg/ml·h in case of the pure drug, while that of PLGA microspheres, it was 3413.14 μg/ml·d. The slower time to maximum plasma concentration of Adefovir microspheres can magnify that the use of PLGA polymer gave a sustained effect for several days not hours as in case of the pure drug (Xie et al., 2014).

4. Conclusion

Adefovir can be formulated as parenteral biodegradable microspheres by emulsion solvent evaporation technique using PLGA polymer. *In-vitro* studies showed that Adefovir microspheres gave a continuous release profile extended for three months. *In-vivo* release studies of the selected formula showed that the AUC of the prepared Adefovir microspheres was higher several times than the injected Adefovir solution ($p < 0.05$). In addition, the half-life of the drug when incorporated in microspheres was thousands of times than that of the pure drug.

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