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Study on physiochemical structure and *in vitro* release behaviors of doxycycline-loaded PCL microspheres

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ABSTRACT: This study aimed to develop drug delivery system of doxycycline-loaded polycaprolactone (PCL) microspheres. The investigated microsphere formulation can be considered for local application in bone infections and degenerative joint diseases, which generally require long-term treatments via systemic drugs. PCL-14 kDa and 65 kDa were used in microsphere preparation. Before release, the microspheres were characterized by scanning electron microscopy, differential scanning calorimetry, and X-ray photoelectron spectroscopy. The mean particle size of microspheres was in the range of 74–122 μm and their drug loadings ranged between 10 and 30%. *In vitro* release profiles were described using the Higuchi and the Korsmeyer–Peppas equations. Diffusion model was applied to experimental data for estimating diffusion coefficients of microspheres; calculated as between 4.5×10^{-10} and 9.5×10^{-10} cm^2/s. Although long-term release from microspheres of PCL-14 kDa obeyed diffusion model, PCL-65 kDa microspheres showed this tendency only for some period. Modeling studies showed that the drug release mechanism was mainly dependent on loading and molecular weight differences. Release behavior of PCL-65 kDa microspheres, however, might be better represented by derivation of a different equation to model for the total release period. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41768

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INTRODUCTION

Controlled release with polymer-based delivery systems has become one of the most interesting topics in pharmaceutical science and technology since 1980s.[1–3] However, it is difficult to foresee the exact release behavior for these systems as the drug release mechanism is governed by several factors like polymer and drug physiochemical properties. Besides polymer-related main factors like molecular weight (Mw), crystallinity, hydrophilicity, and degradation kinetics, drug/bioactive agent-related factors like solubility, Mw, physical state, and stability also necessitate specific modeling of the release behavior for such systems. In addition to these, the drug/polymer couple will form unique characteristics of the formulation as drug loading efficiency, polymer-drug interactions, size distribution, and porosity that cause additional modifications in the release behavior.[4–6]

Poly-ε-caprolactone (PCL) being a hydrophobic, semicrystalline polymer with low glass transition temperature (T_g) of about −60°C and lower degradation rate than other common polyesters such as poly(lactide-co-glycolide) (PLGA) and polylactic acid (PLA) is one of the most challenging polymers for drug delivery studies.[7,8] High permeability for many drugs and nontoxic degradation products are also the major reasons for using PCL as a long-term drug delivery vehicle. Capronor®, for example, is a delivery device in which PCL is used for the long-term zero-order release of levonorgestrel.[9]

Doxycycline, the most stable group member of tetracyclines, is a wide spectrum antibiotic effective for gram positive and negative bacteria and protozoa.[10] It is active against periodontal pathogens such as *Actinobacillus actinomycetemcomitans* (*A.a.*), *Porphyromonas gingivalis* (*P.g.*), and *Bacteroides frosthytus* (*B.f.*).

In this study, it was aimed to develop a long-term doxycycline delivery system in the form of PCL microspheres and investigate it for physicochemical properties and drug release behavior. For this purpose, we designed and optimized an antibiotic/anticollagenase delivery system that can be applied in treatment of

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Table I. Particle Size Analysis Results of Microspheres

MW of PCL (kDa)	Aqueous phase	MS type	*d*(0.1) (μm)	*d*(0.5) (μm)	*d*(0.9) (μm)	SPAN
14	PVA-4%	Empty	16.652	57.439	143.442	2.207
14	PVA-gelatin[a]	Empty	49.625	84.847	137.943	1.041
14	PVA-4%	Loaded	31.421	74.348	151.228	1.611
14	PVA-gelatin[a]	Loaded	52.214	90.331	149.130	1.073
65	PVA-4%	Empty	64.738	104.474	167.744	0.986
65	PVA-gelatin[a]	Empty	50.853	106.343	224.456	1.632
65	PVA-4%	Loaded	46.528	94.010	369.996	3.441
65	PVA-gelatin[a]	Loaded	41.546	121.918	341.314	2.459

[a] PVA-gelatin (1%, each).

several diseases such as bone and joint inflammations. This model is different from those in literature for modeling long-term (3 months) release behavior of the drug delivery system for a long-term treatment approach applied locally in certain diseases. Here, *in vitro* release outcomes were used to determine the diffusion coefficient of the system with a new approach. Taking into account the long term of release, diffusion coefficients were calculated as the average of release results at all time points for each release study.

EXPERIMENTAL

Materials

Doxycycline, PCL (Mw of 14 and 65 kDa), polyvinylalcohol (PVA) (Mw of 27 kDa), and gelatin (Type A) were obtained from Sigma Chemical Co. (St. Louis, MO). Chloroform, tetrahydrofuran (THF), methanol, acetonitrile, and ethanol (all HPLC grade) were purchased from Merck.

Methods

Preparation of Doxycycline Encapsulated PCL Microspheres. Doxycycline encapsulated microspheres were prepared by single emulsion-solvent evaporation method as described elsewhere.[11] Briefly, doxycycline powder was added into PCL solution (7% in chloroform) at drug : polymer ratio of 1 : 2 (w/w) and mixed homogeneously. This oil phase was then added drop-wise into the aqueous phase; PVA or PVA-gelatin solutions, while stirring with a magnetic stirrer. The organic phase was then evaporated under a hood with continued stirring. After the preparation of microspheres they were separated from the aqueous phase by filtration[12] and then centrifugation. Polymeric microspheres were collected by centrifugation of the aqueous medium at 6000 rpm for 10 min. The microspheres were then washed with distilled water several times for the removal of PVA remaining on their surfaces and centrifuged again.[13] At the end of these procedures, the microspheres were placed in a vacuum oven to dry completely and then stored in a desiccator at 4°C until use.[14]

Different sets of microspheres were prepared using PCL sources with two different average Mws (14 and 65 kDa). To optimize the properties of PCL microspheres, such as surface properties and average size and shape, different concentrations, and formulations of PVA and PVA-gelatin combinations were applied in aqueous phase (Table I).

Determination of Loading and Encapsulation Efficiency. To determine doxycycline loading and encapsulation efficiency of the microspheres, they were dissolved in THF-methanol solution (1/1, v/v). For complete extraction of the drug, the mixture was stirred at 37°C and then centrifuged for 20 min at 6000 rpm. The collected supernatant was analyzed by high performance liquid chromatography (HPLC, Shimadzu Prominence Model, Japan) for determining the doxycycline content of microspheres. The system consisted of a C_{18} column (Inertsil ODS-3, 5 μm, 250 × 4.6 mm; GL Sciences, Japan), UV-Vis absorbance detector (set at 275 nm). The mobile phase consisted of methanol, acetonitrile, and THF (50 : 40 : 10, v/v).

The doxycycline content of the extracted samples was converted to drug amounts using a calibration curve of the free drug. Percentages of drug loading and encapsulation efficiency were then calculated with the following equations:

$$\text{Drug Loading } (\%) = \frac{\text{Weight of Drug in Microspheres}}{\text{Weight of Microspheres}} \times 100 \quad (1)$$

$$\text{Encapsulation Efficiency } (\%) = \frac{\text{Actual Drug Loading } (\%)}{\text{Theoretical Loading } (\%)} \times 100 \quad (2)$$

***In Vitro* Doxycycline Release.** The release profiles of doxycycline from microspheres were determined by incubating the microspheres in dialysis bags (Mw cutoff: 12 kDa) placed in 100 mL of phosphate buffered saline (PBS, 0.01 *M*; pH 7.4).[12,15] The whole setup was kept at 37°C in a shaking water bath throughout the release period. Aliquots taken from the release medium at certain time intervals were used to measure the doxycycline amounts at 275 nm[16] using UV-spectrophotometer (Hitachi, U2800A, Japan). An average of three separate release experiments was used to obtain the release profile of doxycycline.

Microsphere Particle Size and Surface Analysis. Particle size distribution of the microspheres was determined by the wet method; microspheres were dispersed in liquid, and the measurement principle was based on the Mie Theory. Measurements were carried out in a Malvern Mastersizer 2000 in the Central Laboratory (Middle East Technical University, METU, Turkey).

