Poly(vinyl alcohol) hydrogels as hydrophilic matrices for the release of lipophilic drugs loaded in PLGA nanoparticles

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Poly(vinyl alcohol) (PVA) hydrogels prepared by a freeze-thawing procedure were evaluated as matrices for the release of water-insoluble drugs such as dexamethasone. As it is impossible to directly entrap a lipophilic drug into a hydrophilic matrix, a novel mechanism has been designed based on producing biodegradable nanoparticles loaded with the drug, that could then be entrapped into the hydrogels. Nanoparticles were prepared by a solvent evaporation technique using a biodegradable copolymer of poly(lactic acid)-poly(glycolic acid) (PLGA). The effects of several processing parameters on particle properties were investigated. The drug release from free nanoparticles was compared to that from the nanoparticles entrapped into the PVA matrices. It was observed that the release profile of the drug is not significantly affected by the PVA matrix. A correlation was found between the amount of drug released and the PVA concentration in the hydrogels: the percentage of drug released, as a function of time, decreased by increasing PVA concentration, indicating that PVA concentration can be used as a tool in modulating the release of the drug.

1. Introduction

Most available drugs exhibit little specificity following systemic administration and often a drug has limited or no access to its intended site of action or is prematurely metabolized or excreted. The selective delivery of drugs to their site of action should increase therapeutic effectiveness and reduce harmful systemic effects. An alternative method of drug administration is to introduce a polymeric delivery system directly into the diseased site, either during a surgical procedure or by direct injection. These devices are capable of releasing a high drug load for prolonged time periods.

Research in this field focuses on developing carrier systems for the controlled release into the target tissue or the cell compartment. Controlled release may be defined as formulations of drug polymer composites, in which drug administration can be sustained through the use of polymeric materials.

Hydrogels are potentially useful materials as drug delivery systems. They are three-dimensional polymeric networks with high permeability to small molecules and have good mechanical properties.

Many synthetic polymers can be used in preparing hydrogels. Among them, poly(vinyl alcohol) (PVA) is widely employed to make hydrogels for biomedical applications, including localized drug delivery systems. They are prepared using a physical cross-linking method consisting of repeated freeze-thawing cycles of PVA aqueous solutions [1].

Hydrogels based on both pure PVA and blends of PVA with natural polymers such as collagen, hyaluronic acid, chitosan and dextran, [2, 3] have been previously evaluated as polymeric matrices for the release of water soluble drugs such as human growth hormone (GH) [4–7].

These hydrogels showed an ability to release the drug in a dose-dependent manner and the concentrations of GH released were within a physiological range and sufficient to have a local effect on cellular proliferation [4].

Water soluble drugs can be easily loaded into PVA-based hydrogels because these agents can be dissolved into the PVA aqueous solution used for hydrogel preparation.

The aim of the present work was the development of a method that also allows the use of such hydrophilic matrices for the release of lipophilic drugs having a very low water solubility that, because of their nature, cannot be directly dissolved into the initial PVA solution. For this purpose, biodegradable nanoparticles [8–10], loaded with lipophilic drugs, were produced and then entrapped into the PVA hydrogels. In particular nanoparticles

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containing dexamethasone were prepared. Dexamethasone is a highly potent synthetic glucocorticoid that has powerful anti-inflammatory activity but unwanted side effects during chronic systemic therapy. The particles were produced using a biodegradable copolymer of poly(lactic acid)-poly(glycolic acid) (PLGA), by a solvent evaporation procedure based on a single oil-in-water (O/W) emulsion, and then entrapped into the PVA hydrogels.

The effects of several processing parameters on particles properties were investigated.

Size, morphology and surfaces characteristics of the nanoparticles were evaluated using scanning electron microscopy (SEM). The *in vitro* release and the mechanism of drug release from free nanoparticles were investigated and compared with that from the nanoparticles entrapped into the hydrogels.

2. Materials and methods

2.1. Materials

Poly(DL-lactide-co-glycolide 50/50) of average molecular weight M_w 40–75 000, poly(vinyl alcohol) of M_w 86 000–146 000 and dexamethasone were supplied by Sigma Aldrich; poly(vinyl alcohol) of M_w 15 000 and poly(vinyl alcohol) of M_w 10 000 were supplied by Fluka Chemika; dichloromethane and Tween 80 were supplied by Carlo Erba Reagenti.

2.2. Preparation of the nanoparticles loaded with dexamethasone

PLGA nanoparticles were prepared using the following O/W emulsification process. The optimal preparation parameters were then chosen.

