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Poly(lactide-*co*-glycolide)/cyclodextrin (polyethyleneimine) microspheres for controlled delivery of dexamethasone

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ABSTRACT

Water-in-oil-in-water ($w_1/o/w_2$) solvent evaporation method is a technique for encapsulation and protection of water soluble and chemically sensitive bioactive molecules.
One of the most important disadvantages of this method is the diffusion of bioactive molecule, during synthesis, from the primary to the secondary aqueous phase, reducing dramatically the encapsulation yield. Therefore, dexamethasone sodium phosphate (DM), a corticosteroid water soluble drug, which is sensitive to degradation, was first complexed with hydroxypropyl cyclodextrin (HPCD), γ-cyclodextrin (γ-CD) or polyethyleneimine (PEI) and then entrapped in poly(lactic-*co*-glycolic acid) (PLGA) microspheres obtained by $w_1/o/w_2$ solvent evaporation method. Association equilibrium constants for the formation of the HPCD/DM and γ-CD/DM inclusion complexes were also calculated, being $1.420 \times 10^3\ M^{-1}$ and $1.447 \times 10^4\ M^{-1}$, respectively. PEI was proved to be the most efficient DM trapper, retaining the highest amount of the drug in microspheres, followed by γ-CD and HPCD. Despite the high values of the association equilibrium constants for DM binding to HPCD and γ-CD, both cyclodextrins are not able to protect the drug against UV irradiation. The morphology of the microspheres as well as the drug entrapment efficiency and the release rates are influenced by complexing agents and the ratio between the primary aqueous phase and the organic phase.

1. Introduction

Controlled drug delivery systems based on polymeric micro- and nano-spheres have seen a tremendous development over the last twenty years [1–3]. The most important advantages of these systems are the prolonged delivery of drugs, maintaining a therapeutic concentration and reduce side effects; moreover, they are easy to prepare and handle and can be administered by any route in the organism [4]. Furthermore, drug binding to a polymeric matrix protects the drug against premature degradation. Drugs are usually linked covalently [5], by electrostatic and hydrogen bonds [6], or they are physically embedded in the polymeric matrix [7]. Many drugs and bioactive molecules are very sensitive to temperature and to organic solvents; therefore, during encapsulation, they should be kept in aqueous solution at low temperatures.

Water-in-oil-in-water ($w_1/o/w_2$) solvent evaporation method is the most used technique for the encapsulation in microspheres of water soluble drugs or biologically active molecules [8,9]. The drug is dissolved in a low amount of water and the aqueous solution (w_1) is dispersed in a polymeric solution in organic solvents (o). The first emulsion is then dispersed in a second aqueous solution containing a stabilizer (w_2). During the evaporation of the volatile solvent and at the end of preparation the drug remain solubilized in water. Finally, the water is eliminated by lyophilization and the drug is encapsulated as free powder after the volatile solvent is completely removed. However, the most important drawback of this method is the diffusion of a large amount of the drug from the first to the second aqueous phase, thus resulting in low encapsulation efficiency [10,11]. In fact, the first emulsion, consisting of a huge number of small droplets (micro- or nano-dispersion) with a very large surface comes in contact with the second aqueous phase facilitating the diffusion of the water soluble drug. This phenomenon is emphasized when the ratio between the first aqueous phase and the amount of the polymer in the organic phase is high [12]. In fact, although the 1:10 ratio of the aqueous/organic phase (obtained dispersing 0.5 mL aqueous drug solution in 5 mL organic phase with 0.2 g dissolved polymer [12]) is acceptable, after complete evaporation of the volatile solvent, 0.2 g dried polymer should entrap ~0.5 g aqueous polymer solution. The final microcapsules should display a cellular structure with very thin walls similar with a honeycomb, facilitating a rapid diffusion of the drug towards the secondary aqueous phase during the preparation process as well as a high release rate in physiological fluids. Several water soluble bioactive molecules were encapsulated by

Abbreviations: ESEM, environmental scanning electron microscopy; PBS, phosphate buffer solution at pH = 7.4; PLGA, poly(lactide-co-glycolide); HPCD, 2-hydroxypropyl-β-cycodextrin; γ-CD, γ-cyclodextrin; PEI, polyethyleneimine; PVA, poly(vinyl alcohol); DCM, dichloromethane; DM, dexamethasone sodium phosphate.

