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Preparation and Release Characteristics of Dexamethasone Acetate Loaded Organochlorine-Free Poly(lactide-*co*-glycolide) Nanoparticles

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ABSTRACT: Dexamethasone-loaded poly(lactide-*co*-glycolide) (PLGA) devices are commonly used as model systems for controlled release. In this study, PLGA nanoparticles containing dexamethasone acetate were prepared by a nanoprecipitation technique in the absence of organochlorine solvents and were characterized by their mean size, ζ potential, scanning electron microscopy, and differential scanning calorimetry to develop a controlled release system. The analytical method for the quantification of dexamethasone acetate by high-performance liquid chromatography was validated. The results show that it was possible to prepare particles at a nanometric size because the average diameter of the drug-loaded PLGA particles was 540 ± 4 nm with a polydispersity index of 0.07 ± 0.01 and a ζ potential of −2.5 ± 0.3 mV. These values remained stable for at least 7 months. The drug encapsulation efficiency was 48%. *In vitro* tests showed that about 25% of the drug was released in 48 h. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41199.

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INTRODUCTION

Controlled release devices have attracted considerable interest in recent years because these systems can deliver a drug to a specific site in a specific time and with a release pattern.[1] One of the most studied systems is dexamethasone-loaded poly(lactide-*co*-glycolide) (PLGA) particles.[2–4] Dexamethasone is a corticosteroid often used because of its anti-inflammatory properties in the treatment of arthritis and skin, blood, kidney, eye, thyroid, and intestinal disorders.[5–7] On the other hand, PLGA is a biodegradable polymer, well-known for its biocompatibility, predictable release kinetics and mechanical strength.[8]

A widespread method for the preparation of dexamethasone-loaded PLGA nanoparticles is solvent evaporation.[3,4] In summary, dexamethasone and PLGA are dissolved in a proper solvent, usually a mixture of acetone and dichloromethane. This organic solution is dropped into an aqueous phase containing a surfactant. The emulsion is subjected to a different temperature and/or pressure to favor organic solvent evaporation and particle formation.[2] The aforementioned procedure presents a huge inconvenience because of the use of the organochlorine solvents frequently associated with the drug dissolution in the organic medium and directly related to the encapsulation efficiency.[9] However, these solvents have significant toxicity. They are difficult to remove from the emulsion, and their inappropriate disposal causes environmental damage.[10] The development of alternative techniques to avoid their use is interesting for safety reasons and future commercial applications.

The aim of this study was to develop an alternative preparation method for dexamethasone-loaded PLGA nanoparticles, which do not contain organochlorine solvents. This alternative route was based on the nanoprecipitation technique, in which the solvent was quickly removed. The evaluation of the effects of the ethanol, PLGA, and Tween 80 contents and the phase ratio on the particle mean size and ζ potential was conducted by means of a reduced factorial design, 2^{4-1}, with replicates in the central point.

Table I. Range of Variables Evaluated by the 2^{4-1} Experimental Plan

Variable	Minimum (−1)	Central (0)	Maximum (1)
Volumetric fraction of ethanol in the ethanol/water solution	0.5	0.65	0.8
Surfactant concentration (% w/v)	2	4	6
Polymer concentration (g/L)	3.7	5.55	7.4
Volumetric ratio of D/C phase	0.25	0.375	0.5

EXPERIMENTAL

Materials

PLGA (lactide to glycolide molar ratio, L:G, 75:25, molecular weight = 66–107 kDa) and Tween 80 were purchased from Sigma Aldrich. Dexamethasone acetate (Tianjin Tianyao Phar, China), analytical-grade acetone (Synth), anhydrous ethanol (Synth), and high-performance liquid chromatography (HPLC) grade acetonitrile (Tedia) were used as received. Ultrapure water from a Milli-Q system (Millipore) was used in the HPLC analysis.

Nanoparticle Preparation

The PLGA nanoparticles were prepared by a nanoprecipitation technique. The desired amount of PLGA was dissolved in acetone. This was the dispersed phase. It was dribbled out into a continuous phase containing water, ethanol, and Tween 80 with various compositions. The system was stirred by means of a magnetic bar for 5 h at 25°C. The resulting mixture was centrifuged (at 15,700 g for 40 min) to separate the nanoparticles. After the removal of the supernatant, the settled nanoparticles were resuspended in 1 mL of distilled water. The evaluation of the operational conditions (Table I) was carried out by means of a 2^{4-1} factorial design, with replicates in the central point. The particle mean size and ζ potential were used as the response. These results were analyzed and the significance of each parameter was determined by means of an empirical model.

