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Paclitaxel-loaded poly(lactic-co-glycolic acid) microspheres: preparation and *in vitro* evaluation

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Poly(lactic-co-glycolic acid) (PLGA) microspheres containing paclitaxel were prepared by an oil-in-water (o/w) emulsification solvent evaporation method. Box Benhken experimental design was used for studying the parameters that influence the microsphere characteristics and for calculating the optimal formulation. The resulting microspheres were characterized regarding their physicochemical properties, drug content and in vitro drug release. The stirring rate had the greatest influence on particle size and particle size distribution. PLGA and polyvinyl alcohol concentrations had an important influence on drug loading: drug loading increased linearly with PLGA concentration and decreased linearly with PVA concentration. The differential scanning calorimetry proved that there are interactions between paclitaxel and polymer. The release behavior of paclitaxel from the polymer matrix exhibited a biphasic pattern characterized by a slow initial release during the first 26 days (less than 10% paclitaxel), followed by a faster and uniform release (the accumulative amount of paclitaxel released in 92 days was between 17 and 44%).

Key words: Microspheres – Paclitaxel – Poly(lactic-co-glycolic acid) – Solvent evaporation method – Experimental design – Optimization – Drug release

Paclitaxel, which is one of the best antineoplastic drugs found in nature, has been praised by the National Cancer Institute (NCI) as the most significant advance in chemotherapy in the past 15-20 years. Paclitaxel was isolated in the early 1960s from the bark of the Pacific Yew tree (*Taxus brevifolia*). It was obtained in a pure form in 1969 and its structure was published in 1971. Its mechanism of action is unique and was discovered in 1979. It inhibits the growth and separation of cancer cells by blocking cells in the late G2-mitotic phase of the cell cycle. The microtubule formed in the presence of paclitaxel is very stable and dysfunctional, thereby causing the death of the cell by disrupting the normal tubule dynamics required for cell division and for the vital interphase process. Paclitaxel has neoplastic activity particularly against primary epithelial ovarian carcinoma, breast cancer, colon, head, non-small cell lung cancer and AIDS related Kaposi's sarcoma [1]. In 1992 paclitaxel was approved by the FDA as second line treatment of ovarian carcinoma and in 1999 as first line treatment in various difficult cancers [2].

A major problem of paclitaxel is its low water solubility (less than 1 μg/mL) [3]. The commercially available formulation uses a 1:1 cosolvent mixture of ethanol and Cremophor EL (polyethoxylated castor oil). However, Cremophor EL is biologically and pharmaceutically active, causing hypersensitivity reactions, nephrotoxicity and neurotoxicity [2, 4].

In an attempt to overcome the low water solubility of paclitaxel as well as the undesirable toxicity of Cremophor EL, numerous alternative formulations have been investigated including emulsions, liposomes and particulate delivery systems [2, 5-7].

Polymeric delivery systems have been used over the years in the delivery of drugs. The delivery of chemotherapeutic agents using polymeric microspheres has become one of the most popular areas of research because of the possibilities of reducing toxicity, enhancing controlled release activity and also localizing the drug delivery [8, 9].

PLGA is a biocompatible and biodegradable polymer that has developed tremendous interest involving the development of microparticule formulations [10]. PLGA microspheres have been reported as carriers for site-specific delivery of various drugs [11].

It has long been known that microspheres properties may be controlled by the processing conditions employed in their preparation. Conditions such as stir speed and solvent ratio impact morphology and drug release behavior because they affect the rate of solvent efflux from forming microspheres [12]. Release profiles of drugs from microspheres are also controlled by PLGA properties such as molecular weight, lactide/glycolide ratio and terminal functional groups [13-15].

The main objectives of this study were: to prepare paclitaxel-loaded PLGA microspheres by an o/w emulsification evaporation method, to evaluate the influence of certain formulation and process parameters on microsphere properties and to optimize these parameters using an experimental design, to characterize the microspheres regarding their physicochemical properties, drug content and *in vitro* drug release.

I. MATERIALS AND METHODS

1. Materials

Paclitaxel was a gift from Sindan Company (Romania). PLGA copolymer (50:50; Resomer RG 502H; MW 13100) was purchased from Boehringer Ingelheim (Germany), polyvinyl alcohol 49 000 (PVA) from Fluka AG (Switzerland), dichloromethane (DCM) from Merck (Germany). All the other reagents are of analytical grade and were used without further purification.

2. Microsphere preparation

PLGA microspheres were prepared using an oil-in-water (o/w) emulsification solvent evaporation method [15,16]. Briefly, PLGA and paclitaxel were dissolved in 10 mL DCM. The organic phase was added to 100 mL of an aqueous solution of PVA and stirred at 25 ± 2°C with a mechanical stirrer equipped with a tree-bladed propeller (Heidolph, Germany), until DCM evaporation. Upon contact with the outer aqueous phase the solvent diffuses into the water. Thus the DCM concentration in the inner organic phase decreased with time and the polymer concentration increased. At a certain time point, the PLGA precipitated and encapsulated the drug; the microspheres were formed.

The microspheres were separated by centrifugation, washed with distilled water, dried at room temperature and preserved in a dessicator until the time of evaluation.

3. Experimental design

We used a Box Benhken experimental design, with three variables (independent variables) and three levels, for studying the following parameters that can influence the microsphere properties: stirring rate (200, 900, 1,600 rpm), PVA concentration in the aqueous phase (0.5, 1.5, 2.5% w/v) and APLG concentration in the organic phase (1, 3 and 5% w/v) (*Table I*). The dependent variables were: particle size (mean diameter), particle size distribution (polydispersity index, PI) and drug loading (*Table II*). The initial loading level of paclitaxel in all formulations was 2% (w/w). The matrix of experimental design is presented in *Table III*.