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this procedure. Among them, dexamethasone sodium phosphate (DM) is a synthetic adrenocortical steroid that is frequently used in ophthalmology, for the treatment of acute and chronic inflammatory diseases of the posterior segment of the eye (i.e. uveitis) [13]. However, it has been reported that the application of eye drops of 0.1% dexamethasone for prolonged periods of time can produce glaucoma accompanied by optic nerve damage and defects of vision [14,15]. The controlled delivery of DM from poly(lactide-*co*-glycolide) (PLGA) microspheres may overcome this inconvenience. Of note, PLGA is a biocompatible and biodegradable copolymer, can exhibit a wide range of degradation time, displays tuneable mechanical properties and, most importantly, is a FDA approved polymer. Moreover, PLGA nanoparticles are currently used for ocular drug delivery due to their low ocular toxicity [16,17].

Here, with the aim to hinder the drug diffusion during sample preparation and to control the release rate as well as to increase the drug stability, DM was included in the cavity of hydroxypropyl cyclodextrin (HPCD) or γ-cyclodextrin (γ-CD) or bound ionically on polyethyleneimine (PEI) and then entrapped in PLGA microspheres obtained by the solvent evaporation method from double emulsion ($w_1/o/w_2$). The association equilibrium constants between DM and HPCD (γ-CD), as well as the chemically stability to UV irradiation of free and complexed drug were evaluated. Finally, the influence of the complexation and of the ratio between the primary aqueous phase and PLGA on drug entrapment efficiency, drug release rates and microspheres morphology were investigated.

2. Experimental

2.1. Materials

Poly(lactide-*co*-glycolide) (PLGA) (M_w = 100,000 g/mol) was purchased from BioMatPol Ltd. (Gliwice, Poland). 2-Hydroxypropyl-β-cycodextrin (HPCD) (M_w = 1380 g/mol), γ-cyclodextrin (γ-CD), poly(ethyleneimine) (PEI) solution, 50% (*w*/*v*) in water (M_w = 750,000 g/mol), poly(vinyl alcohol) (PVA) (Mw = 31,000 g/mol), hydrolysis mole = 88%, and dichloromethane (DCM) were supplied from Sigma-Aldrich Co. (St. Louis, USA). Dexamethasone sodium phosphate (DM) (Fig. 1) was kindly supplied from Rompharm Company SRL (Otopeni, Romania). Standard phosphate buffer solution at pH = 7.4 (PBS, 50 mM Na_2HPO_4 + NaOH) was prepared in our laboratory. All chemicals were of analytical or reagent grade and were used without purification.

2.2. Job's plot

The association complexes between DM and HPCD or γ-CD were formed at a constant volume by addition of different concentration of each CD at several molar ratios (R), which varies from 0.1 to 0.9, to get a final concentration of 2.0×10^{-4} M. After stirring for 24 h, the UV absorption at 241 nm was measured and the difference between the absorption values obtained in the presence (A) and absence of CD (A_0), $\Delta A = A - A_0$, was plotted versus the molar fraction R.

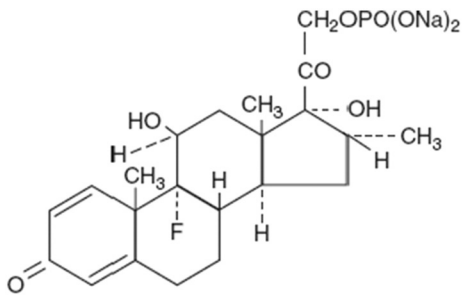

Fig. 1. Chemical structure of dexamethasone sodium phosphate.

