Microencapsulation of Doxycycline into Poly(lactide-*co*-glycolide) by Spray Drying Technique: Effect of Polymer Molecular Weight on Process Parameters

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ABSTRACT: Poly(lactide-*co*-glycolide) (PLGA) polymers with three different molecular weights were prepared, and microparticles were produced by spray drying and water-in-oil-water (w/o/w) double emulsion techniques to encapsulate 86% of doxycycline (DXY), an antibiotic drug, for the use of periodontitis. Placebo and drug-loaded microspheres and pristine DXY were analyzed by Fourier transform infrared spectroscopy, which indicated no chemical interactions between DXY and PLGA. X-ray diffraction of drug-loaded microspheres confirmed the molecular level dispersion of DXY in PLGA. Scanning electron microscopy confirmed spherical nature and smooth surfaces of the microspheres. Mean particle size as measured by laser light scattering technique ranged between 10 and 25 μm. *In vitro* release of DXY performed in 7.4 pH media continued up to 72 h and depended on molecular weight of PLGA and extent of DXY loading. Antimicrobial studies performed on one formulation and placebo microspheres suggested that drug concentrations during *in vitro* release are above the minimum inhibitory concentration (MIC) for *Staphylococcus aureus* growth. Overall, the release studies depended on the molecular weight of PLGA, extent of drug loading, and the method used to prepare microspheres. Statistical analyses of release data performed using the analysis of variance (ANOVA) method agreed well with experimental observations. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 4038–4046, 2008

Key words: PLGA; doxycycline; microspheres; antimicrobial study

INTRODUCTION

Biodegradable polymers are the most widely studied systems in drug delivery applications.[1,2] In the previous literature, the biodegradable polymers, viz., poly(ε-caprolactone) (PCL), poly(3-hydroxybutyrate) (PHB), poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(lactide-*co*-glycolide) (PLGA) have been explored in controlled release (CR) studies.[3–6] Of these, PLGA has been the most widely used polymers in CR applications, despite its slow degradation rate, extending to months or years, depending on its composition, and molecular weight. There are other examples wherein, Goodson et al.[7] developed delivery system comprising ethylene vinyl acetate fiber incorporated with tetracycline, which exhibited *in vitro* drug release up to 9 days. Kenawy et al.[2] employed electrospinning technique to develop PLA, poly(ethylene-*co*-vinyl acetate) (PEVA)/tetracycline fiber systems that exhibited *in vitro* drug release up to 6 days. Mundargi et al.[8] reported the development and clinical studies on doxycycline (DXY)-loaded PLGA : PCL blend microspheres in periodontal applications.

With respect to the limited amount of data published on the effect of molecular weight of PLGA on formulation variables such as the amount of drug, encapsulation efficiency, solvent type, etc., in this study, in addition to water-in-oil-water (w/o/w) double emulsion techniques, we have undertaken a detailed study to prepare microspheres by spray-drying technique, a method that has been widely used in large-scale production of drug-loaded microspheres.[9–12] This one-step method has good control on process parameters with excellent scale-up possibility. The mixture to be sprayed can be solvent, emulsion, suspension, or dispersion. The feed is atomized into millions of individual droplets by a nozzle giving an increased surface area of the sprayed solution, and the solvent is vaporized immediately by passing hot air or N_2. The product obtained can be powdered to evenly sized particles in just few minutes. An advantage of this method is that it requires only about 50–100 mL of solvent or suspension to produce particles of uniform size.

This study aims at investigating the effect of process parameters like PLGA molecular weight and drug loading of DXY-loaded microspheres. DXY is a

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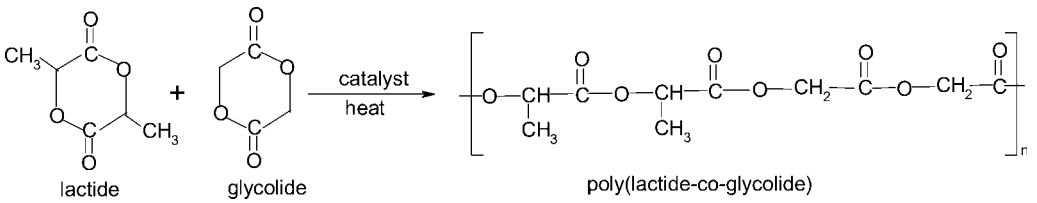

Figure 1 Formation of PLGA from lactide and glycolide.