PLGA nanoparticles loaded with dexamethasone acetate were prepared by the same procedure, except for the addition of 2 mg of the drug into the dispersed phase. The drug content was set at 200 μg/mL.

Particle Size and ζ Potential

The particle size distribution, polydispersity index (PDI), and ζ potential were determined with a Malvern Zetasizer Nano ZS (Malvern Instruments, United Kingdom) on the basis of quasi-elastic light scattering. These tests were performed in triplicate with a 1:3 v/v dilution of the nanoparticles in distilled water at 25°C.

Particle Characterization: Scanning Electron Microscopy (SEM) and Differential Scanning Calorimetry (DSC)

Before the DSC and SEM characterization, the samples were freeze-dried with a Modulyo D Freeze Dryer (Thermo Electron Corp.).

SEM was performed with a JEOL JSM-6360LV instrument operating at 10 kV. Samples were prepared by means of gold sputtering on their surface.

For DSC analysis, about 10 mg of the sample was analyzed in an aluminum pan on a differential scanning calorimeter (Poly Science) equipped with an Electric Cooler Unit PS. There were two heating stages. In the first stage, the samples were heated from 30 to 80°C at a rate of 10°C/min and cooled to 10°C at 10°C/min. Then, a second heating step was conducted from 10 to 80°C also at 10°C/min. The glass-transition temperature (T_g) was analyzed by the second heating step.

Validation of the Analytical Method Used for Quantification of Dexamethasone Acetate

A Waters high-performance liquid chromatograph equipped with a Waters 515 pump, a Waters 486 ultraviolet–visible detector, a Waters 717 autosampler, and Waters Empower Login software was used to quantify the dexamethasone acetate. The analysis was performed at 235 nm with a Chromolith Reverse Phase C18 column (Merck, Germany, 100×4.6 mm) with a mobile phase composed of acetonitrile and water in gradient mode at 1 mL/min. The injection volume was 20 μL, and the analysis was conducted at room temperature. The method was validated in terms of selectivity, linearity, precision, accuracy, and limits of detection and quantification in accordance with FDA guidelines.[11]

Encapsulation Efficiency

The efficiency of drug encapsulation was quantified on the basis of the initial amount of dexamethasone acetate [eq. (1)]. The nanoparticles were separated by centrifugation. An aliquot of supernatant was removed for HPLC analysis. The concentration of the residual dexamethasone acetate was used to calculate the encapsulation efficiency:

$$\text{Efficiency}(\%) = \frac{\text{Drug added to nanoparticles-Free drug}}{\text{Drug added to nanoparticles}} \times 100 \quad (1)$$

Drug Release

In vitro release tests for the dexamethasone acetate-loaded nanoparticles were carried out under sink conditions with a phosphate buffer solution (pH 7.4 at 37°C). At set intervals of time, 2 mL of the sample was collected from the medium, and the dexamethasone acetate content was determined by HPLC. Nanoparticles were previously removed from the system by ultrafiltration with a centrifugal cellulose acetate membrane (3 kDa, Amicon Ultra, at 1,431 g, for 10 min). The filtrate was collected, and the free drug concentration was analyzed by HPLC. For each sample, an equal volume of phosphate buffer solution was restored to the system to ensure that the process conditions were not changed. The volume was kept at 20 mL. The calculations of the fraction of drug released were performed on the basis of the total concentration encapsulated. Experiments were performed in triplicate.

RESULTS AND DISCUSSION

Mean Size, PDI, and ζ Potential

We noticed a turbid solution immediately after dropping the dispersed phase into the continuous one during particle preparation; this was typical of the nanoprecipitation technique.