Two hundred and fifty milligrams of dexamethasone was dissolved in 14 ml acetone, then 750 mg of PLGA and 16 ml of dichloromethane were added to obtain the ''oil phase''. This solution was added dropwise to an aqueous 5% PVA solution which was 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, then Tween 80 was added and solvent evaporation was performed overnight, by gentle magnetic stirring, at room temperature.

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

2.3. Optimizing preparation parameters

In an attempt to elucidate the conditions for the production of particles with a uniform size distribution and with a medium diameter of 100–300 nm, several preparation parameters (PLGA concentration, organic solvent, stirring rate, stirring time, and PVA concentration) were considered.

Three different concentrations of PLGA (50/50) were evaluated and from these a 2.5% (w/v) concentration was chosen as the optimal one.

The selection of the organic solvent was made by preparing 2.5% PLGA solutions in different solvents such as: dichloromethane, acetone, chloroform, benzene and ethanol. A mixture of dichloromethane and acetone was selected as the optimal one.

A stirring rate of 23 500 rpm was selected after performing a series of O/W emulsions at different rates: 400, 1000, 2000, 10 000, 17 800, 23 500 and 33 000 rpm. Several stirring times (5, 10, 15 min) were taken into consideration and the time of 10 min was chosen as the optimal time.

With regard to PVA concentration, a 2.5% PLGA solution was emulsified in either 2.5, 5, and 10% PVA solutions and the 5% PVA solution was selected as the best.

2.4. Scanning electron microscopy (SEM)

The morphological characteristics of the nanoparticles were observed using a scanning electron microscope (Jeol T300). The samples were prepared on aluminum stubs and coated with gold prior to examination.

2.5. Preparation of PVA hydrogels loaded with PLGA nanoparticles

Four PVA (M_w 86 000–146 000) aqueous solutions, (2.5, 5, 7.5 and 10% w/v) were prepared in an autoclave for 1 h at 120 °C. Each solution was poured into five wells of a 12-well plate (2.5 ml/well).

Fifteen milligrams of PLGA nanoparticles loaded with dexamethasone was added to each well.

After nanoparticle addition, samples underwent eight freeze-thawing cycles to obtain the 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.

2.6. *In vitro* release of dexamethasone from the nanoparticles

The release of dexamethasone was evaluated from both free nanoparticles and nanoparticles entrapped into PVA hydrogels.

The release of dexamethasone from free nanoparticles was evaluated using a side by side diffusion chamber (Crown Glass, Somerville, NJ, USA).

This chamber consists of two identical glass cells separated by a Millipore membrane LPVP 0.1 μm thick. The volume of each cell is 1.5 cm^3 and the surface area for material exchange is 0.64 cm^2. A nanoparticle suspension in phosphate-buffered saline (PBS) was placed in one of the two cells (donor side) while pure PBS was placed in the other cell (acceptor side). The apparatus was maintained at 37 °C and was stirred at 70 rpm.

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

With regard to the release of dexamethasone from

nanoparticles entrapped into PVA hydrogels, these were each placed 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 to 37 °C. Elution fluids were assayed for dexamethasone spectrophotometrically at $\lambda = 238.5$ nm.

3. Results and discussion

It is well known that the properties of particulate systems are highly dependent upon the production method and conditions employed [11, 12]. Although the solvent evaporation process is conceptually simple, many variables can influence the final product.

The influence of PLGA concentration was investigated and it was observed that the particle size increased with increasing PLGA concentration, according to the results reported by Scholes *et al*. [12]. A PLGA concentration of 2.5% was chosen as the optimal concentration for subsequent studies.

Choosing the appropriate solvent for PLGA is a quite complicated process, as many competing factors have to be considered [13]. The water/solvent miscibility is the controlling factor regarding the particle shape and their drug content.

The use of highly water miscible solvents like acetone and methanol produced agglomerated particles instead of regular spheres. On the other hand, low water miscible solvents such as benzene and chloroform signified a low diffusion rate of the solvent in the aqueous phase. This phenomenon implied a great loss of the drug in the aqueous phase and thus the spheres produced had a very low content of loaded drug. In addition, it was observed [13] that the amount of drug loaded depended greatly on the rate of PLGA precipitation onto the external surface of the particles.

To satisfy all these requirements a blend of two solvents, dichloromethane and acetone (completely miscible in the ratios used in this work) was used to prepare the oil phase. Dichloromethane allows the formation of spherical drops during the emulsification process and at the same time it induces a rapid PLGA precipitation, while acetone represents one of the best solvents for dexamethasone.

It was observed that particle size decreased significantly with increasing stirring rate. A stirring rate of 23 500 rpm was found to be the optimum value to produce 100–300 nm particles.

Stirring rate also affected the particle size distribution. SEM images showed a more homogeneous particle size distribution when the stirring rate was above 1000 rpm. Increasing stirring time from 5 to 10 min decreased particle size. However, for a stirring time of more then 10 min, particle size started to increase again due to aggregation processes.