broad-spectrum antibiotic, derived from oxytetracycline group and is bacteriostatic, inhibiting bacterial protein synthesis, due to the disruption of transfer RNA and messenger RNA at the ribosomal sites.[13,14] Its use in periodontal applications has been studied before,[8] since the microorganisms associated with periodontal disease are susceptible to DXY at concentrations of 6.0 μg/mL.[15] However, systemic administration of antibiotics used in the treatment of periodontitis has shown many drawbacks including inadequate antibiotic concentration at the site of periodontal pocket[16,17] and rapid decline of plasma antibiotic concentration to subtherapeutic levels.[18,19] We, therefore, felt it is necessary to develop the localized drug delivery systems that would provide an effective concentration of DXY, needed in periodontal applications with minimum side effects.

The principal objective of this article is to identify the optimum formulation parameters for DXY-loaded PLGA microspheres to achieve its maximum therapeutic efficacy. The spray drying method used for particle production is compared with the water-in-oil-water (w/o/w) double emulsion solvent evaporation technique for the medium molecular weight PLGA. Formulation and process variables affecting the production of microspheres and their *in vitro* release characteristics have been investigated. Chemical stability of the DXY-loaded microspheres was confirmed by Fourier transform infrared spectroscopy (FTIR). X-ray diffraction (XRD) was used on drug-loaded microspheres to investigate drug's dispersion in PLGA. Scanning electron microscopy (SEM) was employed to assess the nature of microspheres. *In vitro* release of DXY was performed on these formulations in pH 7.4 phosphate buffer at 37°C. Antimicrobial efficacy of pristine DXY, placebo microspheres, and DXY-loaded microspheres have been tested on *S. aureus* pathogen.

EXPERIMENTAL

Materials and methods

DXY HCL was procured from Vetcare R and D Centre (Bangalore, India). Analytical reagent grade samples of poly(vinyl alcohol) (M_w = 125,000 and 98% hydrolyzed) and dichloromethane (DCM) were purchased from S.D. Fine Chemicals (Mumbai, India). Dialysis membrane-110 was purchased from Himedia Laboratories (Mumbai, India). Double-distilled water was used throughout the work. All the chemicals were used without further purification.

Preparation of PLGA

The PLGA polymers of three different molecular weights were prepared as per the reaction scheme shown in Figure 1 by ring-opening polymerization using freshly prepared and purified D,L-lactide and glycolide monomers. In the above reaction, tin 2-ethylhexoate (tin octoate) was used as a catalyst and the polymerization was carried out at 180°C for 6, 12, and 18 h under inert atmosphere as per the procedure described in the literature.[20] Polymerized yellow colored products were purified by dissolving in DCM and re-precipitating in excess of methanol. The resultant purified products were vacuum-dried in oven at 60°C for 12 h and stored in an inert atmosphere before further study. GPC analysis was carried out at SICART (Anand, Gujarat, India) using Perkin–Elmer, Series-200 equipped with RI detector using THF solvent at 30°C. Molecular weight and polydispersity index data are summarized in Table I.

Preparation of microspheres by spray drying method

First step consisted of the preparation of w/o emulsions in which aqueous solutions containing 5 or 10% DXY were prepared in 5 mL of water. PLGA (1 wt %) was dissolved in 200 mL of ethyl acetate.[12] The aqueous phase was added drop-wise to the polymer solution under stirring at 500 rpm speed, which was continued for 5 min. The second step consisted of spraying w/o emulsion in a spray-drier (LU-222 Advance Spray Drier, Labultima, Mumbai, India), cocurrent flow type, equipped with standard

TABLE I
GPC Data of PLGA Polymers At 25°C

Polymer	M_w	M_n	M_w/M_n
PLGA-High	91,342	54,374	1.67
PLGA-Med	75,857	45,379	1.67
PLGA-Low	46,257	29,780	1.55

TABLE II
Results of % Encapsulation Efficiency and Mean Particle Size of Various Formulations

Formulation code	Polymer	% DXY loaded	% Encapsulation efficiency	Mean particle size (μm)
F1	PLGA-High	5	86.8	25.0 ± 1.01
F2	PLGA-High	10	67.1	19.6 ± 0.64
F3	PLGA-Med	5	69.2	21.2 ± 0.97
F4	PLGA-Med	10	51.0	16.2 ± 0.50
F5	PLGA-Low	5	58.7	12.2 ± 0.50
F6	PLGA-Low	10	40.0	10.2 ± 0.61
F7[a]	PLGA-Med	10	19.0	26.2 ± 0.25