For particle size distribution width, SPAN values were calculated using the following equation:

$$\text{SPAN} = \frac{d[0.9] - d[0.1]}{d[0.5]} \quad (3)$$

where $d[0.9]$, $d[0.1]$, and $d[0.5]$ are the particle diameters determined, respectively, at the 90th, 50th, and 10th percentile of particles.[17]

Surface and morphological properties of microspheres were analyzed by scanning electron microscopy, SEM, after gold sputter coating (Jeol JSM 6400, Tokyo, Japan) in the Department of Metallurgical and Materials Engineering (METU, Turkey).

Differential Scanning Calorimetry Analysis. The thermal characteristics of PCL microspheres were measured using Perkin Elmer Diamond differential scanning calorimetry (DSC) in N_2 atmosphere in the Central Laboratory (METU, Turkey). Two temperature cycles were applied for commercial PCL. The first run from −65 to 100°C (40°C/min) was followed by a cooling step (−10°C/min), allowing to see the crystallization (T_c) and melting (T_m) temperatures. One temperature cycle was applied for microspheres for observing T_m shifts and to calculate crystallinity. The measurements were carried out at a scan rate of 10°C/min between −40 and 100°C. The melting point was determined at the maximum of the melting endotherm. Crystallinity ($\mathcal{X}_C$) was calculated assuming proportionality to the experimental heat of fusion ($\Delta\mathcal{H}_m$)using the reported heat of fusion of 139.5 J/g for the 100% crystalline PCL.[18]

$$\mathcal{X}_c(\%) = \frac{\Delta\mathcal{H}_m}{136.5} \times 100 \quad (4)$$

Degradation Analysis of PCL Microspheres. Microspheres were placed in dialysis bags in 100 mL of phosphate buffered saline (PBS, 0.01 *M*, pH 7.4; 0.02% sodium azide, at pH 7.4). PBS was refreshed at determined time intervals.[19] They were placed in a shaking water bath and kept at 37°C for 1 year.

The surface chemistry properties of PCL microspheres were determined by X-ray photoelectron spectroscopy (XPS), carried out on an ESCA System with Mg/Al dual anode (SPECS, Germany) using an Al Kα (monochromatic) radiation in the Central Laboratory (METU, Turkey). In the literature, degradation of PCL microspheres has also been reported to be analyzed using the XPS method.[20]

THEORY

Empirical and Semi-Empirical Mathematical Models

There are numerous model equations that predict drug release profiles. Takeru Higuchi proposed the "square root of time" equation and several theoretical models for the release of water-soluble and low-soluble drugs incorporated in semisolid and solid matrixes.[21,22] The Higuchi model is defined as:

$$M_t/M_\infty = \mathcal{K}_\mathcal{H} . t^{0.5} \quad (5)$$

Widely known as the power law model, Peppas and coworkers developed a much simpler and more comprehensive semiempirical model to describe drug release from polymeric devices such as microspheres.[23,24] This model is also named as the Korsmeyer–Peppas model and the equation is defined as:

$$M_t/M_\infty = \mathcal{K}_\mathcal{KP} . t^n \quad (6)$$

In eqs. (5) and (6), M_t/M_∞ is the fractional release of the drug over time t, $\mathcal{K}_\mathcal{H}$ and $\mathcal{K}_\mathcal{KP}$ are the Higuchi and the Korsmeyer–Peppas constants characterizing the drug-polymer system, respectively, and n is the diffusion exponent characterizing the release mechanism. The geometric shape of the system influences the diffusion exponent (n). In Fickian diffusion, n is 0.50 for slab, 0.45 for cylinder, and 0.43 for sphere. In non-Fickian (anomalous) diffusion, n is between the Fickian diffusion value and zero-order value ($n = 1$) for nonswellable systems. For swellable systems, n is between the above and case-II transport ($n = 1$ for slab, 0.89 for cylinder and 0.85 for sphere).[24]

Some of the other most relevant and more commonly used empirical and semi-empirical mathematical models describing the release kinetics are the zero order, the first order, and the second order, the Weibull model, etc.[25]

Mechanistic Realistic Theories

Usually, the drug release mechanisms from micro/nanoparticles might be assumed to regard the following factors; surface desorption, diffusion through pores, diffusion through intact polymers, diffusion through water swollen polymers, and surface or bulk erosion of the polymeric matrix.[26]

A mechanistic realistic mathematical model is based on equations that describe real phenomena, for example, mass transport by diffusion, dissolution of drug and/or excipient particles, and/or the transition of a polymer from the glassy to the rubbery state.[27]

The main mechanism determining drug release profile from a polymer matrix is diffusion. Drug dissolution or dispersion is the other factor that affects release profiles. Each of these represents the release of a drug by a single mechanism, that is, either by dissolution or dispersion. In various cases, drug diffusion is the predominant step, in others it "only" plays a major role, for example, in combination with polymer swelling or polymer degradation/matrix erosion.[28,29]

According to the "diffusion model," the release of the drug from the polymeric matrix follows Fick's second law of diffusion, which requires the concentration gradient of the spherical particles obey

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r}\frac{\partial c}{\partial r}\right) \quad (7)$$

where $c(r, t)$ is the local drug concentration at time t and at the distance r, from the center of the particle and $\mathcal{D}$ is the diffusion coefficient of the drug in the polymer matrix.

The initial condition is

$$c(r, 0) = c_0 \quad (8)$$

and the boundary conditions are

$$\mathcal{V}\frac{\partial c_1}{\partial t} = \frac{3\mathcal{V}_s}{\mathcal{R}} D\left(\frac{\partial c}{\partial r}\right)_{r=R} \quad (9)$$

$$c(r, t)_{r=0} = \text{finite} \quad (10)$$

c_0 is the initial concentration of drug in microspheres, $\mathcal{V}$ is the bulk liquid volume of the surrounding medium, $\mathcal{V}_s$ is the total

volume of the particles, and $\mathcal{R}$ is the diameter of the microspheres.

The solution of the initial-boundary value problem given in eqs. (7–10) can be expressed in concentration ratio as[26,30,31]:

$$\frac{c_1}{c_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{n^2\pi^2}{R^2}D\,t\right) \quad (11)$$

where c_1 is the time-dependent concentration of the drug in the medium and c_∞ is the equilibrium concentration of drug in medium. Equation (11) is used to quantify drug release from nondegradable controlled release microparticles.[32]

To avoid infinite series of exponential functions, short time, and late time approximations are used in calculations.[33] Equation (12) given below represents the short time approximation and it is used to calculate the diffusion coefficient from experimental results;

$$c_1/c_\infty = 6.\left(D.t/\pi.\mathcal{R}^2\right)^{1/2} - \left(3.D.t/\mathcal{R}^2\right) \quad (12)$$

This equation is inverted for the diffusion coefficient ($\mathcal{D}$) and the optimum value of $\mathcal{D}$ is found by averaging the diffusion coefficients calculated at each time step. This is novel for diffusion coefficient measurements and not be found in the literature. However, in another study, eq. (12) was further simplified and obtained an alternate equation to calculate the diffusion coefficient for microspheres:[34]

$$c_1/c_\infty = 6.\sqrt{\left(\mathcal{D}.t/\pi.\mathcal{R}^2\right)} \quad (13)$$

