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# The influence of technological parameters on the physicochemical properties of blank PLGA nanoparticles

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In this work, PLGA nanoparticles were prepared by an emulsification-diffusion technique. The main objective was to optimize the preparation of formulations by evaluating the influence of the technological parameters on the physicochemical properties of PLGA nanoparticles. The effects of variations in polymer and emulsifier concentrations, and homogenization duration, rate and type on the particle size distribution, surface charge and morphology of nanoparticles were assessed. The smallest nanoparticles ($177.53 \pm 2.78$ nm) were obtained with a 2% PLGA (w/v) concentration in the organic phase and 3% PVA (w/v) in the aqueous phase and were prepared by an emulsification-diffusion method via ultrasonic homogenization at a power of 80 W applied for 30 s. It was observed that nanoparticles prepared by Ultra Turrax® were more spherical but larger. In addition, increasing the PVA concentration in the aqueous phase, increasing the PLGA concentration in the organic phase and increasing the homogenization rate decreased the zeta potential values of PLGA nanoparticles.

## 1. Introduction

Recent studies indicate considerable interest in the nanoparticle field. Theoretical understanding of nanoparticle structure and related characteristics is very important to achieve pharmaceutical properties and functionalities for new applications not available with conventional materials (Venugopal et al. 2009). Nanoparticle structure, size distribution, polydispersity, composition, stability and agglomeration present a number of challenging problems for drug delivery systems (Torchilin 2000).

However, technological parameters such as polymer concentration, polymer composition, and homogenization rate, duration and method have significant influences on the characteristics of nanoparticles. These parameters lead to variations in nanoparticle size and zeta potential which are very important parameters for their trans-membranal passage and tissue targeting properties (Gaumet et al. 2007; Quintanar-Guerrero et al. 1998).

In addition, the physicochemical properties of nanoparticles are very important in brain drug targeting (Kilic et al. 2005; Pinarbasli et al. 2009). Due to the particle size and surface charge of nanoparticles the amount of active substance transferred may vary (Aktas et al. 2005 a,b; Cetin et al. 2007). In this study we evaluated the technological parameters influencing PLGA nanoparticles in order to design more effective formulations for brain drug targeting (Cetin et al. 2008; Karatas et al. 2009; Yemisci et al. 2006). PLGA, a copolymer which is a United States Food and Drug Administration (FDA) approved polymer, is particularly suitable for drug delivery applications. PLGA has been studied for many years as a suitable drug delivery material, mainly due to its chemical biocompatibility, total biodegradability, and non-toxic degradation products (Anderson and Shive 1997).

A common method for the sustained release of drugs is the production of a PLGA microsphere formulation by the oil-in-water emulsion and solvent evaporation technique, where the drug is evenly distributed throughout a soluble PLGA matrix and then emulsified. Once the organic solvent evaporates off, a suspension of solid microparticles is left. The active substance can be co-dissolved in the PLGA if hydrophobic, or prepared as a double emulsion or suspension if not (Kilic et al. 2005). The hydrophilicity of this polymer is defined by the lactide:glycolide ratio and can be used to alter the release rate in a microparticulate formulation (Janoria and Mitra 2007). In this study, Resomer® RG 503 H is used, being rich in free carboxylic groups (intrinsic viscosity in chloroform: 0.4 dL/g) and having a polymer composition with molar ratios (D,L-lactide:glycolide) of 48:52 to 52:48. The polymer molecular weight also influences the drug release rate. The rate of degradation is inversely proportional to the proportion of lactide monomer in the polymer strands, and the rate of diffusion of drugs through the polymer matrix is inversely proportional to the molecular weight of the PLGA polymer (Kim et al. 2005).

The main goal of this work is to optimize PLGA nanoparticle preparation so that the efficiency of PLGA nanoparticle formation is high (Lamprecht et al. 1999). Here we report the particle size distribution, morphology and surface charge of PLGA nanoparticles in order to show the effect of technological parameters on their physicochemical properties.