[a] Particles prepared by solvent evaporation method.

nozzle of 0.7 mm inner diameter. Process conditions used were as follows: inlet air temperature, 59°C ± 5°C; outlet air temperature, 45°C ± 5°C; and feed spray rate, 10 mL/min. The time required to spray 220 mL of emulsion was about 22 min. Emulsions were kept under magnetic stirring during the entire spraying process at ambient temperature (25°C). Solid microspheres obtained were harvested and kept under vacuum for 48 h before use. Placebo microspheres were prepared in the same manner to be used as control for characterization studies. The compositions of various formulations along with formulation codes are summarized in Table II.

Preparation of microspheres by solvent evaporation method

A w/o/w double emulsion solvent evaporation method[6] with minor modifications was adopted to formulate DXY-loaded PLGA microspheres. In this method, DXY equivalent to 10% (w/w) dry weight of PLGA was dissolved in 5 mL distilled water as the aqueous phase; 1 g of PLGA dissolved in 100 mL of ethyl acetate was the oil phase; and 5 mL of aqueous drug solution was added to the PLGA solution and emulsified using a high-speed stirrer (Remi Lab Stirrer, Remi Motors, Mumbai, India) for about 5 min to form stable w/o emulsion. This stable w/o emulsion was slowly added to 100 mL aqueous solution containing 1 wt % PVA emulsified using Remi Lab Stirrer at 500 rpm speed to form w/o/w emulsion under ambient conditions. Solvent removal and hardening of the microspheres was done by continuously stirring the mixture for about 3 h at 25°C. Microspheres were isolated by filtration and washed with distilled water several times to remove PVA. The produced microspheres were dried at ambient temperature (25°C) for 24 h and dried in vacuum chamber at 25°C for 12 h to remove any residual solvent.

Determination of encapsulation efficiency

To estimate DXY content, drug-loaded microspheres were dissolved in DCM and drug were extracted in phosphate buffer (pH 7.4)[21] followed by UV analysis. Briefly, 10 mg of each batch of DXY-loaded microspheres was dissolved in DCM and 15 mL of phosphate buffer was added to extract DXY. The above suspension was vigorously mixed by vortexing and allowed to stand until clear solution was obtained; it was separated and filtered through 0.45-mm filter (Sartorius, Germany) to remove any polymeric debris. The clear solution obtained was analyzed for DXY content using UV spectrophotometer (UV-1650 PC, Shimadzu, Duisburg) at λ_{max} value of 275 nm. The % drug loading and % encapsulation efficiency of PLGA microspheres were as follows:

$$\%\ \text{Drug loading} = \left(\frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}}\right) \times 100 \quad (1)$$

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\%\ \text{Drug loading}}{\%\ \text{Theoretical loading}}\right) \times 100 \quad (2)$$

Particle size measurements

Particle size was measured by laser light scattering technique (Mastersizer 2000, Malvern, UK). The sizes of the completely dried microspheres of different formulations were measured by dry sample technique using dry sample adapter. The completely dried particles were placed on a sample tray and a compressed air system with inbuilt vacuum was used to suspend the microparticles. The laser obscuration range was maintained between 1 and 2% and volume-mean diameter (V_d) was recorded. After measuring particle size of each sample, the dry sample adopter was cleaned thoroughly to avoid any cross contamination. Each batch was analyzed in triplicate, and its average value was considered in data analysis.

FTIR spectral studies

FTIR spectra were taken on Spectrum GX, Perkin–Elmer (USA) instrument to investigate the possible

chemical interactions between DXY and PLGA. Samples were crushed with KBr and pellets were made at 300 kg/cm^2 pressure. FTIR spectra of placebo microspheres, pristine DXY, and DXY-loaded microspheres were scanned in the range between 4000 and 400 cm^{-1} with scanning resolution 4 and number of scans 10.

XRD studies

Crystalline nature of the pristine DXY and DXY-loaded microspheres were evaluated by powder XRD technique using Philips model PW-1710, UK diffractometer attached to the digital graphical assembly, and a computer with Cu—NF 25 KV/20-mA tube as Cuα-radiation source in the range of 0°–50° of 2θ.

SEM studies

SEM images of 10% DXY-loaded microspheres were taken using JEOL model JSM-840A (Japan). Microspheres were sputtered with gold to make them conducting and placed on a copper stub. Thickness of the gold layer accomplished by gold sputtering was about 15 nm.