RESULTS AND DISCUSSION

Optimization Studies of PCL Microspheres

Here, it was aimed to prepare doxycycline-loaded microspheres with a smooth surface, having homogenous size distribution and spherical morphology in order to minimize variations in release behavior of the samples. Hence, to optimize these properties of microspheres, different preparation conditions were studied. Spherical forms could be obtained with 1, 2, 4, and 6% PVA, but not with 0.5%. The particle size homogeneity and smoothness were improved and mean particle diameters decreased by increasing the surfactant (PVA) concentration as also observed by other researchers.[14,35,36] Smaller microspheres have been previously reported with the use of surfactant during the preparation of microspheres due to the amphiphilic behavior of the surfactant (PVA).[37–40] Further optimization involved use of gelatin in the preparation of microspheres, from 1 to 3% (w/v) and at different temperatures (37 and 45°C) for preventing gellation. SEM images of PCL microspheres at different evaporation temperatures (data not shown) showed that evaporation at 37°C resulted in a more homogeneous shape and size distribution than at 45°C. Uniform and spherical microspheres were obtained with 1% gelatin concentration but increasing the concentration resulted in more roughness and caused the formation of aggregates. SEM images of empty and doxycycline-loaded PCL (14 and 65 kDa) microspheres are presented in Figures 1 and 2. The surfaces of empty microspheres were observed as rough and irregular in Figures 1(A,B) and 2(A,B). Doxycycline-loaded PCL (14 kDa) microspheres prepared with PVA-gelatin (1%, each) were more spherical, less porous, and had smoother surface than microspheres prepared with PVA (4%) surfactant [Figure 1(C–F)]. However, size distribution of latter was more homogeneous. Drug-loaded microspheres prepared using two optimum conditions according to above outcomes (PVA-4% and PVA-gelatin-1%, each) had larger size than the empty ones. In addition, mean particle size of microspheres of PCL (14 kDa) was smaller than those of PCL (65 kDa). In Table I, the particle size results of empty and loaded microspheres have been listed with SPAN values to compare the distribution width between microspheres. When high Mw PCL (65 kDa) was used, smaller microspheres were obtained with PVA (4%) than with PVA-gelatin (1%, each) (Table I). Hnaien *et al.* reported that high viscosity of the organic phase resulted in an increase in the particle size.[41] It was indicated that the viscosity of the organic phase of high Mw PCL was higher than that of low Mw PCL.[37]

Microspheres prepared with PVA-gelatin were smoother and more homogeneous than those prepared with PVA (4%). Similar positive effects of gelatin use have been reported in other studies.[42] It is reported that PVA produced the smallest microspheres whereas gelatin resulted in microspheres with smooth surfaces.[43]

Loading and Encapsulation Efficiency

As the Mw of polymer was increased, both encapsulation efficiency and loading values were increased (Table II). This was mainly due to the higher viscosity of the PCL (65 kDa) solution than that of the PCL (14 kDa). In another study describing felodipine-loaded PCL microspheres, PCL (80 kDa) had the most viscous organic phase, so the encapsulation efficiency was the highest.[37] In our study, this trend was more pronounced for high Mw PCL microspheres in which gelatine was also used.

Highest loading and encapsulation efficiency were obtained with PCL (65 kDa) microspheres prepared in PVA-gelatin (1%, each) aqueous phase (Table II). In another study, etoposide was entrapped in different polymers such as PLGA (50 : 50), PLGA (75 : 25), and PCL. Among them, PCL (the most hydrophobic polymer) had the highest entrapment efficiency.[40] In a recent study, two model drugs *p*-Nitroaniline and rhodamine B with water solubilities of app. 1 and 10 mg/mL, respectively, were encapsulated in PCL (10 kDa). Drug loading of *p*-Nitroaniline was 47.14%, whereas that of rhodamine B was 7.84%[6] thus, indicating the importance of drug hydrophobicity on the loading parameter.

In Vitro Doxycycline Release

Drug encapsulated in the polymer is released from the system depending on many parameters of the system. Hydrophilic drugs localize near the surface when used with hydrophobic polymers.[43] Doxycycline is a water-soluble drug with enhanced dissolution and diffusion in aqueous environment. However, PCL is a hydrophobic polymer which potentially retains its content for extended time periods. Besides this, it was indicated that a highly porous matrix releases drug at a higher rate than a less porous one.[5] In SEM results, the PCL (14 kDa) microspheres were observed to have less-porous structure. Yet, for both Mw microspheres, the surface structure after the release

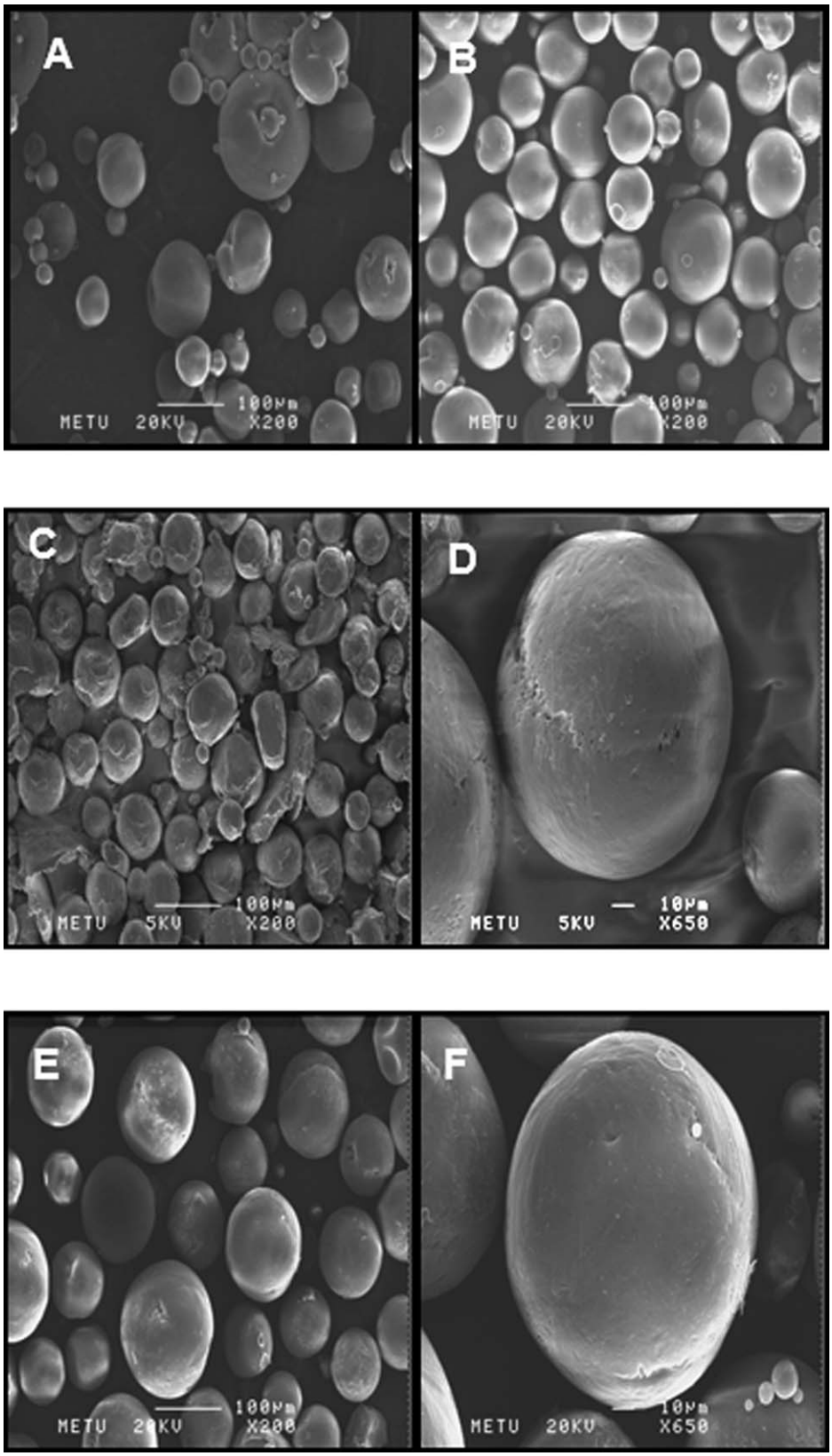

Figure 1. SEM images of empty PCL (14 kDa) microspheres prepared by aqueous phase of (A) 4% PVA, (B) PVA-gelatin (1%, each) and doxycycline loaded PCL (14 kDa) microspheres (shown at two different magnifications), (C) 4% PVA (200×), (D) 4% PVA (650×), (E) PVA-gelatin (1%, each) (200×), and (F) PVA-gelatin (1%, each) (650×) solutions.

