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Brinzolamide Loaded-Polymeric Nanoparticles

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ABSTRACT

The objective of the present study was to investigate the ability to formulate brinzolamide in the form of polylactic-co-glycolic acid (PLGA) nanoparticles. In this study brinzolamide-loaded nanoparticles were formulated according to the emulsification/solvent evaporation technique using the biodegradable PLGA. The effect of surfactant type and its percentage in the preparation were investigated. The investigated PLGA polymer with lactide: glycolide monomers' ratio of 75:25 was able to develop PLGA vesicular system using the investigated surfactants. Brinzolamide-loaded nanoparticles prepared using PLGA with Pluronic acid F68 in the aqueous phase and 1 % Brij 97 in the organic phase showed the smallest particle size value (441.80 ± 72.97 nm). Brinzolamide-loaded nanoparticles prepared using PLGA with Pluronic acid F68 in aqueous phase and 2 % polysorbate 80 in organic phase had the largest encapsulation efficiency and drug loading values (47.86 ± 0.97 % and 38.76 %, respectively).

Key words: Brinzolamide, polylactic-co-glycolic acid (PLGA), nanoparticles

Introduction

Brinzolamide is a selective carbonic anhaydrase II inhibitor which is commercially available as a 1% ophthalmic suspension, Azopt® (Alcon Laboratories, Inc, Ft. Worth, Texas, USA), to treat glaucoma by reducing intraocular pressure (IOP) (Iester, 2008). Blurred vision (3–8%), ocular discomfort (1.8–5.9%) and eye pain (0.7–4.0%) are the most common ocular adverse effects upon using brinzolamide eye drops. Other ocular adverse effects were found at a prevalence of less than 3% included hyperemia, pruritus, tearing, discharge, blepharitis, keratitis, foreign body sensation, dry eye, conjunctiva inflammation and lid margin crusting (Iester, 2008).

Antiglaucoma agents may be administered topically or systemically for the treatment of increased IOP. Traditional dosage forms for topical application of drugs have remained the most ideal method because they are easily administered and less expense. Anatomical and physiological barriers prevent drugs from reaching posterior segment of eye mainly at choroid and retina. A large percent of drug, more than 95 %, is lost by lacrimation, tear dilution, nasolacrimal drainage and tear turnover following topical administration resulting in very low ocular bioavailability. In order to maintain minimum effective concentrations in aqueous humor the agents need to be frequently dosed resulting in poor patient compliance. Repeated application of topical medications for patients with chronic conditions such as glaucoma may lead to significant accumulation of preservative in ocular tissue and increased risk of ocular tissue damage (Lee and Robinson, 1986 ; Hughes *et al.*, 2005).

Alternative dosage forms for topical administration of ocular drugs are recommended to overcome lack of adequate bioavailability and failure to deliver therapeutic amounts of drugs into the eye as well as increase patient compliance.

One of the colloidal systems that have been most widely studied over the past few decades for improving drug targeting of tissues and organs therefore increasing drug bioavailability inside biological membranes is polymeric nanoparticles (Nair and Laurencin, 2007). Nanoparticulate systems are able to encapsulate, improve tolerance, penetration efficiency and increase corneal uptake of the drug (Lallemand *et al.,* 2003). One of the most commonly used biodegradable polymers is PLGA (Kreuter, 1994). PLGA is approved by the FDA for use in drug delivery application as it is upon hydrolysis leads to formation of lactic and glycolic acids which are two endogenous metabolite monomers (Jain, 2000) and proved to have ocular tolerance and biocompatibility (Gupta *et al.,* 2010 ; Gupta *et al.,* 2011).

In this study, brinzolamide loaded nanoparticles (NPs) were formulated according to the emulsification/solvent evaporation technique using the biodegradable polymer PLGA. The influence of

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surfactant type and its ratio was investigated. The formulated nanoparticles were investigated in terms of mean particle size, encapsulation efficiency and drug loading.

Materials and Methods

Brinzolamide was purchased by Shijiazhuang Apopharm, China. Poly (lactic-co-glycolic acid) with ester terminal copolymer of DL-lactide and glycolide in a 75/25 molar ratio was purchased from Lactel polymers Co., USA. Pluronic acid F68 (F68) was obtained from MP Biomedicales, LLC, France. Polysorbate 80 (Tween 80) was purchased from El Nasr Pharmaceutical Chemicals Co., Egypt. Brij 97 was purchased from Sigma–Aldrich, Germany. Dicholoromethane and acetonitrile with HPLC grade were obtained from Sd fine-Chem. Limited, Mumbai, India. Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from SISCO Research Laboratories Pvt. Ltd., Mumbai, India.