## 2. Investigations, results and discussion

In order to determine the influences of the technological parameters on the final nanoparticles, the physicochemical properties

**Table 1: Effect of PLGA concentration and ultrasonic homogenization power on particle size of nanoparticles (nm) (mean ± standard error)**

| Ultrasonic homogenization power (W) | PLGA concentration (%, w/v) | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| 40 | 184.98 ± 3.79 | 185.98 ± 3.60 | 186.30 ± 3.00 |
| 80 | 177.53 ± 2.78 | 180.01 ± 1.86 | 182.03 ± 2.92 |

PVA concentration in aqueous phase 3% (w/v)

**Table 3: Effect of PLGA concentration and Ultra Turrax® homogenization duration on particle size of nanoparticles (nm) (mean ± standard error)**

| Homogenization duration (min) | PLGA concentration (%, w/v) | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| 2 | 265.55 ± 4.02 | 304.01 ± 1.27 | 325.27 ± 8.24 |
| 4 | 263.72 ± 4.94 | 300.75 ± 8.87 | 317.68 ± 2.74 |

Ultra Turrax® homogenization rate 11000 rpm and PVA concentration in aqueous phase 3% (w/v)

of the nanoparticles were evaluated in terms of their particle size distribution, morphology and surface charge.

### *2.1. Particle size distribution*

With respect to particle size analysis, as demonstrated in Tables 1–3, all the systems prepared were on a nano scale (mean diameter <400 nm), exhibiting various size distributions (polydispersity index <0.80) (Fig. 1). It was observed that increasing PVA concentration in the aqueous phase resulted in smaller particle sizes and narrower particle size distributions ($p < 0.05$), because PVA in the water phase enhances the stabilization of water droplets against coalescence and results in a more uniform particle size distribution as the viscosity of the aqueous phase increases (Capan et al. 1999; Yang et al. 2001; Zambaux et al. 1998). Also higher homogenization rates and longer durations with the Ultra Turrax® decreased the mean diameter of the nanoparticles ($p < 0.05$). Using an ultrasonic homogenizator instead of the Ultra Turrax® yielded smaller particles, and with a rise of ultrasonic power from 40 W to 80 W smaller nanoparticles were also obtained ($p < 0.05$) (Abismail et al. 1999). No statistically significant difference was observed when the PLGA concentration in the organic phase was increased from 2% (w/v) to 4% (w/v) when using ultrasonic homogenization, but a statistically significant difference was observed when the Ultra Turrax® was used. Further, increasing the polymer concentration in the organic phase with increasing duration of homogenization did not affect the particle size of the nanoparticles. The smallest nanoparticles (177.53 ± 2.78 nm) were obtained with a 2% (w/v) PLGA concentration in the organic phase and 3% (w/v) PVA in the aqueous phase, and were prepared by the emulsification-diffusion method using an ultrasonic homogenizator at a power of 80 W applied for 30 s.

### *2.2. Morphology*

TEM micrographs of nanoparticles prepared using 2% (w/v) PLGA in organic phase and 3% (w/v) PVA in aqueous phase homogenized by both Ultra Turrax® (at 11000 rpm for 4 min.) and ultrasonic homogenizator (at a power of 40 W applied for 30 s) are presented in Fig. 2. Nanoparticles which were prepared by ultrasonication exhibit smaller but nonspherical shaped particles probably because of the high power applied. It is observed that nanoparticles prepared by Ultra Turrax® were better separated and were spherical with larger sizes.

### *2.3. Surface charge*

All nanoparticle formulations exhibited a net negative charge with zeta potential values ranging from $-0.59 \pm 0.52$ to $-18.81 \pm 0.23$ mV. It was observed that increasing PVA concentration in the aqueous phase, increasing PLGA concentration in the organic phase and increasing homogenization rate decreased zeta potential values, as well as affecting the particle size analyses ($p < 0.05$). Homogenization duration was the only variable not to affect the zeta potential of the nanoparticles (Tables 4–6) (Fig. 3). Surface charge is recognized to affect the stability and influence interactions with the biological milieu after *in vivo* administration, as well as affecting the release rate of a loaded substance and interactions with cells of colloidal systems. It is known that both PLGA and PVA have negative zeta potential values and increasing concentrations of both polymers can cause a decrease in the zeta potential of nanoparticles (Kumar et al. 2004; Vandervoort and Ludwig 2002).