Table II. Experimental Conditions and Results for the Mean Size, ζ Potential, and PDI of the Particles

Test	Volumetric fraction of ethanol	Tween 80 concentration (% w/v)	Polymer concentration (g/L)	D/C phase ratio	Size (nm)	ζ potential (mV)	PDI
1	1	1	1	1	302.2 ± 0.5	−27 ± 3	0.099
2	1	1	−1	−1	210 ± 8	−15 ± 2	0.212
3	1	−1	1	−1	202 ± 1	−22.0 ± 0.5	0.085
4	1	−1	−1	1	(36 ± 3) × 10	−9 ± 1	0.330
5	−1	1	−1	−1	(19 ± 3) × 10	−6.7 ± 0.4	0.545
6	−1	1	1	1	241 ± 4	−25 ± 2	0.127
7	−1	−1	−1	1	528 ± 9	−25 ± 2	0.092
8	−1	−1	1	−1	279 ± 1	−43 ± 2	0.088
9	0	0	0	0	342 ± 3	−30 ± 1	0.082
9	0	0	0	0	217 ± 1	−27 ± 2	0.075
9	0	0	0	0	238 ± 3	−8.5 ± 0.5	0.066

The results for the mean size, ζ potential, and PDI are presented in Table II. It is worth noting that the range of each variable [ethanol volumetric fraction, Tween 80, and PLGA contents and dispersed/continuous (D/C) phase ratio] was normalized from −1 to 1, as shown in Table I.

According to Table II, the PDI ranged between 0 and 0.6; this showed that monodispersed systems were formed. With regard to the samples size, we noticed that the smaller particles were formed for the experimental condition of 5, which led to a mean diameter equal to 190 nm, with the highest deviation. The ζ potential values varied from −43 to −6.7 mV (condition 5).

The influence of the variables on the mean size and ζ potential of each sample was evaluated by means of empirical mathematical models. Linear and quadratic models were investigated, including the synergistic effect of variables. Because the variables were normalized, the parameters could be directly compared and used to infer the relevance of each one to the response.

Two variables were significant for explaining the mean particle size: the Tween 80 concentration and the D/C phase ratio. The adjusted model [eq. (2)] showed a correlation coefficient (R^2) of 0.61. In eq. (2), the size is the particle mean size (nm), the surfactant is the Tween 80 content, and the D/C is the ratio between the dispersed and continuous phases:

$$\text{Size} = (28 \pm 2) \times 10 - (5 \pm 2) \times 10 \times [\text{Surfactant}] + (7 \pm 2) \times 10 \times \text{D/C} \quad (2)$$

Upon the analysis of eq. (2), the D/C phase ratio showed a higher significance than the surfactant content because the estimated parameters were 70 and 50, respectively, in the module. It is worth noting that these variables showed different behaviors: the increase in the surfactant content caused a decrease in the particle mean size, and the decrease in phase ratio led to a decrease in the sample diameter. This could be reasoned in terms of the interfacial properties of the system. The increase in the Tween 80 content decreased the interfacial tension between the phases because it is an amphiphilic molecule. As a nonionic surfactant, Tween 80 was placed in the interface, and it caused the stabilization of the system, which prevented nanoparticle clustering. It is believed that the stabilization mechanism was based on the steric hindrance caused by the polysorbate molecule in each particle.[12] On the other hand, when the D/C phase ratio decreased, the system was diluted, and this decreased the probability of collision between two particles because they were far from each other. This also should have decreased the productivity of such particles in each cycle.

With regard to the ζ potential of the samples, the model showed a low correlation coefficient ($R^2 = 0.39$), and it is not presented here.

The volumetric ratio of ethanol and water showed significance neither for the mean size nor for the ζ potential of the samples.

By considering the results of Table II and the model for the mean size [eq. (2)], we defined a new set of conditions to decrease the particle size and ζ potential. Because the ethanol volumetric ratio showed no significance, it was fixed at 0.5, the minimum value in the range. The Tween 80 content was 6% w/v, the PLGA concentration was 7.4 g/L, and the D/C phase ratio was 0.25. The results of the particles prepared under these conditions are shown in Table III, as are the ones for the dexamethasone acetate-loaded particles.

