Release of dexamethasone from PLGA nanoparticles entrapped into dextran/poly(vinyl alcohol) hydrogels

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Hydrogels based on blends of poly(vinyl alcohol) (PVA) with dextran were prepared by a physical cross-linking procedure and used as matrices for the entrapment of biodegradable nanoparticles loaded with dexamethasone.

The nanoparticles were prepared, by a solvent evaporation technique, using biodegradable copolymers of poly(lactic acid)–poly(glycolic acid) (PLGA).

Size, morphology and surface characteristics of the nanoparticles were evaluated by scanning electron microscopy. The mechanism of drug release from the nanoparticles entrapped into the PVA-based matrices was studied and compared to that from free nanoparticles. The effect of dextran on the *in vitro* release profile of dexamethasone from the hydrogels was investigated.

The obtained results indicate that PLGA nanoparticles are able to release dexamethasone following a diffusion-controlled mechanism. The entrapment of the nanoparticles into the hydrogels affects only slightly this mechanism of drug release.

In addition, dextran/PVA hydrogels release a higher amount of drug with respect to pure PVA hydrogels and by increasing dextran content in the hydrogels, the amount of drug released increases.

Introduction

Poly(vinyl alcohol)-based hydrogels prepared by a physical cross-linking method based on freeze–thawing cycles [1, 2] are potentially useful as delivery devices for water soluble drugs.

Hydrogels based on blends of PVA with biological macromolecules such as collagen [3, 4], hyaluronic acid [3–6], dextran and chitosan [7, 8] showed to be able to release human growth hormone. In additon it was observed that the content of the biological component could be used as a tool to modulate the release of the drug.

In order to allow the use of such hydrophilic matrices for the release of lipophilic agents, a novel mechanism was designed [9] based on producing biodegradable nanoparticles, loaded with water insoluble drugs, that were then entrapped into PVA-based hydrogels. PLGA nanoparticles loaded with dexamethasone were entrapped into pure PVA hydrogels [9] and the *in vitro* release of the drug was investigated. It was observed that the release profile was not significantly affected by the PVA matrix which however represented an additional resistance to drug diffusion. In addition a correlation was found between the amount of drug released and the PVA concentration in the hydrogels.

In the present work PLGA nanoparticles containing dexamethasone were produced by a solvent evaporation procedure based on a single oil-in-water emulsion, and then entrapped into hydrogels based on blends of PVA with dextran. The *in vitro* release of the drug was studied with the aim to evaluate the effect of the biological polymer on dexamethasone release from the dextran–PVA hydrogels.

The mechanism of drug release from the nanoparticles entrapped into the hydrogels was investigated and compared with that from the free nanoparticles.

Materials and methods

Materials

Poly(DL-lactide-co-glycolide 50/50) with average molecular weight M_w 40–75 000 (PLGA 50/50), poly(DL-lactide-co-glycolide 75/25) with M_w 90–126 000 (PLGA 75/25), poly(vinyl alcohol) with M_w 86 000–146 000 (PVA_H), dextran (average molecular weight 78 000,

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produced by *Leuconostoc mesenteroides*) (Dx), dexamethasone and phosphate buffered saline (PBS) were supplied by Sigma Aldrich; poly(vinyl alcohol) with M_w 15 000 (PVA_L) was supplied by Fluka Chemika; dichloromethane and Tween 80 were supplied by Carlo Erba Reagenti.

Preparation of nanoparticles loaded with dexamethasone

Nanoparticles were prepared by a solvent evaporation process based on a single oil-in-water (O/W) emulsification process as reported earlier [9]. Briefly, the process is as follows. Dexamethasone was dissolved in acetone, then PLGA and dichloromethane were added to obtain the ''oil phase''. This solution was added dropwise to an aqueous 5% PVA_L solution whilst being mixed with a high speed homogenizer (Art. Miccra-D8, Falc Instruments) at a speed of 23 500 rpm. The whole process was carried out in a beaker cooled in an ice bath. Mixing was continued for a total of 10 min. The resulting oil-in-water emulsion was stirred overnight under room conditions to allow solvent evaporation and nanoparticle formation.

Nanoparticles were cleaned by centrifuging and resuspending in distilled water for a total of three times. The final product was dried under vacuum at room temperature to obtain a fine white powder.