The results obtained show that the technological parameters selected in this study permitted reproducible formation of nanometric, almost spherical and homogenous PLGA nanoparticles. Moreover, it is shown that increasing homogenization rate and duration; increasing ultrasonic homogenization power and increasing PVA concentration in the aqueous phase decrease the mean diameter of PLGA nanoparticles. Based on these results, it can be concluded that the technological experimental conditions developed in this study can be used to design specific drug delivery systems in which the physicochemical properties of the nanoparticles are important and for scale-up studies of nanoparticle formulations.

**Table 2: Effect of PVA concentration, Ultra Turrax® homogenization rate and duration on particle size of nanoparticles (nm) (mean ± standard error)**

| Homogenization duration (min) | PVA concentration (%, w/v) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 3 | | 5 | |
| | Homogenization rate (rpm) | | | | | |
| | 11000 | 16000 | 11000 | 16000 | 11000 | 16000 |
| 2 | 329.00 ± 5.81 | 241.32 ± 2.43 | 265.55 ± 4.02 | 238.68 ± 7.28 | 256.77 ± 2.88 | 217.43 ± 2.20 |
| 4 | 282.21 ± 8.51 | 227.64 ± 4.59 | 263.72 ± 4.94 | 209.78 ± 3.31 | 250.62 ± 1.42 | 209.35 ± 1.73 |

PLGA concentration in organic phase 2% (w/v)

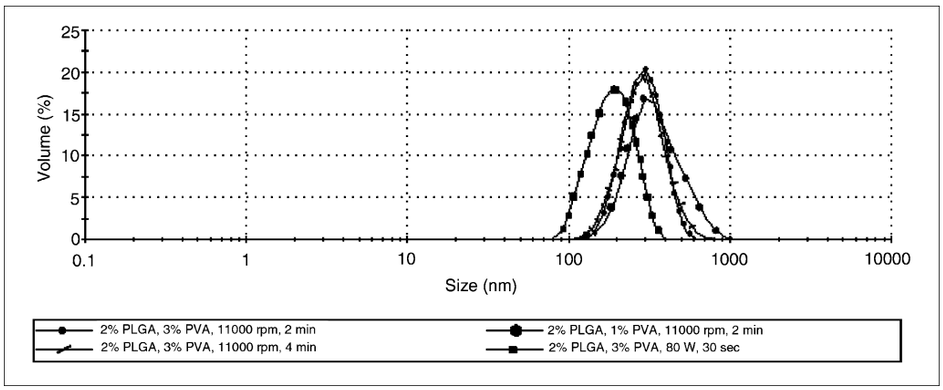


Fig. 1: Effects of PVA concentration, homogenization type and duration on particle size distribution of PLGA nanoparticles