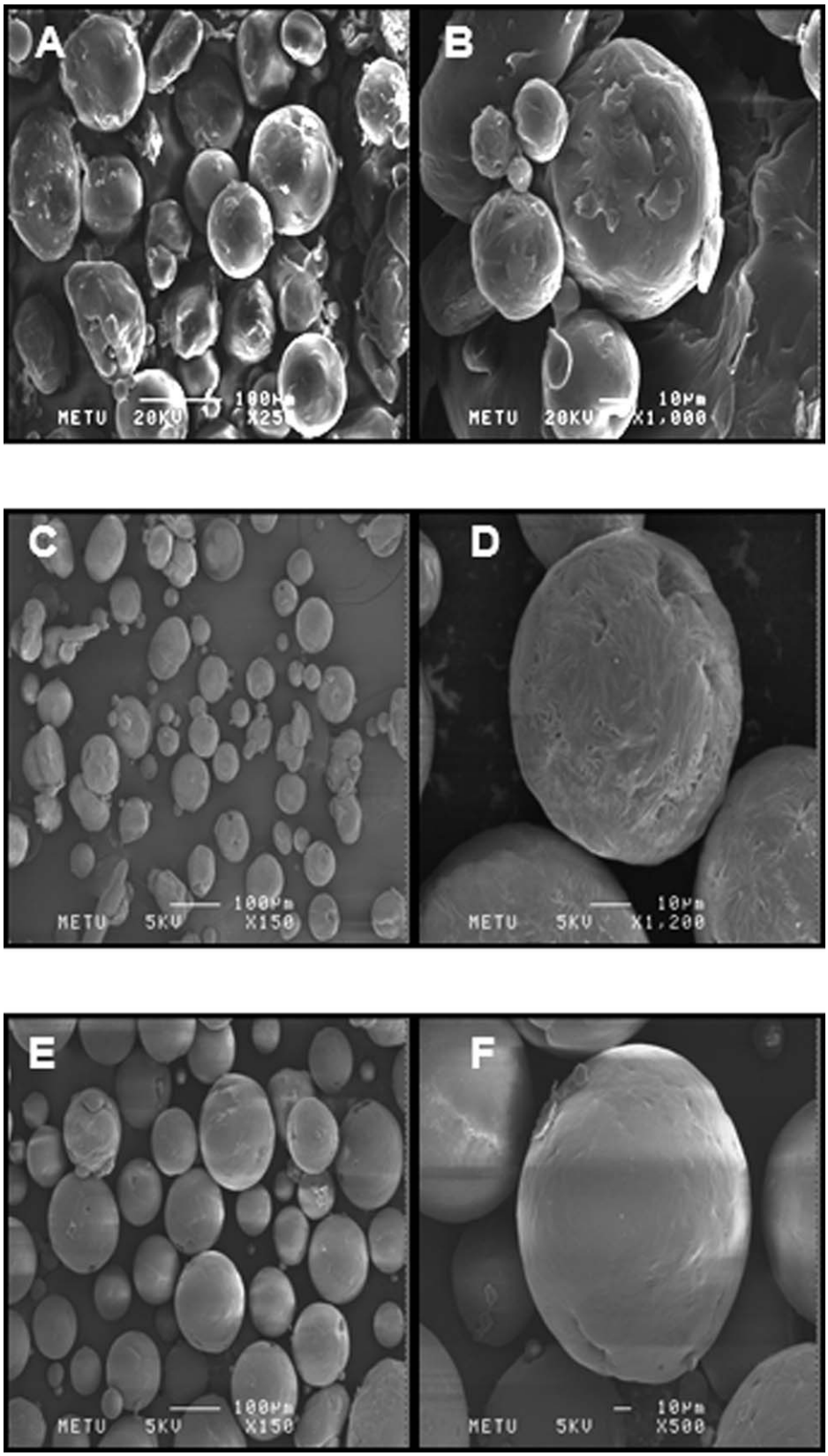

Figure 2. SEM images of empty PCL (65 kDa) microspheres prepared by aqueous phase of (A) 4% PVA, (B) PVA-gelatin (1%, each) and doxycycline loaded PCL (65 kDa) microspheres (shown at two different magnifications), (C) 4% PVA (150×), (D) 4% PVA (1200×), (E) PVA-gelatin (1%, each) (150×), and (F) PVA-gelatin (1%, each) (500×) solutions.

Table II. Loading and Encapsulation Efficiency Percentages of Doxycycline Loaded PCL Microspheres

MS	Aqueous phase	MW of PCL (kDa)	Drug loading (%)	Encapsulation eff. (%)
1	PVA (4%)	14	17.58 ± 0.33	52.79 ± 0.99
2	PVA-gelatin (1%)	14	10.80 ± 0.46	32.43 ± 1.38
3	PVA (4%)	65	24.50 ± 1.30	73.57 ± 3.89
4	PVA-gelatin (1%)	65	29.42 ± 2.12	88.35 ± 6.36

experiments was observed to be similar to the surface structure before the release (Figure 3 vs. Figures 1 and 2).

The cumulative release of doxycycline from the two PCL microsphere preparations had similar curves for each Mw group (Figure 4). During the first few days, a small burst effect was seen which then slowed down at a constant rate. The initial rapid release phase was thought to be caused by the high water solubility of doxycycline molecules at the superficial regions of the microspheres. PCL (14 kDa) microspheres prepared using PVA-gelatin solution encapsulated less doxycycline (10.80 ± 0.46%) than microspheres prepared with only PVA (17.58 ± 0.33%). Therefore, the slight difference in the amount of doxycycline release curves was thought to be related to either the amount of drug loaded or with differences in particle sizes of the microspheres. It is known that a decrease in the total surface area directly reduces the total amount of contact with water.[4] Moreover, an increase in area/volume ratio increases drug release by enhancing the diffusion of the drug molecules. In 1 month

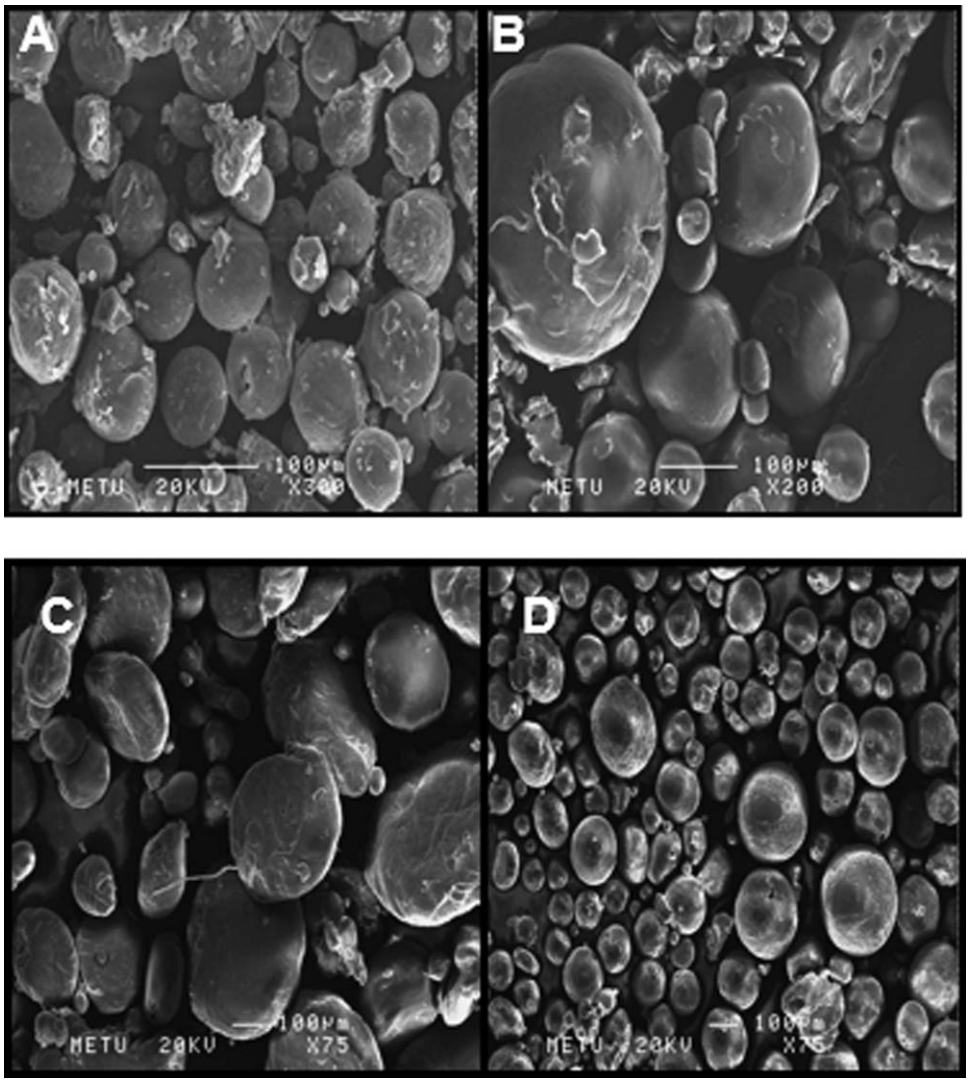

Figure 3. SEM images of microspheres after release prepared by (A) PCL (14 kDa) with 4% PVA (B) PCL (14 kDa) with PVA-gelatin (1%, each) (C) PCL (65 kDa) with 4% PVA (D) PCL (65 kDa) with PVA-gelatin (1%, each) solutions.