Particle size analysis by scanning electron microscopy (SEM)

Size and morphological characteristics of the nanoparticles were analyzed by SEM. The samples were mounted onto metal stubs using double-sided adhesive tape, vacuum-coated with a gold film and directly analyzed by a SEM (Jeol T300).

Preparation of PVA hydrogels loaded with PLGA nanoparticles

A PVA_H aqueous solution (10% w/v) was prepared in an autoclave for 1 h at 120 °C. This solution was mixed with a dextran aqueous solution to obtain Dx/PVA blends with the following compositions (w/w): 0/100, 10/90, 20/80, 30/70, 40/60. The blends were poured in wells of 12-well plates (2.5 ml/well).

Fifteen milligrams of PLGA nanoparticles loaded with dexamethasone were added to each sample. After nanoparticles addition, samples underwent eight cycles of freeze–thawing to obtain hydrogels. Each cycle, with the exception of the first one, consisted of 1 h at − 20 °C and 30 min at room temperature. The first cycle differed from the others due to a longer standing time (over night) at − 20 °C.

In vitro releasing test

Dexamethasone release from free nanoparticles

The release of dexamethasone entrapped in the free nanoparticles was evaluated using a special diffusion chamber previously described [9].

At regular time intervals, an aliquot of the eluate was removed from the acceptor side of the chamber and stored. The eluate was replaced with fresh PBS. The amount of drug contained in the eluates was measured spectrophotometrically at $\lambda = 238.5$ nm.

Dexamethasone release from hydrogels

The hydrogels were placed each in 10 ml PBS in individual containers at 37 °C. The elution fluid was removed at regular time intervals (every hour for the first day, every day for the following six days, and every two days for the last three weeks). The eluate was replaced with fresh PBS and the containers returned at 37 °C. Elution fluids were assayed for dexamethasone spectrophotometrically at $\lambda = 238.5$ nm.

Results and discussion

Nanoparticles are colloidal polymer particles with size below 1 μm that are widely employed as drug carriers [10–18]. In this work PLGA nanoparticles were produced by a solvent evaporation method based on a single oil-in-water emulsion. Electron microscopy analysis showed particles with a mean diameter of 200–300 nm, spherical shape and smooth surface (Fig. 1). Fig. 2 shows the nanoparticles entrapped into a Dx/PVA hydrogel.

The release curves for dexamethasone from nano-

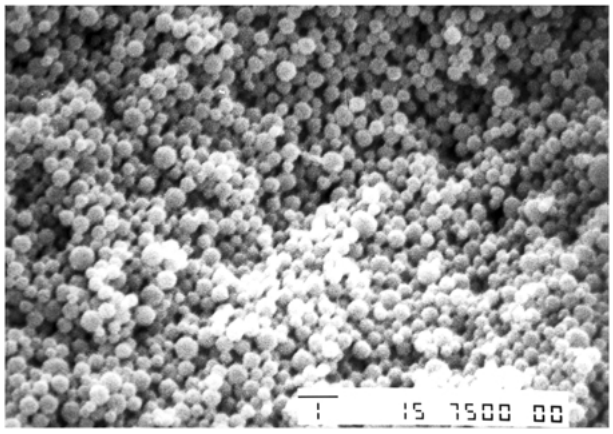

Figure 1 SEM image of free PLGA nanoparticles loaded with dexamethasone (bar = 1 μm).

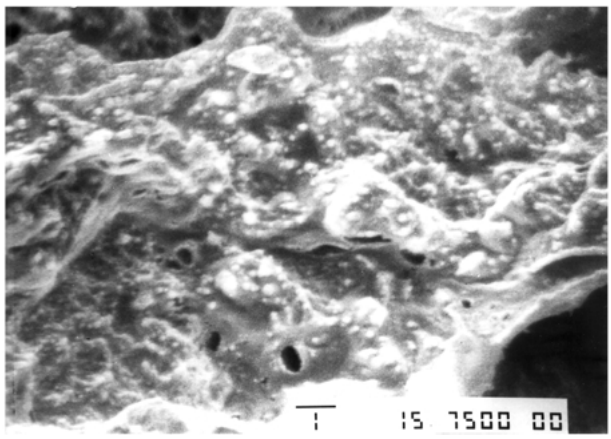

Figure 2 SEM image of PLGA nanoparticles entrapped into a Dx/PVA = 20/80 hydrogel (bar = 1 μm).