According to Table III, the low values of PDI showed that the systems were monodispersed. It was possible to prepare particles

Table III. Results of the Mean Size, ζ Potential, and PDI of Both the Unloaded and Loaded Nanoparticles Prepared with the Conditions Selected by the Experimental Plan

Sample	Mean size (nm)	ζ potential (mV)	PDI
PLGA nanoparticles	208 ± 9	−10.2 ± 0.4	0.137
Dexamethasone acetate-loaded PLGA nanoparticles	540 ± 4	−2.5 ± 0.3	0.07

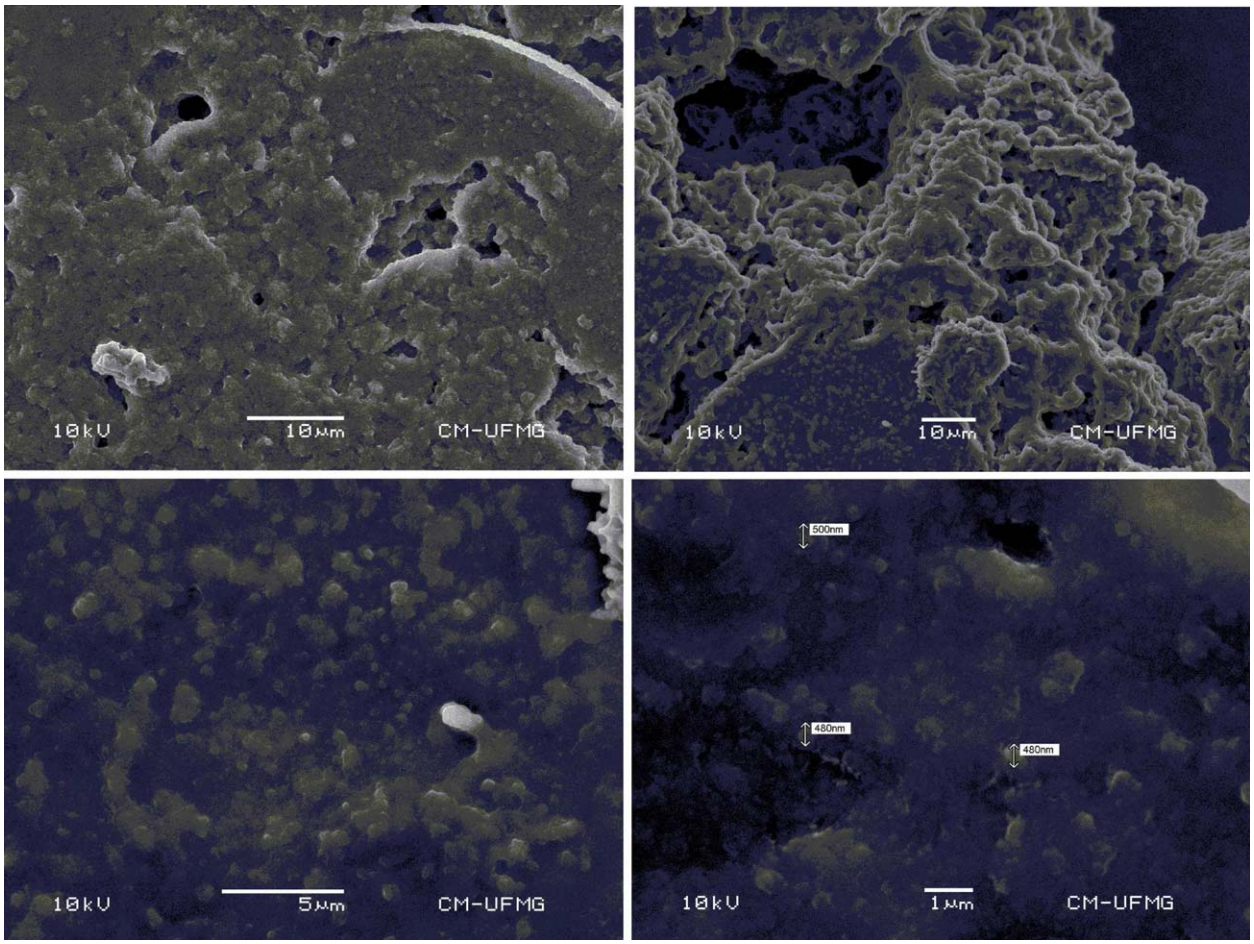

Figure 1. SEM micrographs for the dexamethasone acetate-loaded PLGA particles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the nanometric size with the proposed methodology in the absence of organochlorine solvents.

With the unloaded dexamethasone acetate PLGA nanoparticles taken as an example, the mean size (208 ± 9 nm) was in the range of the experimental results presented in Table II (condition 5) and slightly lower than the values predicted by the model [eq. (2)], which was $(30 \pm 6) \times 10$ nm. The surface potential also showed a smaller value compared to condition 5; this could be explained by the increase in the PLGA content.