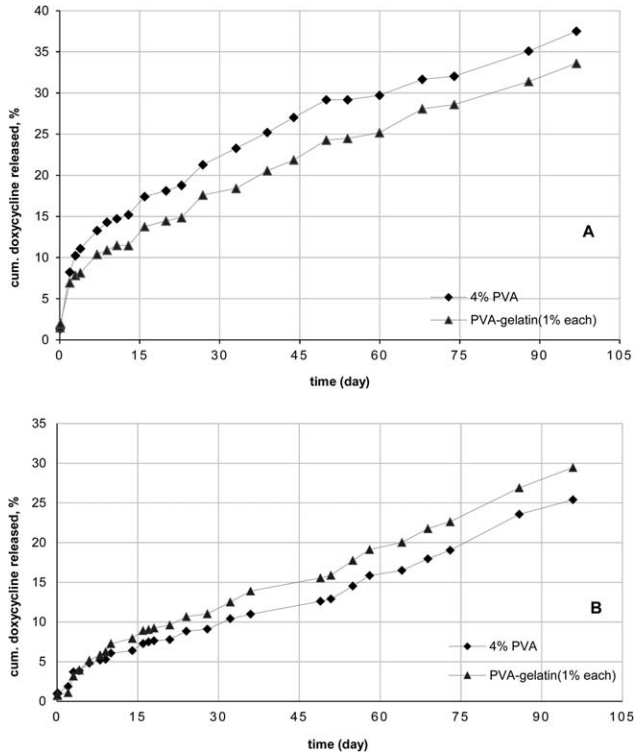

Figure 4. *In vitro* release profiles of doxycycline-loaded (A) PCL (14 kDa) and (B) PCL (65 kDa) microspheres prepared with two aqueous environment ($n = 3$).

period, microspheres prepared with PVA and PVA-gelatin could release about 20% of the drug and in 3 months about one-third of their contents (33 and 37%, respectively). The water solubility of doxycycline (50 mg/mL) is high, and this might be the reason for its faster release profile compared to other PCL microsphere systems. Researchers also observed a relatively high burst release of cytochrome c from microsphere preparations with the more hydrophilic polymer.[44] Hydrophobic polymers have low amounts of surface adsorbed drugs compared to hydrophilic polymers. In this study, despite the solubility of doxycycline in water, hydrophobic characteristics of PCL decreased the burst effect compared to those observed with more hydrophilic polymers.

The release profiles of PCL (65 kDa) microspheres of two different preparation conditions are compared in Figure 4(B). Drug release was sustained throughout the release period for both sets (PVA and PVA-gelatin). Unlike low Mw polymer microspheres, they had a constant release rate with similar amounts of drug being released in each time interval starting from an earlier time point (app. first day). So, for PCL (65 kDa) microspheres, a burst release was not as apparent as for PCL (14 kDa) microspheres, which might be another indication of the increased hydrophobic properties. On the third day of the experiment, the released doxycycline amount was only 3% of the total amount. This was probably due to the lower water permeability for high Mw PCL. Previous reports with similar Mw PCL (50 kDa) also observed a limited burst release.[45] Overall, the percentage of the drug released from the microspheres prepared with PCL (65 kDa) was lower than PCL (14 kDa). After the release studies, PCL (65 kDa) microspheres prepared with only PVA surfactant showed increased surface roughness, and they appeared more porous. Surfaces of PVA-gelatin group, however, were almost the same as before release [Figure 3(C,D)]. The overall release results indicated that large modifications of release behavior and rates were governed by polymer properties; smaller effects were dependent on the preparation condition.

Modeling of Doxycycline Release from Microspheres

The release behaviors and related constants of doxycycline-loaded PCL microspheres were calculated for the Higuchi and Korsmeyer–Peppas model equations. The model parameters are tabulated in Tables III and IV, respectively. The results indicated that the kinetics of doxycycline release from PCL (14 kDa) microspheres can be described effectively by the Higuchi Model (linear relationship of release amount with the square root of time), that is, the release was governed by Fickian diffusion. Thus, a decrease in doxycycline release over time is expected as the drug is depleted in the matrix and diffusion path length is increased. The release rate was boosted by raising the drug loading in the matrix, correlating with the response of diffusing species to a higher concentration gradient. The value of the release exponent (n) in the Korsmeyer–Peppas model was calculated as 0.37 and 0.43 for PCL (14 kDa) microspheres for two different preparation conditions (Table IV), providing further support that the release mechanism was governed by Fickian diffusion.

Determination of an exact diffusion coefficient was quite difficult for the total duration of the experiment due to several factors such as variations in water conduction into the polymer matrix or changes in the microenvironment with the release of drug molecules. Therefore, diffusion coefficients of the microspheres were calculated at each time step of the measurements using eq. (12) (Figure 4) and averaged in order to find the optimum diffusion coefficient for each microsphere group. These average values, tabulated in Table V, were very close to each other and ranged between 8.1×10^{-10} and 9.2×10^{-10} cm^2/s except for the PCL (65 kDa) microspheres prepared from PVA (4%). The number of experimental studies available in the literature on the diffusion coefficients of doxycycline through PCL is limited. Here, we suggest a new method for the calculation of diffusion coefficient obtained from the release data. The diffusion coefficient of doxycycline in water through PVDF membrane was reported as 6.59×10^{-6} cm^2/s with Franz diffusion cell.[46] Conversely, the diffusivity of another antibiotic, gentamicin sulfate, in the porous PCL matrix (with 8.3% loading) was calculated as 1.5×10^{-9} cm^2/s.[47] In another study, the diffusion coefficient of lidocaine in PLA nanoparticles was very low, of

Table III. Higuchi Model of Doxycycline Release from PCL Microspheres

Aqueous phase	MW of PCL (kDa)	K_H	R^2
PVA (4%)	14	3.968	0.970
PVA-gelatin (1%)	14	3.360	0.988
PVA (4%)	65	2.081	0.936
PVA-gelatin (1%)	65	2.484	0.951

Table IV. Korsmeyer–Peppas Model of Doxycycline Release from PCL Microspheres

Aqueous phase	MW of PCL (kDa)	K_{KP}	n	R^2
PVA (4%)	14	7.508	0.374	0.986
PVA-gelatin (1%)	14	5.003	0.432	0.989
PVA (4%)	65	1.652	0.550	0.965
PVA-gelatin (1%)	65	1.652	0.600	0.988

the order of 10^{-20} m^2/s.[31] Small diffusion coefficients were mainly due to dense polymer matrices.[34] Indeed, PCL (14 kDa) microspheres had lower drug loadings than PCL (65 kDa) microspheres (Table II), and hence, it is seen that the diffusion model correlated better with the experimental data for the particles with lower loadings. It has been indicated that drug release is diffusion controlled at low loadings (at least 10%) in PLA nanospheres.[31]

The experimental results were then compared with the diffusion model, based on Fick's second law. A diffusion model is dependent on mass transfer of the substance caused by random molecular motion and associated with a concentration gradient.[26] The diffusion model was applied for description of the experimental release data and for identification of the release mechanisms. In Figures 5 and 6, the experimental release profiles were compared with the diffusion model equation, eq. (11), in which the calculated diffusion coefficients were used. The experiments were carried out for a period of more than 3 months, at the end of which complete release of the loaded drug was still not achieved. As a result, it was assumed in the modeling studies that all loaded doxycycline was released into the media.