In vitro drug release studies

In vitro drug release from different formulations was investigated in phosphate buffer solution (PBS) of pH 7.4 (without enzymes). Microspheres (10 mg) were suspended in 1 mL of PBS and placed within the dialysis bag.[21] The sample within the dialysis bag was kept in a conical flask containing 50 mL of PBS as the dissolution medium on a shaker at 100 rpm at 37°C (New Brunswick Scientific Innova 4230, MN, USA). The amount of drug released was determined by withdrawing 2 mL aliquots at the selected specific time intervals. The volume withdrawn was replenished with an equal volume of fresh and prewarmed PBS at 37°C. Samples were analyzed by UV spectrophotometer (UV-1650 PC, Shimadzu, Duisburg) at λ_{max} value of 275 nm using PBS as the blank.

Antimicrobial studies

Antimicrobial activities of DXY-loaded, placebo microspheres, and pristine DXY were evaluated by performing the experiments in triplicate. Pristine DXY at different concentrations of 1–7.5 μg/mL in PBS (pH 7.4) was tested against *E. coli*, *Pseudomonas aeruginosa, S. aureus*, and *Klebsiella pneumoniae* isolates found in periodontitis patients.[22,23] For formulation F2, samples collected from *in vitro* release study of DXY-loaded and placebo microspheres at different time intervals (2, 4, 6, 24, 30, 48, 50, and 72 h) were also tested against *S. aureus*.[22] Molten nutrient agar media (1%, 25 mL) was cooled to 40°C, mixed thoroughly with 1 mL of 24-h grown *S. aureus* culture with an absorbance of 0.1 at 600 nm and transferred to sterile petri-dishes for solidification. Wells of diameter of 0.75 cm, equidistant from one another, were made in solidified medium using sterilized well borer. The solutions (100 μL) collected (*in vitro* drug release aliquots and pristine drug solutions) were filtered through sterilized millipore membrane filters (0.45 μm) and added into the wells. Samples were allowed to diffuse for 30 min at 25°C and plates were incubated for 24 h at 37°C. The diameter (cm) of zone of growth inhibition surrounding each well was measured.

Statistical analyses

Statistical analyses were done using SPSS statistical package. Analysis of variance followed by least significant difference (LSD) procedure was used for comparison of drug release rates from different formulations and with $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Preparation and characterization of spray-dried formulations

The solvent evaporation or spray-drying methods described here are able to effectively encapsulate DXY into PLGA microspheres. The spray drying technique has produced microparticles in the size range of 10–25 μm for different formulations. However, in case of microparticles prepared from water-in-oil-water (w/o/w) double emulsion solvent evaporation method, the particle size was 26 μm, which is slightly higher than those of the particles prepared by spray drying technique. Notice that in the w/o/w method, PLGA was dissolved in ethyl acetate, whereas DXY was dissolved in water. The dispersed organic phase was then emulsified into aqueous phase to produce droplets of regular shape and uniform size. In case of spray drying technique, the droplets remained stable over the entire process of spray drying without the use of surfactants, while producing the microparticles. Moreover, during the spraying of the emulsion, more of ethyl acetate was evaporated, giving solid microparticles.[24] To understand more about finding the optimum formulation protocols, we have investigated the effect of PLGA molecular weight on size and encapsulation efficiency of the DXY-loaded microparticles.

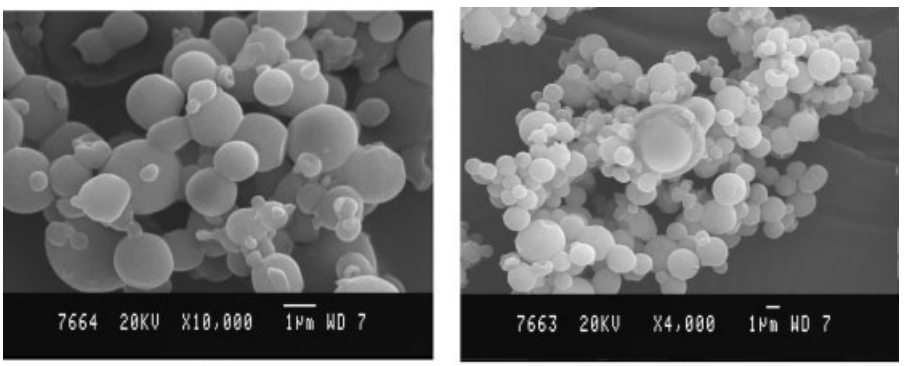

Figure 2 SEM image of DXY-loaded PLGA microspheres.