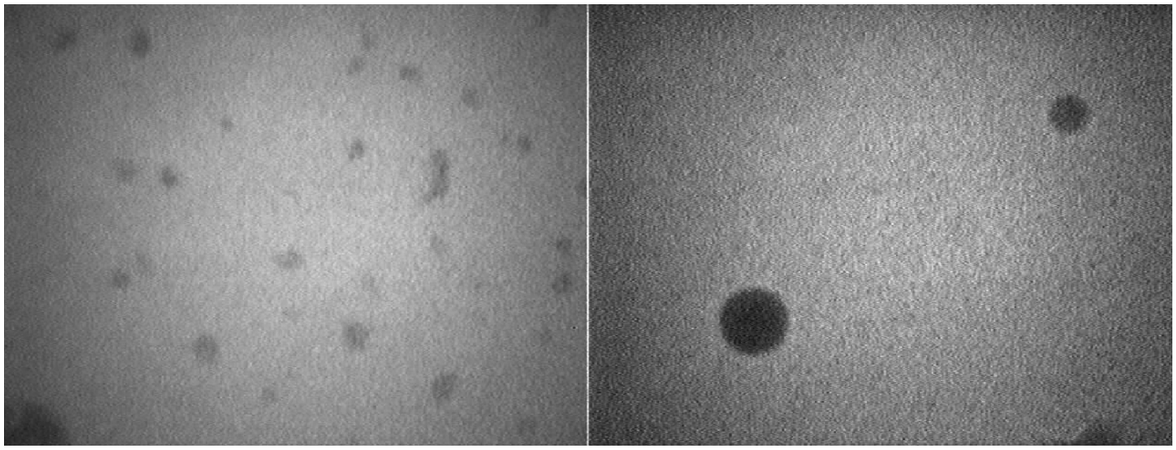

Fig. 2: Transmission electron micrographs of nanoparticles prepared using (*a*) ultrasonic homogenization (40 W for 30 s) with 2% (*W/V*) PLGA concentration in organic phase and 3% (w/v) PVA concentration in aqueous phase (x100000 magnification); (*b*) Ultra Turrax® (11000 rpm for 4 min) with 2% (w/v) PLGA concentration in organic phase and 3% (w/v) PVA concentration in aqueous phase (x16700 magnification)

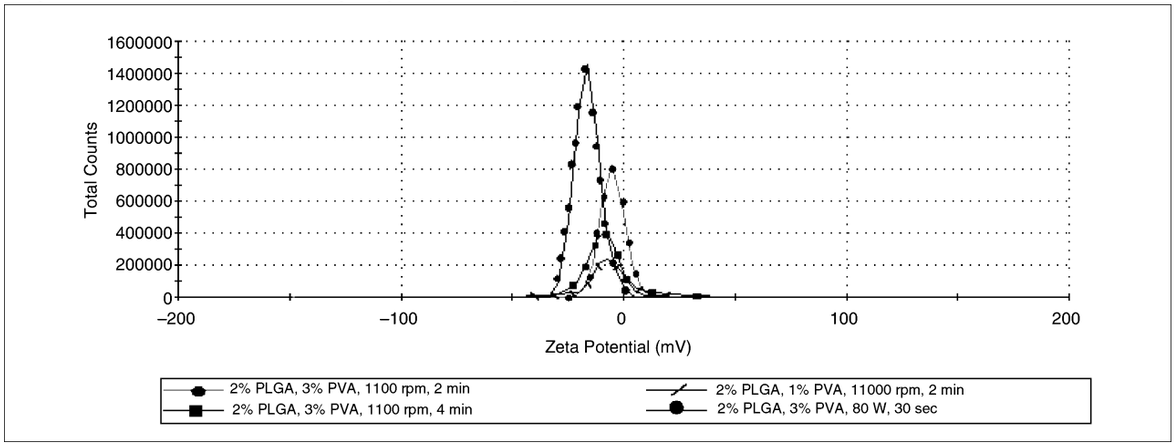


Fig. 3: Effects of PVA concentration, homogenization type and duration on zeta potential values of PLGA nanoparticles

**Table 4: Effect of PVA concentration, Ultra Turrax® homogenization rate and duration on zeta potential of nanoparticles (mV) (mean ± standard error)**

| Homogenization duration (min) | PVA concentration (%, w/v) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 3 | | 5 | |
| | Homogenization rate (rpm) | | | | | |
| | 11000 | 16000 | 11000 | 16000 | 11000 | 16000 |
| 2 | −2.09 ± 1.52 | −7.59 ± 0.35 | −5.02 ± 0.82 | −16.25 ± 2.15 | −10.93 ± 1.19 | −18.81 ± 0.23 |
| 4 | −0.59 ± 0.52 | −6.95 ± 0.47 | −5.87 ± 1.60 | −14.86 ± 0.54 | −11.29 ± 0.38 | −17.64 ± 0.20 |

PLGA concentration in organic phase 2% (w/v)

**Table 5: Effect of PLGA concentration and ultrasonic homogenization power on zeta potential of nanoparticles (mV) (mean ± standard error)**

| Ultrasonic homogenization power (W) | PLGA concentration (%, w/v) | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| 40 | −7.15 ± 1.87 | −10.53 ± 3.37 | −12.13 ± 0.35 |
| 80 | −6.05 ± 1.28 | −10.45 ± 0.51 | −11.70 ± 0.50 |

PVA concentration in aqueous phase 3% (w/v)