Preparation of brinzolamide PLGA nanoparticles

PLGA nanoparticle formulations of brinzolamide were prepared adopting the emulsification/solvent evaporation technique (Nagavarma *et al.*, 2012). A specific weight of PLGA (100 mg) and pluronic acid F68 with a constant weight of the drug (20 mg) were dissolved in 50 ml dicholoromethane by the use of a bath sonicator (Ultrasonic bath sonicator, Model SH 150-41, MTI Corporation, USA) for 10 min. The organic phase was then added drop wise into 200 ml of phosphate buffer (pH 7.4) (Digital pH-meter, JENWAY 350, UK) as the aqueous phase which contain polysorbate 80 or Brij 97 in concentrations of 1 or 2 % (w/v). The addition of organic phase to the aqueous phase was done with continues stirring (Hot plate magnetic stirrer, MSH-30D, WiseStir, Korea) and then the mixture was left under the stirring condition for 15 min. The resulting solution was then transferred into a rotary evaporator to evaporate the organic solvent under reduced pressure using rotavapor (Heidolph VV 2000, Germany).

Characterization of brinzolamide PLGA nanoparticles

Determination of PLGA nanoparticles size

Size of brinzolamide nanoparticles were determined by the laser scattering method using a ZetaSizer Nano ZS (Malvern Instruments, UK). Three samples for each formula were used for nanoparticle size determination.

Determination of brinzolamide entrapment efficiency

The brinzolamide loaded nanoparticles were separated from the unentrapped brinzolamide by centrifugation of the prepared nanoparticles suspensions at 30000 rpm and at – 5°C (Sigma 3–30K, Spincontrol Comfort, Germany). The nanoparticles precipitates were then mixed with acetonitrile and sonicated for 10 min to obtain clear solutions. Concentrations of brinzolamide in the acetonitrile solutions were determined spectrophotometrically (Shimadzu UV spectrophotometer (2401/PC), Japan) at 254 nm.

Drug entrapment efficiency was determined according to the following equation:

$$\%\,E = \frac{ED}{TD}\ X100$$

where, % E is the percentage of drug entrapped, ED is the amount of entrapped drug and TD is the amount of total drug.

Determination of brinzolamide loaded in PLGA nanoparticle

Fixed amount of the nanoparticle suspension was dried using lyophilizer (Christ Freeze dryer ALPHA 2-4 LD plus, Germany) then weighted. Each experiment was performed in triplicate.

The drug loading of brinzolamide was calculated as the actual amount of brinzolamide incorporated into nanoparticles *versus* the total amount of the freeze dried brinzolamide PLGA nanoparticles.

Drug loading was determined according to the following equation:

$$\%\,DL = \frac{DW}{TW}X100$$

where, % DL is the percentage of drug loaded, DW is the brinzolamide weight in sample and TD is the total weight of sample.

Results and Disscussion

Size of brinzolamide polymeric nanoparticles

Figure 1 shows the values of mean diameter for brinzolamide particles prepared using F68 in the aqueous phase with/without using polysorbate 80 or Brij 97 in the organic phase (F1 – F10). Results show that increasing F68 concentration from 1 to 2 % (F1 & F4, respectively) led to a significant increase in the mean diameter of NPs ($p<0.05$). It is obvious that adding 1 or 2% polysorbate 80 to the organic phase of the preparations containing 1 % F68 (F2 & F3, respectively) led to a significant decrease in the mean diameter of PLGA NPs ($p<0.05$) compared to that of F1. Data reveal also that adding 1 % polysorbate 80 to the organic phase of the preparation containing 2 % F68 (F5) led to a significant decrease in the mean diameter of the PLGA NPs compared to that of F4 ($p<0.05$), while further increasing polysorbate 80 concentration to 2 % (F6) resulted in a non significant change in the NP mean diameter ($p>0.05$) compared to that of F4. It is also obvious that adding 1 % Brij 97 to the organic phase of the preparation containing 1 % F68 (F7) led to a significant decrease in the mean diameter of NPs compared to that of F1 ($p<0.05$), while further increasing Brij 97 concentration to 2 % (F8) led to a significant increase in the mean diameter of the PLGA particles ($p<0.05$) compared to that of F1. It is also found that adding 1 or 2 % Brij 97 to the organic phase of the formulations containing 2% Plx 188 (F9 & F10, respectively) resulted in a significant increase in NP mean diameter ($p<0.05$) compared to that of F4. The preparation of PLGA NPs using F68 in the aqueous phase led to adsorption of F68 molecules into the outer surface of the PLGA NPs with its bulk copolymer's hydrophilic moieties (poly (ethylene oxide); PEO) dangled in water modifying the particle size diameter resulting in NPs with larger size (Vandervoort and Ludwing, 2002 ; Ninham *et al.,* 1984 ; Huibers, 1997 ; Moreno *et al.,* 2003).