On the basis of these considerations, a stirring time of 10 min was chosen.

It was observed that it was not possible to produce nanoparticles in the absence of a specific surfactant agent that reduces the interfacial tension between the aqueous and the organic phase. PVA was chosen as a surfactant because it is one of the few stabilizers that avoids nanoparticle aggregation during the postpreparative steps and enhances the yield of dry nanoparticle product. In addition, it is a low cost commercial product with good biocompatibility properties.

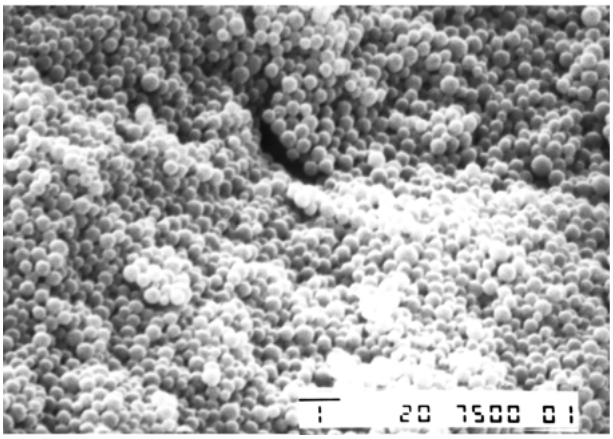

Figure 1 SEM image of free PLGA nanoparticles loaded with dexamethasone.

It was found that particle size also depended on PVA concentration [11]. Increasing the stabilizer concentration had a biphasic effect on particle size, first causing a decrease for concentrations of PVA between 1 and 8%, followed by an increase for concentrations up to 15%. Other works [11] reported that particle size could be decreased by lowering the PVA molecular weight. A PVA with a molecular weight of 15 000 and a concentration of 5% was chosen as the optimal polymer for preparing the aqueous phase. In order to further reduce the risk of nanoparticle aggregation, a second surfactant, Tween 80, was added to the PVA solution.

Using the above mentioned parameters, nanoparticles with an average diameter of 200–300 nm were obtained, as shown by the results of the SEM analysis (Fig. 1). PLGA nanoparticles entrapped in a PVA hydrogel are shown in Fig. 2.

Fig. 3 shows the release curve for dexamethasone from free nanoparticles. A burst phase is seen during the first 70–80 h, releasing about 50% of the loaded drug. This massive release can be related to the drug adsorbed on

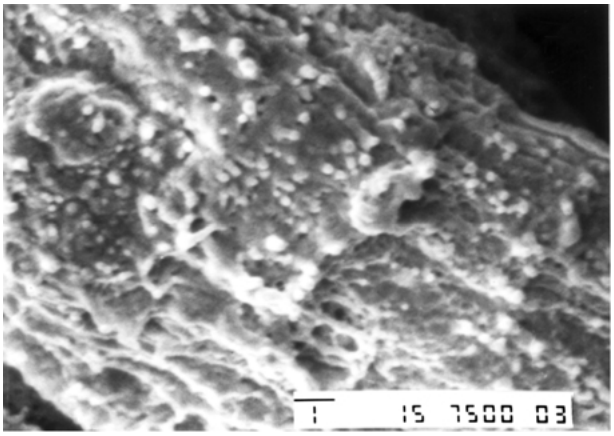

Figure 2 SEM image of PLGA nanoparticles loaded with dexamethasone, entrapped into a PVA (2.5%) hydrogel.

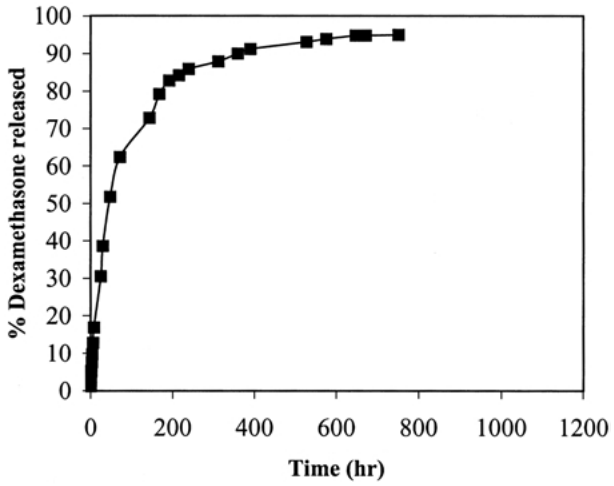

Figure 3 In vitro release of dexamethasone from free PLGA nanoparticles.