**Table 6: Effect of PLGA concentration and Ultra Turrax® homogenization duration on zeta potential of nanoparticles (mV) (mean ± standard error)**

| Homogenization duration (min) | PLGA concentration (%, w/v) | | |
|---|---|---|---|
| | 2 | 3 | 4 |
| 2 | −5.02 ± 0.82 | −10.90 ± 0.76 | −13.70 ± 3.09 |
| 4 | −5.87 ± 1.60 | −10.40 ± 1.00 | −14.30 ± 2.12 |

Ultra Turrax® homogenization rate is 11000 rpm and PVA concentration in aqueous phase 3% (w/v)

## 3. Experimental

### *3.1. Materials*

PLGA (50:50; Resomer® RG 503 H, $M_W$: 28000 Da) was purchased from Boehringer Ingelheim Pharma GmbH (Ingelheim, Germany). Polyvinyl alcohol (PVA) ($M_W$: 30000–70000 Da) was purchased from Sigma-Aldrich Co. (St. Louis, USA). Ethyl acetate was purchased from Merck KGaA (Darmstadt, Germany). Deionized water was obtained by a Millipore Milli-Q® System (Bedford, USA).

### *3.2. Preparation of blank PLGA nanoparticles*

Nanoparticles were prepared by the emulsification-diffusion method (Kilic et al. 2005). We used PVA, since it is one of the most commonly used polymer surfactants to stabilize the dispersed phase. Briefly, 10 mL of PLGA solution in ethyl acetate was added into 20 mL of aqueous PVA solution. Then, the emulsion was homogenized with an IKA® T25 Basic Ultra Turrax® (Staufen, Germany) at 11000 rpm for 2 min, and under magnetic stirring conditions 20 mL of deionized water was added into the dispersion in 2 min. to achieve diffusion of the organic phase into the aqueous phase. The organic phase was then evaporated at room temperature with magnetic stirring for 24 h. The nanosuspension obtained was centrifuged at 13000 rpm for 20 min to collect nanoparticles. To remove the residual PVA adsorbed on the nanoparticles, the centrifugate was washed three times with deionized water, since it is well known that PVA remains on the surface of particles where it is very difficult to remove and potentially carcinogenic (Carrio et al. 1995).

In an attempt to investigate the influence of technological parameters on the physicochemical properties of PLGA nanoparticles, different experimental conditions were evaluated. Two distinct methods for the homogenization of emulsion were tested: (*i*) homogenization by Ultra Turrax® (Ding and Shah 2009) and (*ii*) homogenization by a Bandelin Electronic Sonoplus HD 2200 ultrasonic homogenizator (Berlin, Germany) (Lee et al. 2008). When using the Ultra Turrax®, different homogenization rates (11000 and 16000 rpm) and durations (2 and 4 min) were assessed. Different conditions (40 and 80 W of ultrasonic power) of ultrasonic homogenization were also evaluated.

In addition to the experimental conditions, the effects of formulation variables such as PVA concentration in the aqueous phase (1%, 3% and 5%, w/v) and PLGA concentration in the organic phase (2%, 3%, and 4%, w/v) were also investigated.

### *3.3. Physicochemical characterization*

#### *3.3.1. Particle size distribution*

Mean particle diameter and polydispersity index was determined by dynamic light scattering using a Malvern Instruments Zetasizer Nano Series (Malvern, UK). For each sample, the mean diameter of six determinations was calculated. For a monodisperse system, the polydispersity index should be between 0.03 and 0.06 (Zambaux et al. 1998).

#### *3.3.2. Morphology*

The morphology of the nanoparticles was screened using a Sony LEO 906E transmission electron microscope (TEM) (Tokyo, Japan). A drop of the nanoparticle suspension was placed on copper electron microscopy grids and stained with 2% (w/v) uranyl acetate. After 24 h the dried samples were examined.

#### *3.3.3. Surface charge*

Nanoparticles were also characterized with respect to zeta potential using a Malvern Instruments Zetasizer Nano Series (Malvern, UK). Samples of prepared suspensions were diluted in deionized water and placed in disposable measurement cells and then measured.

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