2.3. Determination of association equilibrium constants

The interaction between DM and HPCD (γ-CD) was investigated by UV spectrophotometry. A known volume of aqueous DM solution (0.05 mM) was mixed with increasing amounts of CD to obtain different CD/DM molar ratios, keeping constant the molarity of the drug solution. The solutions were stored at room temperature for 24 h before measuring the absorbance at 241 nm. The association equilibrium constants (K_a) were determined by the modified Benesi-Hildebrand equation (Eq. (1)) [18]:

$$\frac{[CD][DM]_0}{\Delta A} = \frac{K_d}{\Delta\varepsilon} + \frac{[CD]}{\Delta\varepsilon} \quad (1)$$

where $[CD]$ and $[DM]_0$ indicate the CD and DM concentration, respectively; K_d is the dissociation constant ($K_d = 1/K_a$); ΔA is the difference between the absorbance of DM in the absence and in presence of CD; and $\Delta\varepsilon$ is the change in the molar absorption coefficient. Plotting values of $[CD][DM]_0/\Delta A$ versus [CD] yielded a straight line. The slope corresponds to $1/\Delta\varepsilon$ and the intercept indicates $K_d/\Delta\varepsilon$.

2.4. UV stability studies

Open glass containers, each containing 10 mL of the DM solution (0.05 mM) or of the DM/CD (PEI) complex (1:1 ratio) in double distilled water (i.d. = 2.5 cm, solution depth = 2.0 cm), were irradiated at 365 nm (UV-A) and 254 nm (UV-C) for different period of time (from 5 min to 24 h).

The irradiation at 254 nm was performed by a Bactericidal Lamp LBA, 15 W-P situated at 60 cm from the samples. The irradiation at 365 nm was performed by an OSRAM HQE-40 lamp, as an artificial light source, at 6 cm from the samples. The light source power was 100 W. The irradiance value, measured at a distance of 6 cm from the source, was 89.5 W/m^2. A PMA 2100 radiometer provided with a UV-A detector (Solar Light Co., USA) was used to measure the irradiance. The temperature measured inside of the irradiation devices was 20–22 °C and the relative air humidity (RH) was 59%.

After irradiation, the content of DM in solution was determined by high-performance liquid chromatography. The apparatus (Shimadzu HPLC system, Kyoto, Japan) was equipped with an injection valve (sample loop, 20 μL), a C-18 column (l = 250 mm, i.d. = 4.6 mm), and the UV–Vis detector fixed at 241 nm. The mobile phase was acetonitrile–PBS with the 35:65 (*v*/v) ratio and the 0.6 mL/min flow rate.

2.5. Synthesis of PLGA microspheres

PLGA microspheres were synthesized by the solvent evaporation method from double emulsion, water-in-oil-in-water ($w_1/o/w_2$) [19]. The 1:1 DM/HPCD complex was prepared by dissolving 0.268 g HPCD and 0.1 g DM in 1 mL of distilled water. The 1:1 DM/γ-CD complex was obtained by mixing 0.2 g γ-CD and 0.08 g DM in 1 mL of water and the stoichiometric DM/PEI complex was prepared by solubilization of 0.017 g PEI and 0.1 g DM in 1 mL of water. The organic phase was prepared by dissolving 0.4 (0.2) g PLGA in 5 mL DCM. The primary emulsion (w_1) was obtained by dispersing 0.1–0.5 mL of the complex solution in 5 mL organic phase by using a probe sonicator (30 s at 300,000 J). The primary emulsion was added to 25 mL of the PVA aqueous solution (0.5%, *w*/*v*) (w_2), then the mixture was homogenized for 5 min at 10,000 rpm using an Ultra-Turrax T 25 homogenizer (IKA Labortechnik). Subsequently, DCM was evaporated from the droplets under magnetic stirring at 800 rpm for 2 h at room temperature, and for 30 min at 36 °C. Finally, the microspheres were separated by ultracentrifugation at 10,000 rpm, washed two times with 50 mL distilled water, and recovered by freeze-drying.