Although the release of doxycycline from microspheres prepared with PCL (14 kDa) could be explained by the diffusion model as shown in Figure 5, release from microspheres prepared with PCL (65 kDa) could not be completely explained by the same model. This is because the release profiles of the PCL (65 kDa) groups showed a different behavior (Figure 6). It was observed that approximately the first half of the release fitted the diffusion equation, while the second half seemed to fit the zero-order release. Therefore, it can arguably be claimed that the release profile of doxycycline-loaded PCL (65 kDa) microspheres fits non-Fickian diffusion (see Table IV : $n = 0.55$ for PVA and $n = 0.60$ for PVA-gelatin).[24] Consequently, the drug

Table V. Diffusion Coefficients Calculated Using Experimental Data Presented in Figure 4

Aqueous phase	MW of PCL (kDa)	D (cm^2/s)
PVA (4%)	14	8.0989×10^{-10}
PVA-gelatin (1%)	14	9.0073×10^{-10}
PVA (4%)	65	4.5700×10^{-10}
PVA-gelatin (1%)	65	9.1900×10^{-10}

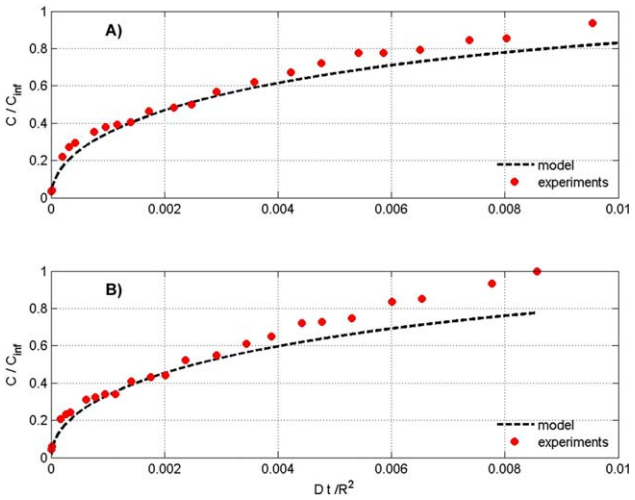

Figure 5. Mathematical modeling of *in vitro* release of doxycycline-loaded PCL (14 kDa) microspheres prepared by (A) PVA (4%) and (B) PVA-gelatin(1%, each) (dashed line: model; points: experimental results). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

release mechanism of PCL can be considered to be Fickian for PCL (14 kDa) microspheres and non-Fickian for PCL (65 kDa) microspheres. To the best of our knowledge, there is no study in the literature that uses diffusion models for analysis of drug release for such long periods (3 months) as in our research. The shift from the model in case of PCL (65 kDa) is mainly due to the characteristic property of the polymer. The release rate increases after the time of the shift. Researchers have studied the correlation between release behaviors of different microspheres and reported that surface porosity affects the release behavior.[48] Although it is clear from the SEM figures that the PCL microspheres did not have a porous structure, water penetration during release may help to increase the rate of released

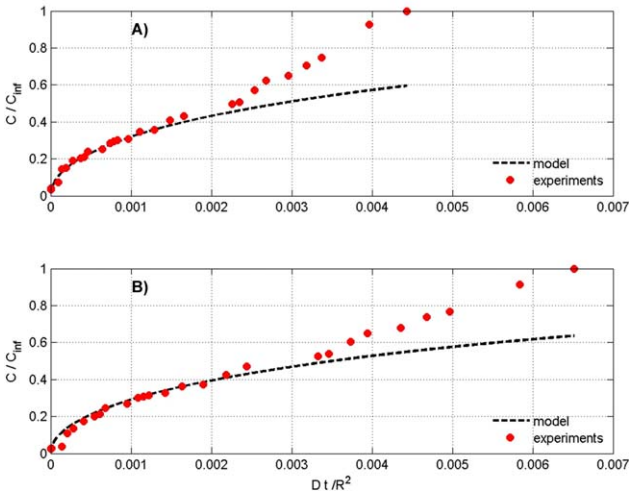

Figure 6. Mathematical modeling of *in vitro* release of doxycycline-loaded PCL (65 kDa) microspheres prepared by (A) PVA (4%) and (B) PVA-gelatin (1%, each) (dashed line: model; points: experimental results). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

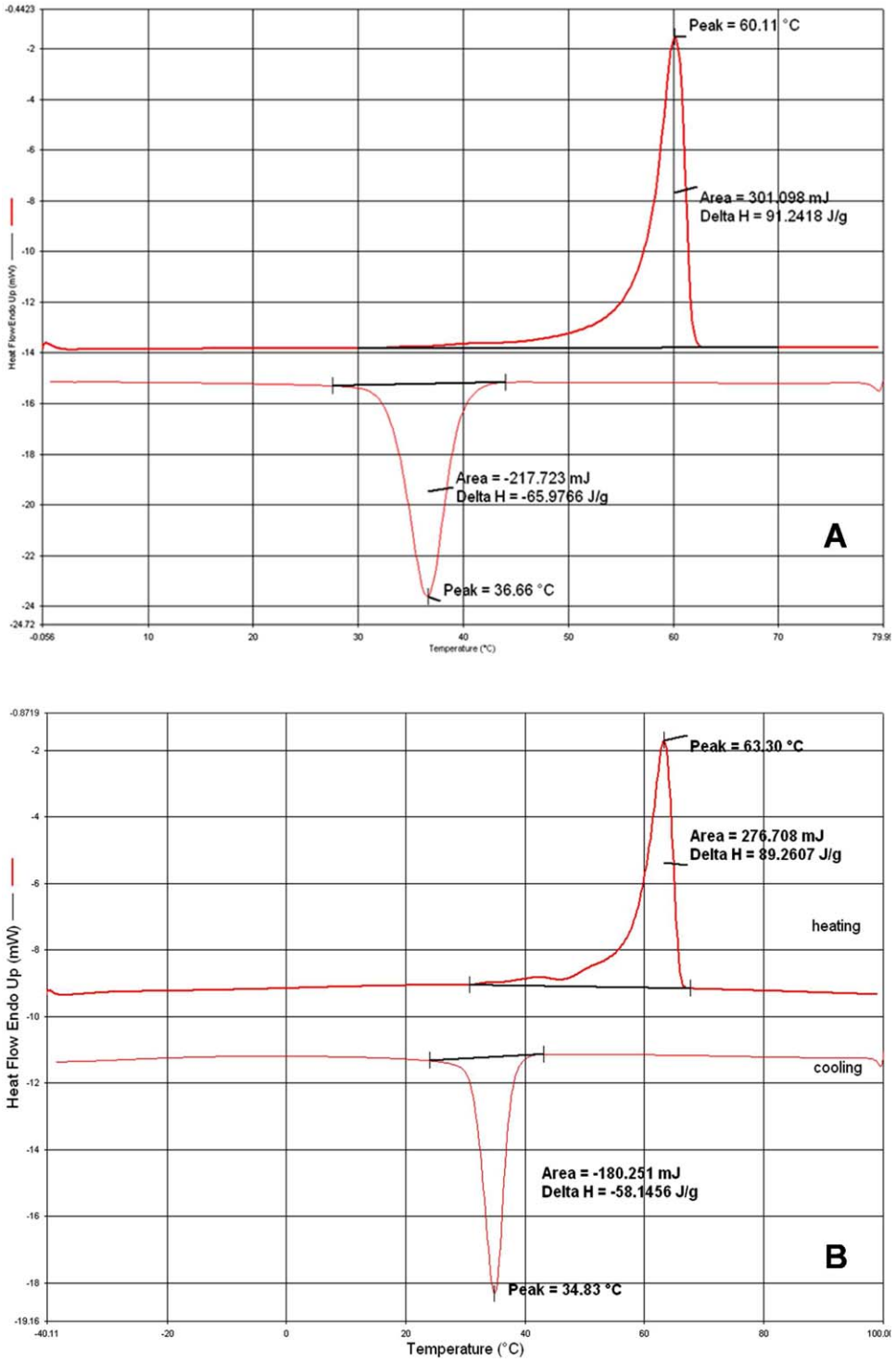

Figure 7. DSC figures of doxycycline-loaded microspheres prepared by (A) PCL (14 kDa) and (B) PCL (65 kDa) with 4% PVA (before release). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

drug. As a result, it is likely that the structure of the carrier did not have similar barrier properties shortly after the start of the release. The particle size, area/volume ratio, encapsulation efficiency, and Mw of the PCL are the other important factors affecting the release rate of the drug. The shift of the model might have arisen from these factors.[26] It has been claimed that differences in release behavior of drugs could be due to water penetration, degradation of the polymer, and drug solubility.[49] Conversely, the degradation of PCL is slow compared to PLGA and other polymers.[39] This makes PCL suitable for long-term delivery. Also, it has been argued that water penetration and degradation were low in PCL, and drug release was only based on diffusion from amorphous regions of the polymer matrix.[4] Drug removal to the surrounding medium is usually governed