Effect of PLGA molecular weight on size of microparticles

The results of mean particle size and size distribution of microspheres as measured by laser light diffraction technique (Mastersizer-2000, Malvern, UK) along with other pertinent data, viz., volume-mean diameter, % encapsulation efficiency, and % drug loading of different formulations are summarized in Table II. It is observed that higher molecular weight PLGA gave higher particle size than lower molecular weight PLGA. With increasing PLGA molecular weight, size of the microspheres increased as 25, 21, and 12 μm for 5 wt % DXY-loaded formulations, viz., F1, F3, and F5, whereas for 10 wt % DXY-loaded formulations, viz., F2, F4, and F6, sizes of the microparticles are 19, 16, and 10 μm, respectively. This is due to the fact that PLGA concentration in the internal emulsion phase would influence the size of microspheres formed, which increases with increasing molecular weight of PLGA. The increased hydrodynamic viscosity of dispersed phase (polymer solution) would result in poor dispersion of PLGA solution into aqueous phase against high viscous resistance shear forces that get balanced during the process of emulsification.[25] These effects would result in a coarse emulsion at higher concentration of PLGA due to the formation of bigger size particles during the diffusion of DXY out of the PLGA matrix.

The sizes are also affected by the amount of drug incorporated in PLGA. It is observed that as the DXY-loading increases the particle size decreases. This could be the effect of lower encapsulation efficiencies produced from the formulations containing higher amount of DXY. This type of anomaly is attributed to water-soluble property of DXY and its reduced interaction with the hydrophobic PLGA chains, leading to the more amount of DXY loss in the surrounding medium. It was also noticed that particles prepared by solvent evaporation technique gave somewhat higher size (26.2 μm) for 10 wt % DXY-loaded formulation than the spray-dried technique. SEM photographs of microspheres prepared by spray drying and solvent evaporation techniques are spherical with smooth surfaces as shown in Figure 2, typically microspheres prepared from spray drying technique.

Effect of PLGA molecular weight on encapsulation efficiency

In the present study, we have taken loadings of DXY, i.e., 5 and 10 wt % in PLGA microspheres. The calculated % encapsulation efficiency data, also included in Table II, decrease with increasing drug loading. For microspheres containing different molecular weight PLGA containing 10 wt % DXY (i.e., formulations F2, F4, and F6), encapsulation efficiencies are 67, 51, and 40%, respectively. On the other hand, for formulations containing different molecular weights of PLGA containing 5 wt % DXY (i.e., formulations F1, F3, and F5), the encapsulation efficiencies are 87, 69, and 59%, respectively. It is clear that % encapsulation efficiency of DXY in the PLGA microspheres decreases with increasing amount of DXY; on the other hand, it increases with increasing PLGA molecular weight.

DXY–PLGA interaction using FTIR

FTIR spectral data were used to confirm the chemical interactions of DXY with PLGA in spray-dried microspheres. FTIR spectra of (a) pristine DXY, (b) placebo microspheres, and (c) DXY-loaded microspheres are displayed in Figure 3. The spectrum of placebo PLGA exhibits characteristic absorption bands at 3000 and 2926 cm^{-1} due to —C—H and —CH_2 stretching vibrations, respectively. Additional characteristic absorption bands of PLGA appear at 1752 and 1629 cm^{-1} due to C=O and O—CO stretching vibrations, respectively. The bands appeared at 1456 and 1386 cm^{-1} are due to C—C

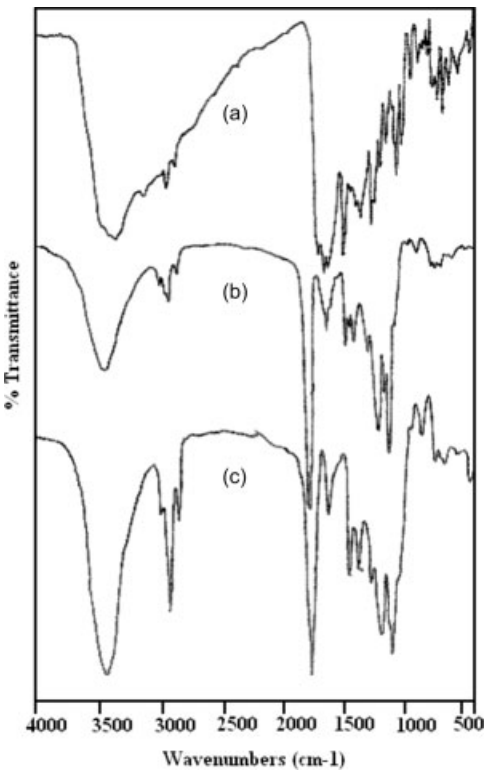

Figure 3 FTIR spectra of (a) placebo microspheres, (b) pristine DXY, and (c) DXY-loaded microspheres.