Experimental design, coefficient calculation, statistical parameters calculation and evaluation of the quality of the fit were performed with Modde 6 software (Umetrics, Umea, Sweden).

4. Microsphere physicochemical evaluation

4.1. Particle size and particle size distribution

Particle size distribution and mean diameters of the prepared microspheres were measured by the image analysis technique using an optical microscope (Optika Microscopes) equipped with camera and computer software for image analysis (each measurement included at least 100 particles).

4.2. Morphology

The shape and the surface morphology of the microspheres were examined with the scanning electron microscopy (SEM) (Jeol 5600 LV, Japan). The results of size and size distribution were further confirmed by SEM. Samples were not metal coated and the examinations were performed in advanced vacuum using electron voltage between 25 and 30kV.

4.3. Thermal analysis

The thermal properties of microspheres were observed by differential scanning calorimetry (DSC). Samples were analyzed with a Mettler Toledo model 822e/700/984 DSC (Suisse). The heat flow rate was recorded from 2 to 300°C at a rate of 10°C/min.

The glass transition temperature (Tg) was evaluated. The samples were heated at a rate of 10°C/min from 0 to 85°C, quench cooled to -20°C (to eliminate any sample history), and then heated again to 85°C at 10°C/min. The results were analyzed using Mettler STARe software. Tg was reported as the onset of the corresponding glass transition.

4.4. Drug content

The measurement of the paclitaxel content in the microspheres was carried out in triplicate using HPLC according to the method given in reference [1] with certain modifications. Five milligrams of paclitaxel-loaded microspheres were dissolved in 1.5 mL DCM. A solution of internal standard was added (carbamazepine). Then a solution acetonitrile-water (50/50 v/v) was added and mixed. A nitrogen stream was introduced to evaporate DCM at room temperature until a clear solution was obtained. The solution was put into a vial for HPLC detection. For HPLC analysis an Agilent 1100 Series chromatograph (Agilent USA), equipped with a Zorbax SB-C18 column (50 mm × 2.1 mm i.d., pore size 3.5 μm Agilent, USA) was used. The column was protected with a Zorbax C18 pre-column (12.5 mm × 4.6 mm i.d., pore size 5 μm Agilent, USA). The mobile phase, a mixture of phosphoric acid 0.1% and acetonitrile, was delivered at a flow rate of 1 mL/min. A 20-μL aliquot of the samples was injected. Paclitaxel was quantified by UV detection at λ = 227 nm and the internal standard (carbamazepine) at λ = 285 nm. The calibration curve was linear over the range of 1.8 and 36 μg/mL (standard concentration of paclitaxel) with a correlation coefficient of $R^2 = 0.999943$.

5. *In vitro* release studies

The *in vitro* release of paclitaxel-loaded microspheres was measured in phosphate buffer pH 7.4, in triplicate, at a temperature of 37°C. Ten milligrams of paclitaxel-loaded microspheres were suspended in 10 mL of buffer containing a surfactant agent (Tween 80 or PVA) (concentrations w/v) in screw capped tubes which were placed in a horizontal shaker bath (80 rpm, 37°C). The microsphere characteristics and the nature of the release mediums used in this study are presented in *Table IV*.

At given time intervals, the tubes were vortexed for 1 min and then centrifuged at 4,000 to 10,000 rpm for 10 min. The supernatants were taken for analysis. The precipitated microsphere pellets were resuspended in 10 mL of fresh buffer. The paclitaxel concentration in the release medium was determined by a HPLC (Agilent 1100 Series, Agilent USA) assay, the samples being directly injected into the chromatographic system. For this analysis, a Zorbax SB-C18 column (50 mm × 2.1 mm i.d., pore size 3.5 μm Agilent, USA) was used. The column was protected with a Zorbax C18 pre-column (12.5 mm × 4.6 mm i.d., pore size 5 μm Agilent, USA). The mobile

Table I - Levels of independent variables.

Process and formulation variables	Symbols	Levels		
		-1	0	1
Stirring rate (rpm)	X_1	200	900	1600
PLGA concentration (%)	X_2	1	3	5
PVA concentration (%)	X_3	0.5	1.5	2.5

Table II - Dependent variables (responses).

Responses	Symbols
Particle size (μm)	Y_1
Polydispersity index (%)	Y_2
Drug loading (%)	Y_3

Table III - Matrix of experimental design.

Exp. No.	X_1 Stirring rate (rpm)	X_2 PLGA conc. (%)	X_3 PVA conc. (%)
1	200	1	1.5
2	1600	1	1.5
3	200	5	1.5
4	1600	5	1.5
5	200	3	0.5
6	1600	3	0.5
7	200	3	2.5
8	1600	3	2.5
9	900	1	0.5
10	900	5	0.5
11	900	1	2.5
12	900	5	2.5
13	900	3	1.5
14	900	3	1.5

Table IV - Microsphere characteristics and release mediums in the release study.

Sample	Mean diameter (μm)	Paclitaxel loading (%)	Release medium
S1	3.49	1.45	Tween 80 0.1 %
S2	20.61	1.93	Tween 80 0.1 %
S3	127.70	1.86	Tween 80 0.1 %
S4	4.75	1.95	Tween 80 0.1 %
S5	5.10	9.58	Tween 80 0.1 %
S6	5.33	26.59	Tween 80 0.1 %
S7	3.49	1.45	Tween 80 0.01 %
S8	8.30	1.90	PVA 0.5 %
S9	8.30	1.90	PVA 1.5 %
S10	8.30	1.90	PVA 2.5 %