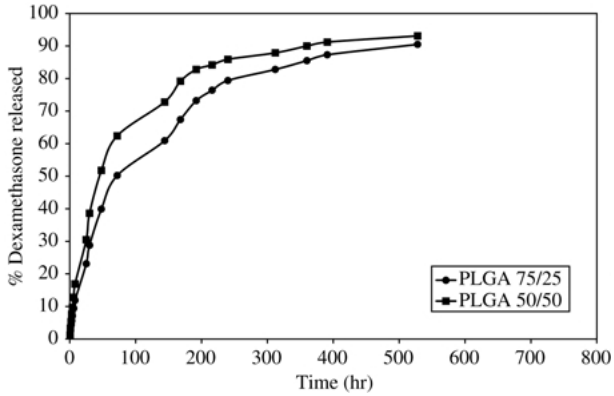

Figure 3 In vitro release of dexamethasone, from free PLGA nanoparticles.

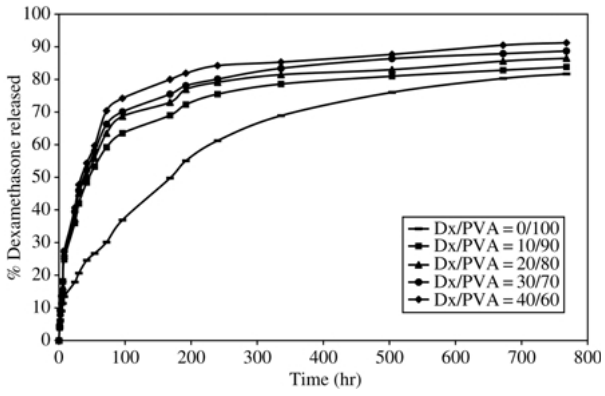

Figure 5 In vitro release of dexamethasone from PLGA 50/50 nanoparticles entrapped into Dx/PVA hydrogels with different composition.

particles produced using PLGA 50/50 and PLGA 75/25 respectively, are reported in Fig. 3. It can be seen that the chemical composition of PLGA does not affect the release profile of the drug. In both cases, after an initial burst phase (first 70–80 h) a decrease in the release rate can be observed followed by a new increase after about 200–220 h. However, the release rate of dexamethasone from PLGA 50/50 nanoparticles resulted higher with respect to that from PLGA 75/25 nanoparticles. This can be attributed to a different diffusion rate of the external aqueous phase into the polymeric matrix due to the different PLGA lactil to glycolyl unit ratio. It is known that glycolil units are more hydrophilic with respect to lactil units. Increasing the content of glycolyl units it increases the absorption rate of water by the PLGA matrix which swells favoring a subsequent faster release of the drug entrapped inside itself.

In Fig. 4 the release curves for dexamethasone from Dx/PVA = 10/90 hydrogels loaded with PLGA 50/50 and PLGA 75/25 nanoparticles are reported. It can be observed that, as in the case of free nanoparticles, the release rate of the drug from the hydrogels containing PLGA 50/50 nanoparticles results higher with respect to that from the hydrogels containing PLGA 75/25 nanoparticles. In addition, by comparing this figure with Fig. 3, it results evident that the entrapment of the particles into the hydrogels causes a reduction in both the releasing rate and the total amount of drug released because of the additional resistance to drug diffusion offered by the PVA matrix.

With regard to the effect on drug release from the hydrogels exerted by dextran, Fig. 5 shows the release curves for dexamethasone from Dx/PVA hydrogels with different composition and loaded with PLGA 50/50 nanoparticles. It can be observed that all the curves have the same shape: the polysaccharide does not affect the release profile of the drug, whilst it affects both the initial release rate and the total amount of drug released. Even an amount of dextran as small as 10% is enough to induce an increase in the release rate of the drug with respect to that from a pure PVA hydrogel, also indicated in the same figure. In addition, as the amount of nanoparticles added to the samples was constant, both the initial release