With regard to the dexamethasone acetated loaded PLGA particles, we observed an increase in the mean size compared to that of the unload ones, although the sizes still remained in the nanometric range. The value was around 2.6 times higher. This could be explained by the fact that a higher mass (PLGA + dexamethasone acetate) required a higher volume. In addition to this, the decrease in the ζ potential module (from 10.2 to 2.5) could be explained by the dilution of the PLGA by dexamethasone acetate.

Gómez-Gaete et al.[3] prepared dexamethasone acetate loaded PLGA nanoparticles with an average size of 230 nm and a ζ potential equal to -4 mV; however, the organic phase was composed of acetone and dichloromethane at a 1:1 ratio in volume. A similar result was also reported by Ali et al.,[5] who obtained drug-loaded PLGA nanoparticles ranging from 140 to 298 nm and with a ζ potential value around -60 mV with sodium taurocholate as the emulsifying agent. By comparing the results of the literature with those of this study, we noticed that the mean size of the PLGA nanoparticles obtained here were higher, probably because of the difference in the preparation technique and because they used an anionic surfactant, which caused electrostatic repulsion and decreased the nanoparticle mean size. With regard to the ζ potential values, this study presented a lower value in the module compared to those found in the literature.[5]

The stability of the loading and unloading system was evaluated by new measures of size, ζ potential, and PDI after 7 months of storage. The dexamethasone-unloaded PLGA particles showed a mean size of 167 ± 1 nm with a PDI of 0.10 ± 0.01 and a ζ potential of -13.3 ± 0.4 mV, whereas the dexamethasone-loaded PLGA particles presented a mean size equal to 532 ± 6 nm with a PDI of 0.13 ± 0.02 and a ζ potential of -0.9 ± 0.1 mV. The decrease or maintenance of the particle size was evidence of the stability of these systems because we expected the nanoparticles to cluster. This would have led to an increase in particle size. The decrease in nanoparticles was

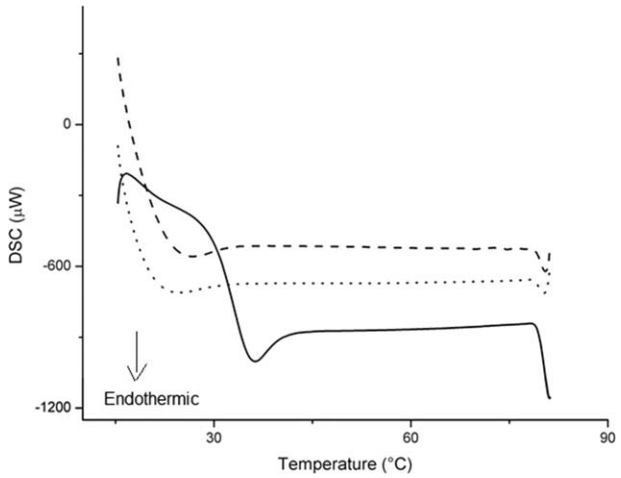

Figure 2. DSC thermograms of the PLGA pellets (solid line), dexamethasone acetate unloaded nanoparticles (dashed line), and dexamethasone acetate-loaded nanoparticles (dotted line).

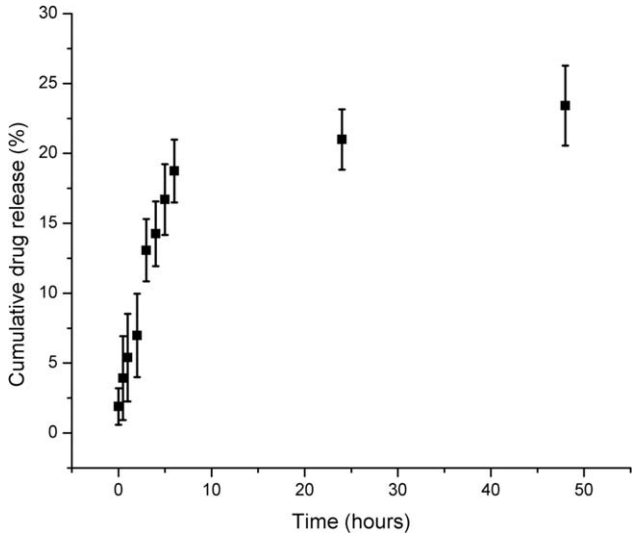

Figure 3. Release profile of the dexamethasone acetate from the PLGA nanoparticles.

reasoned in terms of the fluctuations of the technique used to measure the particle size. This result could be especially important in the case of commercialization; that is, it could increase the shelf life of a product.