Table VI. DSC Analysis Results of PCL Polymer and PCL Microspheres

Sample	T_m (°C)	ΔH_m (J/g)	X_c (%)	T_c (°C)
PCL = 14 kDa	67.61	82.74	59.32	22.19
PCL = 65 kDa	65.16	68.16	48.86	9.34
P MS PCL = 65 kDa before R	63.30	89.26	63.99	34.83
P MS PCL = 65 kDa after R	66.31	92.45	66.27	35.84
P MS PCL = 14 kDa before R	60.11	91.24	65.41	36.66
P MS PCL = 14 kDa after R	62.96	91.60	65.66	38.49
PG MS PCL = 14 kDa before R	60.12	83.65	59.96	36.20
PG MS PCL = 14 kDa after R	64.98	97.81	70.12	38.38
PG MS PCL = 65 kDa before R	63.64	81.96	58.75	31.85
PG MS PCL = 65 kDa after R	66.00	87.00	62.37	36.52
P PCL = 14 kDa empty MS	61.31	97.20	69.67	37.71
P PCL = 65 kDa empty MS	62.78	81.28	58.27	33.34
PG PCL=14 kDa empty MS	61.75	86.14	61.75	36.02
PG PCL = 65 kDa empty MS	63.96	83.03	59.52	33.00

T_m, melting temperature; ΔH_m (J/g), Heat of Fusion; X_c, crystallization degree; T_c, crystallization temperature; P, PVA 4%; PG, PVA-gelatin (1%, each); R, release; MS, microsphere.

by the diffusion process. So, it can be concluded that the rate limiting property of the microspheres regulated the release profile.

DSC Analysis

In polymer drug delivery systems, crystallinity strongly affects both the drug release kinetics and the degradation. Crystallinity hinders diffusion and drug release.[50] From the thermograms, it was observed that PCL (14 kDa) had higher crystallinity value than PCL (65 kDa). The DSC results have been compiled in Table VI (Supporting Information Figures S1–S7).The release profile (Figure 4) and crystallinity (%) of the microspheres analyzed in this study was almost identical for PCLs of the same the Mw. Figure 7 showed DSC images of doxycycline-loaded PCL (14 and 65 kDa) microspheres (before release) prepared by aqueous phase of 4% PVA.

When the Mw of PCL is increased, the crystallinity decreased.[4] Papaverine- and felodipine-loaded microspheres were prepared with different Mws of PCL and DSC results showed that encapsulation did not change the melting and crystallization temperatures of these microspheres.[4,37] Crystallization temperature of PCL (65 kDa) of our samples was also lower than that of PCL (14 kDa) (Table VI). However, DSC results of microspheres did not show much difference in crystallinity degrees between microspheres made with polymers of different Mw when they were prepared in the same aqueous environment. The degree of polymer crystallinity was found to be higher in the microspheres than the original polymers, indicating that polymer chains got more ordered while being transformed into the microsphere form. Also, after the release experiment the microspheres had higher degree of crystallinity, which is a common observation in similar drug delivery studies. This can be explained by the loss of small amorphous polymer chains especially on the surface of the microspheres during release experiments.

Crystallinity of the polymer is also very important in the encapsulation of drugs. The crystalline phase of the polymer is known to be impermeable to water and drug is encapsulated in the amorphous phase of the polymer. In this study, doxycycline-loaded PCL (65 kDa) microspheres were more amorphous than PCL (14 kDa) microspheres. Hence, PCL (65 kDa) microspheres showed higher drug encapsulation than the PCL (14 kDa) microspheres. It has been indicated that low Mw PCL (10 kDa) microspheres with the lowest entrapment efficiency exhibited higher crystallinity than the higher Mw ones (65 and 80 kDa).[37]

Degradation Analysis of PCL Microspheres

PCL is known to degrade very slowly because of its hydrophobic structure which does not allow fast water penetration.[19] Crystallinity is known to play an important role in determining both biodegradability and permeability because of the widely accepted fact that the crystalline phase is inaccessible to water and other permeates. PCL is defined as a semicrystalline hydrophobic polymer, having long *in vivo* degradation times.[19]

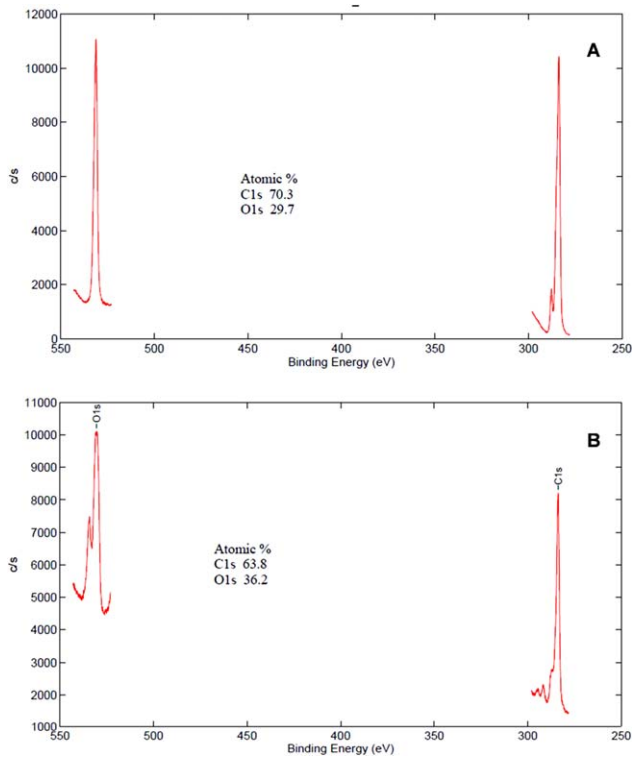

Figure 8. XPS analysis of PCL (65 kDa) microspheres prepared by PVA (4%) (A) before and (B) after 1 year incubation in PBS at 37°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table VII. Quantitative Chemical Composition of Microspheres Before and After 1 year PBS Incubations at 37°C

Element at %	PCL (14 kDa) before release	PCL (14 kDa) after release	PCL (65 kDa) before release	PCL (65 kDa) after release
C	70.7	63.2	70.3	63.8
O	29.3	36.8	29.7	36.2

The change in structure was determined by the composition of microspheres after the release studies. The binding energies for C1s and O1s were obtained at Al monochromatic anode (58.70 eV). Representative XPS survey spectra of PCL microspheres before and after 1 year of incubation in PBS are given in Figure 8. The relative compositions of all the microsphere surfaces calculated from the XPS spectra are given in Table VII. Considering the time period of the degradation study (1 year), no significant decrease in C1s peaks and its atomic percentage was observed while no significant increase in O1s peaks and their percentages were seen for both PCL-14 kDa and 65 kDa microspheres. Its slow degradation property allows PCL to be used in long-term delivery devices, especially for applications lasting more than 1 year.[7] These facts support the SEM micrographs observed after the release studies [Figure 3(A,C)] and release results (Figure 4).

CONCLUSIONS

Polymeric controlled drug delivery systems have many advantages over conventional drug therapies including reduced side effects, possibility of local or targeted application of the treatment and enhanced treatment potency. In this study, doxycycline encapsulated PCL microspheres were prepared and studied for their potency as a new treatment approaches.

The particle size of the microspheres can be controlled by manipulating the aqueous phase and the Mw of the polymer. Two different Mws of PCL (14 and 65 kDa) were used to obtain the desired properties in the developed systems. Modifications of the conditions for the preparation of microspheres were also carried out for this purpose: PVA (4%) and PVA-gelatin (1%, each) were used. Such modifications (e.g., addition of gelatin and temperature optimization) showed improvement in microsphere surface and morphological structures as well as homogeneity in size.