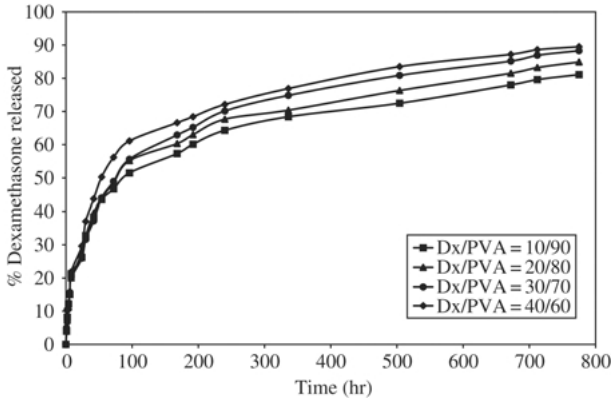

Figure 6 In vitro release of dexamethasone from PLGA 75/25 nanoparticles entrapped into Dx/PVA hydrogels with different composition.

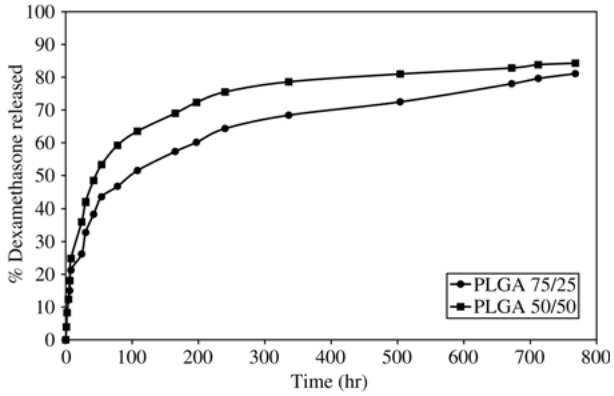

Figure 4 In vitro release of dexamethasone from PLGA nanoparticles entrapped in a Dx/PVA = 10/90 hydrogel.

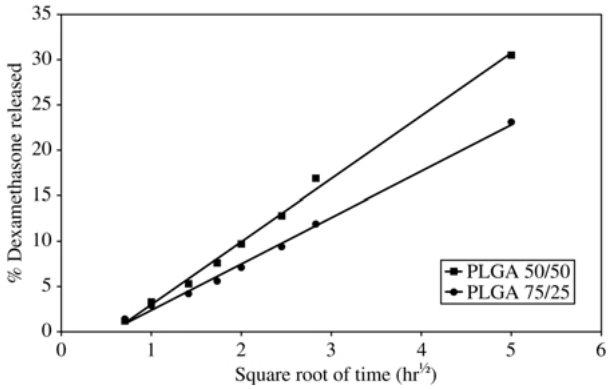

Figure 7 Apparent diffusion-controlled release profiles, according to Higuchi's model for dexamethasone from free PLGA nanoparticles.