SEM

The dexamethasone acetate loaded particles were freeze-dried before the SEM analysis to maintain particle morphological properties after the sample was dried. The SEM micrographs (Figure 1) were generally spherical and presented a mean size of about 500 nm.

It is worth noting that the freeze-drying processes led to an oily product, which probably affected the SEM micrographs. We believed that this sample aspect was caused by the Tween 80 content in the sample. Although the steric hindrance of this surfactant helped in the stabilization of the nanoparticles, the high viscosity also prevented the success of sample lyophilization.

The presence of dexamethasone crystals was not observed in the SEM micrographs.

DSC

DSC analysis was performed for PLGA pellets and for both the dexamethasone acetate unloaded and loaded nanoparticles (Figure 2). The T_g values of the samples are presented in Table IV. The PLGA pellets exhibited a T_g around 37°C within the range reported in the literature,[13] whereas dexamethasone unloaded and loaded PLGA nanoparticles showed T_g values equal to 27 and 25°C, respectively. This result suggested that the method selected to prepare the nanoparticles significantly changed the conformation of the polymer chains. On the other hand, the addition of dexamethasone to the nanoparticles did not affect the polymer chain conformation to a high extent.

Table IV. T_g Values of the Samples

Sample	T_g (°C)
PLGA pellets (75:25)	37
Dexamethasone unloaded PLGA nanoparticles	27
Dexamethasone loaded PLGA nanoparticles	25

The decrease in T_g is usually associated with higher release rates because delivery tests were conducted at 37°C. Before, the T_g of the sample caused the amorphous system to be more easily eroded compared to the unmodified polymer.

Encapsulation Efficiency

Dexamethasone acetate encapsulation efficiency was evaluated for three different samples with eq. (1). The mean value was a 48% drug encapsulation efficiency. Gómez-Gaete et al.[3] reached a concentration of 230 μg/100 mg of PLGA with a mixture of dichloromethane and acetone as a solvent and 10 mg of drug. In this study, it was possible to encapsulate 259 μg/100 mg of PLGA with only acetone as the solvent and, in this case, with 5.4 mg of dexamethasone acetate.

Drug Release

In vitro release studies were performed in triplicate for 48 h. The delivery profile showed a fast release of dexamethasone during the first 6 h of the test, and for the next few hours, the profile was stabilized (Figure 3). The initial burst release is commonly observed for biodegradable polymer systems, as described by Yasukawa et al.[9] The degradation of the polymer played an important role in the release mechanism because it was responsible for the gradual delivery of the drug remaining inside the matrix. About 25% of the drug encapsulated in the nanoparticles was released in the test. Similar systems developed by Gómez-Gaete et al.[3] showed complete drug release in 4 h. We believe that the main difference was the preparation technique used in this study, which caused an increase in the drug load compared to the values in the literature.[3] According to these results, we believe that the nanoparticles prepared in this study

are promising candidates for use in drug-delivery devices for long-term tests.

It is worth noting that *in vitro* and *in vivo* tests usually present quite different release kinetics. We believe that the drug can be 100% delivered in such *in vitro* test, but this result is not directly related to the performance in living systems.

CONCLUSIONS

Dexamethasone acetate loaded PLGA nanoparticles were prepared by a new nanoprecipitation organochlorine-free method. The factorial design 2^{4-1} with replicates in the central point revealed that the D/C phase ratio was the most significant variable for reducing the particle mean size. The drug-loaded samples showed an average diameter of 540 nm, which remained stable for at least 7 months; this may be used to increase the shelf life of the system. The ζ potential was slightly decreased in the module from -2.5 to -0.9 mV.

The encapsulation efficiency of dexamethasone acetate in the PLGA nanoparticles was 48% with 259 μg/100 mg of PLGA. Dexamethasone release studies reached 25% of drug delivery in 48 h. These results show the potential of the proposed method for the treatment of various diseases.

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