Table 1: Composition of brinzolamide-loaded PLGA particles

Organic phase containing:		Aqueous phase containing Pluronic acid F68 in concentration of:	
		1 % w/v	2% w/v
Tween 80	-	F1	F4
	1 % w/v	F2	F5
	2 % w/v	F3	F6
Brij 97	1 % w/v	F7	F9
	2 % w/v	F8	F10

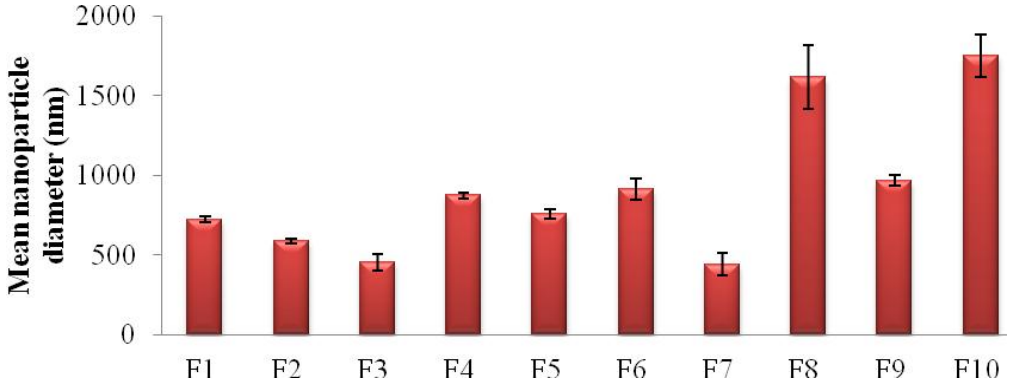

Fig. 1: Mean diameter values for brinzolamide PLGA particles

Entrapment efficiency of brinzolamide in polymeric nanoparticles

Figure 2 shows the values of brinzolamide entrapment efficiency for nanoparticles using F68 in the aqueous phase with/without adding polysorbate 80 or Brij 97 in the organic phase (F1–F10). It is observed that increasing F68 concentration from 1 to 2 % (F1 & F4, respectively) resulted in increasing significantly the brinzolamide entrapment efficiency ($p<0.05$). It is also observed that adding 1 or 2 % Tween 80 to the organic phase of the formulations containing 1% F68 (F2 & F3, respectively) resulted in a significant increase in the drug entrapment efficiency compared to that of F1. Results also show that increasing the concentration of Tween 80 in the organic phase from 1 to 2 % in the preparation containing 2 % F68 in the aqueous phase (F5 & F6, respectively) led to a significant decrease in the drug entrapment efficiency ($p<0.05$)

Results in figure 2 reveal also that adding Brij 97 to the organic phase (F7 – F10) resulted in a significant increase in the drug entrapment efficiency ($p<0.05$) compared to that of F1. Increasing the concentration of Brij 97 from 1 to 2 % in the preparation containing 2 % F68 (F7 & F8, respectively) did not result in significant

difference in the drug entrapment efficiency ($p<0.05$). It is also obvious that adding 1% Brij 97 to the preparation containing 2% Plx 188 (F9) led to a significant increase in the drug entrapment efficiency compared to that of F4. However, further increasing Brij 97 concentration to 2% (F10) led to a significant decrease in brinzolamide entrapment efficiency ($p<0.05$) compared to that of F9.

The drug loading values for brinzolamide PLGA nanoparticles ranged from 9.26 – 38.76. These drug loading values run in parallel with the entrapment efficiency values of brinzolamide in which PLGA nanoparticles with high encapsulation efficiency showed high brinzolamide loading values while those with high low encapsulation efficiency had low brinzolamide loading values.

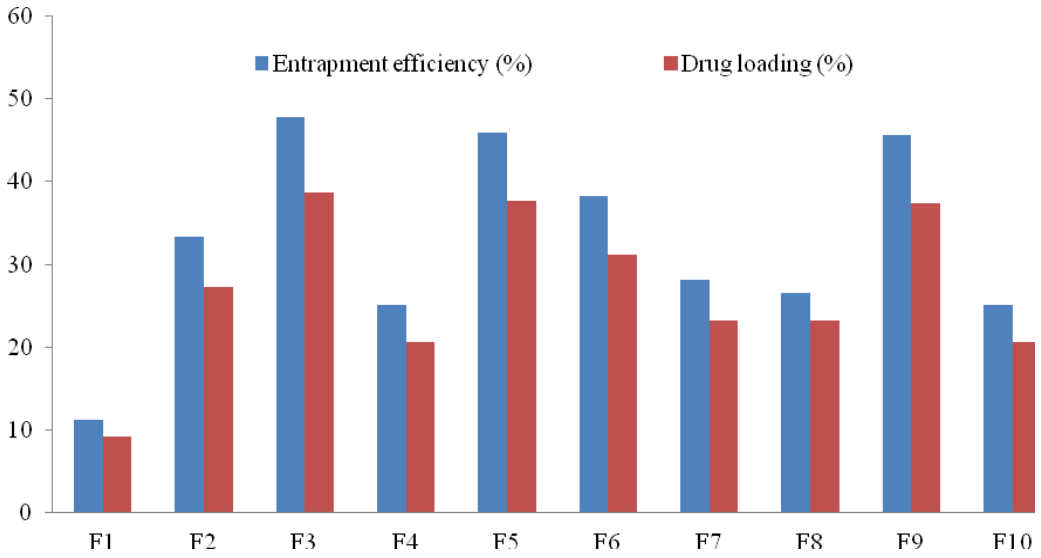

Fig. 2: Brinzolamide entrapment efficiency and drug loading values for PLGA particles

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