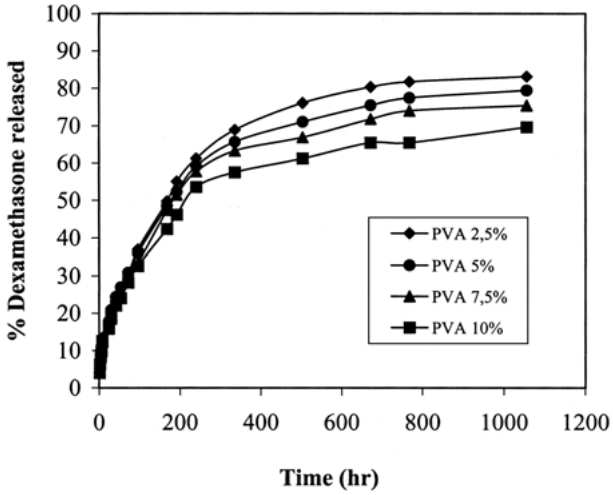

Figure 4 In vitro release of dexamethasone from PLGA nanoparticles entrapped into PVA hydrogels, prepared starting from PVA solutions with different concentrations (2.5, 5, 7.5, 10%).

the external surface of the particles. After this initial phase, a decrease in release rate can be observed, which is then followed by a new increase after about 200–220 h.

It could be that the external aqueous phase diffuses into the polymeric matrix, inducing it to swell and favoring the subsequent release of the drug entrapped inside the matrix itself.

Approximately 95% of the loaded dexamethasone was released within four weeks.

The release of dexamethasone from nanoparticles entrapped within hydrogels with different PVA concentrations showed the same trend (Fig. 4). It can be observed that all the curves have the same shape: the release rate is high at the beginning, then it decreases until a plateau is reached after about one month.

It is evident that, as the amount of nanoparticles added to the samples was constant, a correlation exists between the amount of drug released and PVA concentration in the hydrogels: the percentage of drug released, as a function of time, decreased with increasing PVA concentration.

In addition, comparing Fig. 3 with Fig. 4, it is evident that the entrapment of the nanoparticles into the PVA hydrogels causes a reduction in both the release rate and the total amount of dexamethasone released.

These results can be explained considering that the PVA matrix, which encloses nanoparticles, represents an additional resistance to drug diffusion that increases with increasing PVA concentration and hence hinders the release.

4. Conclusions

Sustained release has been observed from PVA hydrogels. However the low hydrophilicity of the drug entrapped represents one of the most relevant limitations of these system.

In this study, a new method was developed, which was capable of overcoming this problem. Nanoparticles loaded with a water insoluble drug, were entrapped into the PVA hydrogels in order to allow the use of these hydrophilic matrices for the release of lipophilic drugs. It was observed that the drug release is not significantly affected by the PVA matrix. It also seems that the PVA concentration used in preparing the hydrogels can be used as a tool for controlling the amount of drug released.

References

1. M. NAMBU, JP pat. 82–130543, 1982 L.
2. L. LAZZERI, N. BARBANI, M. G. CASCONE, D. LUPINACCI, P. GIUSTI and M. LAUS, *J. Mater. Sci. Mater. Med.* **5** (1994) 862.
3. R. SBARBATI DEL GUERRA, M. G. CASCONE, N. BARBANI and L. LAZZERI, *ibid.* **5** (1994) 613.
4. M. G. CASCONE, L. DI SILVIO, B. SIM and S. DOWNES, *ibid.* **5** (1994) 770.
5. M. G. CASCONE, B. SIM and S. DOWNES, *Biomaterials* **16**(7) (1995) 569.
6. M. G. CASCONE and S. MALTINTI, *J. Mater. Sci. Mater. Med.* **10**(5) (1999a) 301.
7. M. G. CASCONE, S. MALTINTI, N. BARBANI and L. LAUS *ibid.* **10**(7) (1999b) 431.
8. L. BRANNON-PEPPAS, *Int. J. Pharm.* **116** (1985) 1.
9. H. J. KRAUSE, A. SCHWARTZ and P. ROHDEWALD, *ibid.* **27** (1995) 145.
10. J. KREUTER, *Pharm. Acta. Helv.* **53**(2) (1978) 33.
11. R. JALIL and J. R. NIXON, *J. Microencapsulation* **7** (1990) 297.
12. P. D. SCHOLES, A. G. A. COOMBES, L. ILLUM, S. S. DAVIS, M. VERT and M. C. DAVIES, *J. Controlled Rel.* **25** (1993) 145.
13. R. BODMEIER and J. W. MC GINITY, *Int. J. Pharm.* **43** (1988) 179.

Received 21 April 2000
and accepted 27 February 2001