multiple bond stretching and C—H bending vibrations, respectively. The bands in the region 1092–1184 cm^{-1} are assigned to C—O—C stretching vibrations. Pristine DXY has characteristic bands due to different functional groups, but band appearing at 3358 cm^{-1} is due to O—H/N—H stretching vibrations, while those observed at 2924 and 2854 cm^{-1} are due to C—H stretching vibrations. The bands at 1666 and 1581 cm^{-1} are due to the primary amide (N—H) bending and aromatic N—H bending vibrations, respectively. Carbonyl (C—O) stretching vibrations are seen at 1615 cm^{-1}. On the other hand, bands at 1460 and 1329 cm^{-1} are due to $—CH_2$ bending and C—H bending vibrations, respectively. The bands at 1220 and 1173 cm^{-1} are due to C—N stretching vibrations.

The spectra of DXY-loaded microspheres are not characteristically different from the spectra of placebo PLGA microspheres. When DXY is incorporated into spray-dried microspheres, in addition to the characteristic bands of PLGA, some additional bands have appeared due to the presence of DXY in PLGA matrix. Some bands of DXY are not prominent in the DXY-loaded microspheres due to identical stretching of placebo PLGA microspheres as well as that of DXY-loaded microspheres at the same wave number. The peaks appearing at 2926, 1617, 1577, 1456, and 1273 cm^{-1} for DXY are also appearing in DXY-loaded microspheres, indicating chemical stability of DXY in spray-dried formulations; this further indicates that DXY has not undergone any chemical change during the production of microspheres.

X-ray diffraction

The crystalline nature of pristine DXY and DXY-loaded microspheres have been evaluated by XRD data recorded for pristine DXY, placebo PLGA microspheres, and DXY-loaded microspheres using powder XRD technique. XRD diffractograms of (a) pristine DXY, (b) placebo microspheres, and (c) DXY-loaded microspheres are presented in Figure 4. These studies indicated molecular level dispersion of DXY in spray-dried PLGA microspheres. Notice that DXY has characteristic intense bands observed at 2θ of 10°, 11°, 15°, 20°, 22°, 24°, and 25°, suggesting its crystalline nature; but these peaks have disappeared in DXY-loaded PLGA microspheres. The XRD intensities depend on crystal size, but in this study, for DXY-loaded formulations, characteristic intensities of DXY have overlapped with the noise of the coated PLGA itself, indicating that DXY is dispersed at molecular level in PLGA matrix and hence, no crystals were found in DXY-loaded matrices.

In vitro release

In vitro release profiles of PLGA formulations of different molecular weights containing 5 wt % (curve a) and 10 wt % (curve b) of DXY loadings are dis-

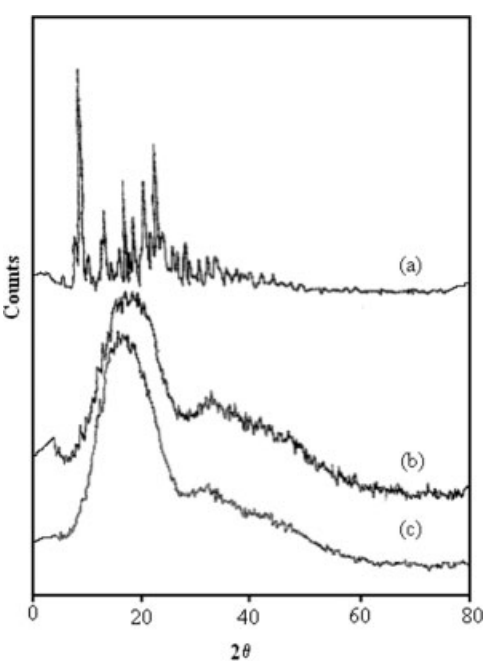

Figure 4 XRD diffractograms of (a) plain DXY, (b) placebo microspheres, and (c) DXY-loaded microspheres.