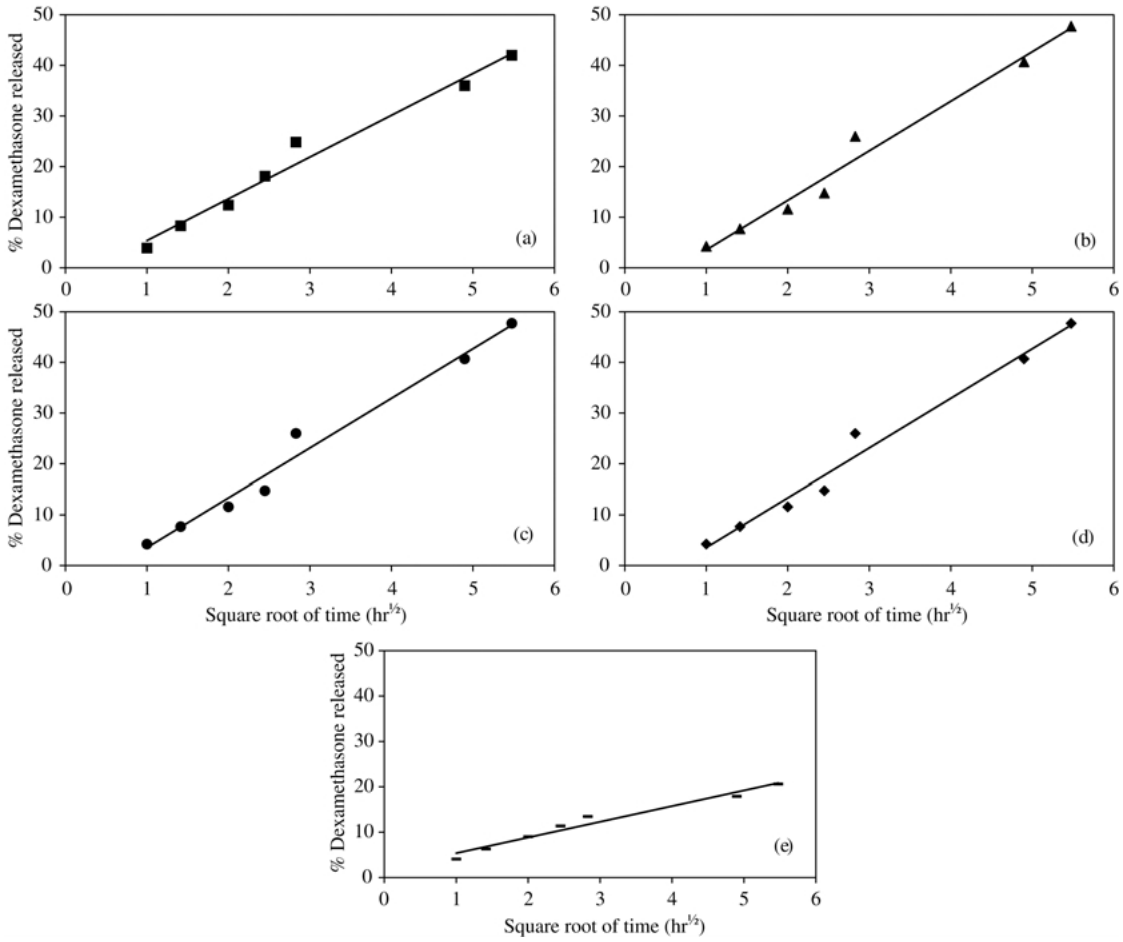

Figure 8 Apparent diffusion-controled release profiles, according to Higuchi's model for dexamethasone from Dx/PVA hydrogels containing PLGA 50/50 nanoparticles (a) Dx/PVA = 10/90; (b) Dx/PVA = 20/80; (c) Dx/PVA = 30/70; (d) Dx/PVA = 40/60; and (e) Dx/PVA = 0/100).

rate and the percentage of drug released increased by increasing dextran content. The same results were obtained in the case of Dx/PVA hydrogels loaded with PLGA 75/25 nanoparticles (Fig. 6). It can be supposed that because dextran is more hydrophilic than PVA, the higher is the dextran content, the higher is the hydrophilicity of the hydrogel as a whole and this in turn allows for an easier release of the drug.

With regard to the mechanism of dexamethasone release, plotting the amounts of drug released from both PLGA 50/50 and PLGA 75/25 free nanoparticles during the first 200 h, against the square root of time, straight lines were obtained with a good approximation (Fig. 7). These linear plots appear to indicate that drug release in these systems is diffusion controlled in accordance with the equation (Higuchi square root of time equation) developed by Higuchi [19] for diffusion controlled release of drugs from solid matrices. Similar results were obtained investigating the mechanism of dexamethasone release from the nanoparticles entrapped into dextran/PVA hydrogels (Fig. 8).

Conclusions

The obtained results indicate that a pure PVA matrix hinders the release of a drug loaded into PLGA nanoparticles by adding an additional resistance to the diffusion: the amount of dexamethasone released and the initial rate of release from PLGA nanoparticles entrapped into pure PVA hydrogels were lower with respect to those observed in the case of free PLGA nanoparticles.

However, it was observed that the addition of a biological macromolecule like dextran to PVA lowers this resistance, in fact the amount of drug released and the initial release rate observed in the case of dextran/PVA hydrogels were higher with respect to those observed in the case of pure PVA hydrogels.

According to results previously obtained [9], it can be concluded that it is possible to employ a PVA-based hydrogel as a hydrophilic matrix for the release of a lipophilic drug by entrapping into the hydrogel biodegradable nanoparticles loaded with the drug. It was observed that the concentration of PVA in the solution used for the preparation of the hydrogels is a parameter that controls the release of dexamethasone [9].

This work has shown that the presence of a biological macromolecule in the structure of these hydrogels offers a further tool in controlling important parameters of release such as the initial rate and the amount of drug released.

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