The estimated diffusion coefficients of doxycycline were calculated from the experimental results with values varying in range of 4.5×10^{-10}–9.5×10^{-10} cm^2/s. PCL (14 kDa) microspheres showed Fickian diffusion and its release results were in good agreement with the diffusion model for the entire course of the experiments. However, the release profiles of PCL (65 kDa) microspheres agreed with the diffusion model only for the first 50 days, while the rest of the release duration did not, suggesting non-Fickian diffusion. This can be justified by previously calculated *n* values of PCL (65 kDa) microspheres, which indicated that the release kinetics of the PCL (65 kDa) microspheres could be explained by non-Fickian diffusion. It can be concluded that these PCL microspheres can be proposed as suitable long-term doxycycline delivery systems. Yet, these outcomes should be investigated under *in vivo* conditions.

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REFERENCES

1. Burger, J. J; Tomlinson, E.; Mulder, E. M. A.; McVie, J. G. *Int. J. Pharm.* **1985**, *23*, 333.
2. Dhanaraju, M. D; Gopinath, D.; Ahmed, M. R; Jayakumar, R.; Vamsadhara, C. *J. Biomed. Mater. Res. A* **2006**, *76*, 63.
3. Jiang, T.; Petersen, R. R; Call, G.; Ofek, G.; Gao, J.; Yao, J. Q. *J. Biomed. Mater. Res. B Appl. Biomater.* **2011**, *97*, 355.
4. Jeong, J. C; Jaeyoung, L.; Cho, K. *J. Control. Release* **2003**, *92*, 249.
5. Freiberg, S.; Zhu, X. X. *Int. J. Pharm.* **2004**, *282*, 1.
6. Wang, X.; Wang, Y.; Wei, K.; Zhao, N.; Zhang, A.; Chen, J. *J. Mater. Process. Technol.* **2009**, *209*, 348.
7. Woodruff, M. A; Hutmacher, D. W. *Prog. Polym. Sci.* **2010**, *35*, 1217.
8. Sackett, C. K; Narasimhan, B. *Int. J. Pharm.* **2011**, *418*, 104.
9. Nair, L. S; Laurencin, C. T. *Prog. Polym. Sci.* **2007**, *32*, 762.
10. Bokor-Bratic, M.; Brkanic, T. *Med. Pregl.* **2000**, *53*, 266.
11. Aydin, O. In Situ, In Vitro and In Vivo Evaluation of Effectiveness of New Treatment Approaches Involving Controlled Drug Delivery Systems in Cartilage Degenerations. Ph.D. Thesis. Middle East Technical University: Ankara, **2011**.
12. Swapna, N.; Jithan, A. V. *J. Young Pharm.* **2010**, *2*, 223.
13. Tomoda, K.; Makino, K. *Colloids Surf. B* **2007**, *55*, 115.
14. Kemala, T.; Budianto, E.; Soegiyono, B. *Arab. J. Chem.* **2012**, *5*, 103.
15. Thompson, C. J; Hansford, D.; Higgins, S.; Rostron, C.; Hutcheon, G. A; Munday, D. L. *Int. J. Pharm.* **2007**, *329*, 53.
16. Mundargi, R. C; Srirangarajan, S.; Agnihotri, S. A; Patil, S. A; Ravindra, S.; Setty, S. B; Aminabhavi, T. M. *J. Control. Release* **2007**, *119*, 59.
17. Vrana, N. E; Erdemli, O.; Francius, G.; Fahs, A.; Rabineau, M.; Debry, C.; Tezcaner, A.; Keskin, D.; Lavalle, P. *J. Mater. Chem. B* **2014**, *2*, 999.
18. Estellés, J. M; Vidaurre, A.; Duenas, J. M. M.; Cortázar, I. C. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 189.
19. Ha, J. H; Kim, S. H; Han, S. Y. *J. Control. Release* **1997**, *49*, 253.
20. Zhang, Y.; Sun, L.; Jiang, J.; Zhang, X.; Ding, W.; Gan, Z. *Polym. Degrad. Stab.* **2010**, *95*, 1356.
21. Higuchi, T. *J. Pharm. Sci.* **1961**, *50*, 874.
22. Higuchi., T. *J. Pharm. Sci.* **1963**, *52*, 1145.

23. Korsmeyer, R. W; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N. A. *Int. J. Pharm.* **1983**, *15*, 25.
24. Ritger, P. L; Peppas, N. A. *J. Control. Release* **1987**, *5*, 23.
25. Costa, P.; Lobo, J. *Eur. J. Pharm. Sci.* **2001**, *13*, 123.
26. Jo, Y. S; Kim, M. C; Kim, D. K; Kim, C. J. *Nanotechnology* **2004**, *15*, 1186.
27. Siepmann, J.; Siepman, F. *Int. J. Pharm.* **2008**, *364*, 328.
28. Arifin, D. Y; Lee, L. Y; Wang, C. H. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1274.
29. Siepmann, F.; Siepmann, J. *Int. J. Pharm.* **2011**, *418*, 42.
30. Crank, J. The Mathematics of Diffusion. Oxford University Press: London, **1956**.
31. Polakovic, M.; Görner, T.; Gref, R.; Dellacherie, E. *J. Control. Release* **1999**, *60*, 169.
32. Hombreiro-Perez, M.; Siepmann, J.; Zinutti, C.; Lamprecht, A.; Ubrich, N.; Hoffman, M.; Bodmeier, R.; Maincent, P. *J. Control. Release* **2003**, *88*, 413.
33. Siepmann, J.; Siepmann, F. *J. Control. Release* **2012**, *161*, 351.
34. Romero-Cano, M. S; Vincent, B. *J. Control. Release* **2002**, *82*,127.
35. Wei, G.; Pettway, G. J; McCauley, L. K; Ma, P. X. *Biomaterials* **2004**, *25*, 345.
36. Bolourtchian, N.; Karimi, K.; Aboofazeli, R. *J. Microencapsul.* **2005**, *22*, 671.
37. Kim, B. K; Hwang, S. J; Park, J. B; Park, H. J. *J. Microencapsul.* **2005**, *22*, 193.
38. Shukla, J.; Bandopadhyaya, G. P; Varma, I. K; Kumar, R.; Maulik, S. K. *Hell. J. Nucl. Med.* **2007**, *10*, 9.
39. Aishwarya, S.; Mahalakshmi, S.; Sehgal, P. K. *J. Microencapsul.* **2008**, *25*, 298.
40. Vivek, K.; Reddy, L. H; Murthy, R. S. *Pharm. Dev. Technol.* **2007**, *12*, 79.
41. Hnaien, M.; Ruffin, E.; Bordes, C.; Marcillat, O.; Lagarde, F.; Jaffrezic-renault, N.; Briançon, S. *Eur. J. Pharm. Biopharm.* **2011**, *78*, 298.
42. Sendil, D.; Gursel, I.; Wise, D. L; Hasirci, V. *J. Control. Release* **1999**, *59*, 207.
43. Dash, T. K; Konkimalla, V. B. *J. Control. Release* **2012**, *158*, 15.
44. Jain, R. A; Rhodes, C. T; Railkar, A. M; Malick, A. W; Shah, N. H. *J. Microencapsul.* **2000**, *17*, 343.
45. Zalfen, A. M; Nizet, D.; Jerome, C.; Jerome, R. F; Frankenne, F. J. M.; Foidart, J. M; Maquet, V.; Lecomte, F.; Hubert, P.; Evrard, B. *Acta Biomater.* **2008**, *4*, 1788.
46. Fan, Q.; Sirkar, K. K; Wang, Y.; Michniak, B. *J. Control. Release* **2004**, *98*, 355.
47. Chang, H. I; Perrie, Y.; Coombes, A. G. A. *J. Control. Release* **2006**, *110*, 414.
48. Yang, Y. Y; Chung, T. S; Bai, X. L; Chan, W. K. *Chem. Eng. Sci.* **2000**, *55*, 2223.
49. Lao, L. L; Venkatraman, S. S; Peppas, N. A. *Eur. J. Pharm.-Biopharm* **2008**, *70*, 796.
50. Carcaboso, A. M; Chiappetta, D. A; Höcht, C.; Blake, M. G; Boccia, M. M; Baratti, C. M; Sosnik, A. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 666.