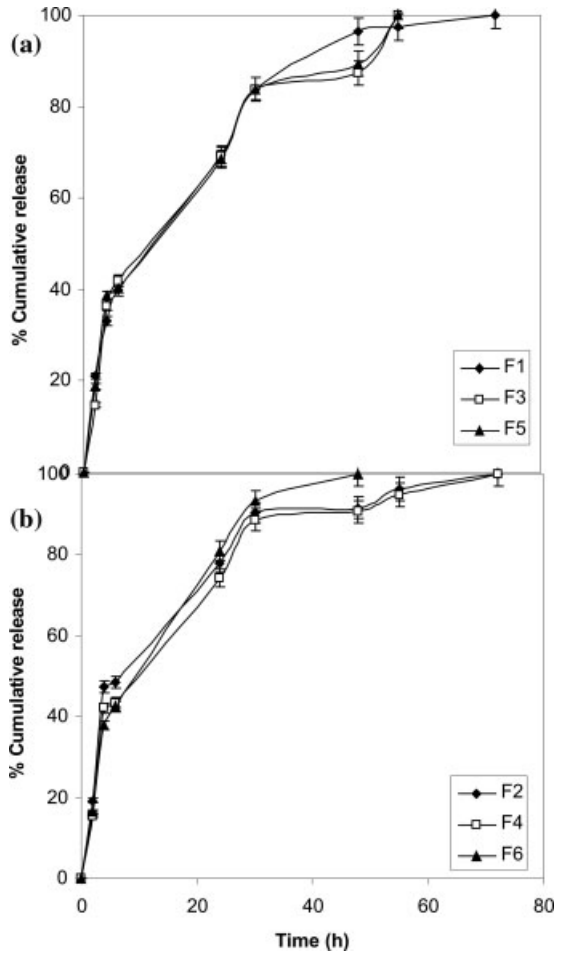

Figure 5 *In vitro* release profiles of formulations with different molecular weight PLGA: (a) with 5% DXY loading and (b) with 10% DXY loading.

played in Figure 5. In general, drug release from biodegradable polymers like PLGA would occur due to the degradation followed by matrix erosion. During the degradation of PLGA microspheres, more acidic groups are generated[26] and hence, the release of DXY depends on the molecular weight of PLGA. In this study, DXY release from PLGA microspheres prepared from spray-dried formulations occurred in 72 h. A comparison of drug release from microspheres with varying molecular weight PLGA was statistically evaluated by ANOVA. The F value was found to be 0.511 (df = 20, P = 0.83), indicating insignificant difference in DXY release characteristics.

Effect of drug loading on release rates for formulations F1, F3, F5 and F2, F4, F6 are compared in Figure 5 for 5 wt % and 10 wt % DXY, respectively. It is observed that the release rates vary depending upon the amount of DXY present in the PLGA matrix, i.e., release is slower for those formulations having lower amount of DXY, while release is higher if higher amount of DXY is present in the microspheres. A comparison of drug release from formulations containing different drug loadings was statistically evaluated by ANOVA. The F value was found to be 0.838 (df = 41, P = 0.670), which indicated no significant difference in drug release rates. However, the initial burst effect remains the same for all formulations, i.e., it occurred within 2 h, suggesting that nearly 15–20% of DXY has released due to hydrophilic nature of DXY as well as the method of preparation of microparticles.

DXY release profile from medium molecular weight PLGA microspheres containing DXY (10 wt %) is compared in Figure 6 for particles prepared by w/o/w and spray drying methods. Microspheres prepared by w/o/w method released 98% of DXY in about 48 h, whereas for spray-dried formulations release was extended to 72 h. For microparticles prepared by w/o/w method, the water-soluble DXY might have migrated to aqueous dissolution medium, thereby sticking to the surface of microparticles,[27,28] which has increased the chance for its initial quick release, indicating that the high solubility of DXY in water would favor its rapid migration to the dissolution medium.

Antimicrobial studies

DXY, a tetracycline-based antibiotic, was primarily used toward prophylaxis and treatment of malaria.[29] However, its potential benefits against standard pathogens have prompted the use for treating various pathologies. Because of its low-dose therapeutic limits, the design of a controlled delivery system has become very essential. Antibacterial efficiency of DXY-loaded PLGA was assessed by determining MIC by standard tube dilution method against four standard pathogenic strains (ATCC) in their mid-log-

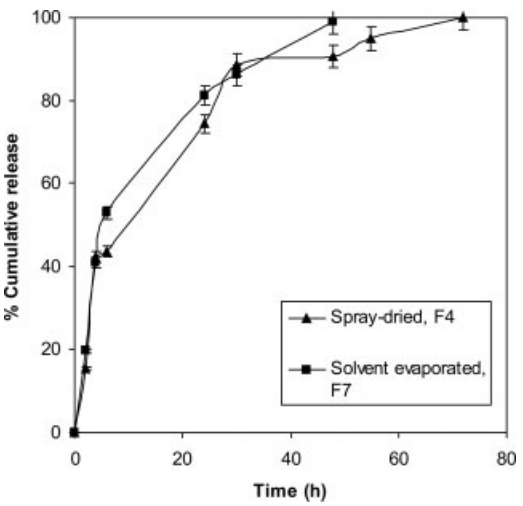

Figure 6 Comparison of *in vitro* release data by spray drying (F4, 1% medium mol wt PLGA, 10% DXY loading) and solvent evaporation (F7, 1% medium mol wt PLGA, 10% DXY loading) methods.

arithmic phase. Observed MICs for *Klebsiella pneumoniae and P. aeruginosa* were 2.5 and 10 μg/mL, respectively. On the other hand, *S. aureus* and *E. coli* exhibited susceptibility at slightly lower concentration levels (1 μg/mL).

In vitro antimicrobial activities of DXY-loaded microspheres, placebo microspheres, and pristine DXY were evaluated in phosphate buffer (pH 7.4) against *S. aureus*, a pathogen found in periodontal pockets of patients with periodontitis. The method used for this study was similar to that used in other dental studies.[30] The results of Table III show that MIC of DXY for *S. aureus* is 1 μg/mL. Our *in vitro* release study (see Table IV) revealed that the concentration of DXY released at each time point throughout the 72-h test period was above 1 μg/mL. In a similar study design,[31] it was shown that the amount of drug released from natamycin chitosan beads over 2 days was above the MIC of 2 μg/mL required for inhibiting the growth of *C. albicans* for periodontal therapy. *In vitro* dissolution samples showed the inhibition of growth of *S. aureas* throughout the test period, confirming the antimicrobial activity of the microspheres. *In vitro* release samples obtained from DXY-free microspheres showed no inhibition of growth of the microorganism.

CONCLUSIONS

This article demonstrates the effective preparation of DXY-loaded PLGA microspheres ranging in size from 10 to 25 μm by spray-dried and solvent evaporation techniques. Particle surfaces as assessed by SEM are smooth with regularly shaped morphologies. FTIR indicated no chemical interactions between DXY and PLGA. XRD suggested molecular level dispersion of DXY in PLGA. Drug release showed the dependence on PLGA molecular weight as well as amount of drug loading and encapsulation efficiency. *In vitro* release data of the spray-dried formulations and of the solvent evaporated formulations indicated quick release of about 40–50% of DXY in about 5–7 h. However, the release of DXY was continued for longer times up to 72 and 48 h, respectively, for both the formulations. Statistical analyses of release data indicated no dependence of DXY release on molecular weight PLGA as well as DXY loading. Antimicrobial studies revealed that the release of DXY over 72 h was above the MIC required for inhibiting the microbial growth, which corroborated well with the *in vitro* results.

TABLE III
Antibacterial activity of Doxycycline (pristine DXY) against *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Klebsiella pneumoniae*

Concentration (μg/mL)	Mean zone diameter (cm)			
	A	B	C	D
75	2.4	1.6	2.8	2.6
50	2.3	1.5	2.7	2.5
25	2.1	1.2	2.5	2.3
10	1.8	1.0	2.0	1.8
7.5	1.7	0	1.8	1.6
5.0	1.6	0	1.5	1.4
2.5	1.4	0	1.4	1.2
1.0	1.2	0	1.3	0

A, *E. coli*; B, *Pseudomonas aeruginosa*; C, *Staphylococcus aureus*; D, *Klebsiella pneumoniae*; 0, no zone of inhibition.

TABLE IV
Antibacterial Activity of *In Vitro* Dissolution Samples against *Staphylococcus aureus*

Time (min)	Mean zone diameter (cm)
0	0
5	1.3
10	1.5
20	2.0
30	2.2
60	2.2
90	2.35
120	2.35
240	2.35
360	2.35
1,440	2.35
1,800	2.48
2,800	2.48

0, no zone of inhibition.

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