International Journal of Drug Delivery 3 (2011) 371-380
http://www.arjournals.org/index.php/ijdd

Original Research Article

ISSN: 0975-0215

Effect of Stabilizers and Process Parameters for Budesonide Loaded PLGA-Nanoparticles

A. Dinda[1]*, I. Biswal[1], D. Das[1], S. Si[1], Sanjay Kumar[2], B. B. Barik[3], M. M. Safhi[4]

***Corresponding author:**

Agnimitra Dinda
1. School of Pharmaceutical Sciences, SOA University, India
2. Institute of Medical Sciences, Department of Pharmacology, SOA University, India
3. University Department of Pharmaceutical Sciences, Utkal University, India
4. College of Pharmacy, Jazan University, Kingdom of Saudi Arabia
e.mail:
agnimitra_d@rediffmail.com

Abstract

The present work is aimed at investigations of the effects of various stabilizers and process parameters on the properties of budesonide (glucocorticoid steroid) loaded PLGA (Poly-dl-lactide-co-glycolide) nanoparticles. Budesonide loaded PLGA nanoparticles were prepared following high pressure emulsification- solvent evaporation technique. The effects of three different stabilizers like polyvinylalcohol (PVA), carbomer (Carbopol 980) and poloxamer (Lutrol F-68) are used during emulsification process were studied. Investigations were also carried out regarding influences of different homogenization parameters (pressure and number of cycles) on the properties of various nanoparticles. For comparison, nanoparticles were prepared without the treatment of high pressure also. Various properties of nanoparticles subjected to investigations include, particle size, polydispersity index, drug loading, encapsulation efficiency and the drug release profile. These properties were found to be strongly influenced by the type of stabilizer, homogenization pressure and the number of cycles. Amongst three stabilizers, PVA found to produce comparatively smallest nanoparticles than poloxamer and carbomer. The nanoparticles prepared without high pressure homogenization found to posse's larger size and high values of polydispersity index especially with the stabilizers carbomer and poloxamer. The low drug loading of nanoparticles found, could be resulted due to high pressure promoted drug diffusion from the protoparticles during the emulsification process and the characteristics of the outer water phase of emulsion. Faster drug release was observed from the nanoparticles obtained after high pressure emulsification as compared to those prepared without pressure homogenization of emulsion.

keywords: Budesonide, nanoparticles, polyvinylalcohol, carbomer, poloxamer, homogenization.

Introduction

Budesonide is a glucocorticoid steroid used for the treatment of asthma, non-infectious rhinitis (including hay fever and other allergies), and for treatment and prevention of nasal polyposis. Additionally, it is used for crohn's disease (inflammatory bowel disease). Budesonide has a high first pass metabolism and half life of 2 to 3 hours (for children plasma half life is still shorter), thus, making it a suitable candidate for particulate drug delivery system.

Biodegradable particulate drug delivery systems have been widely studied mainly for aerosol,

parenteral, oral or ocular applications [1-3]. Several methods for their preparation were developed, but the most popular is the emulsification-solvent evaporation method [4] and its modification, the double emulsion-solvent evaporation method [5]. The main difficulty of this technique is the necessity to optimize various parameters in order to obtain particles of suitable size with narrow size distribution. The stirring speed used during emulsification as well as the nature and concentration of selected stabilizers have been extensively examined [6,7]. However, the high stirring speed and even sonication are not sufficient to achieve a narrow particle size distribution. Therefore, the high pressure homogenization technique was successfully adapted [8]. This method is mainly used for production of microemulsion [9-11] and liposome [12,13], but there are found to be only a small number of studies concerning polymeric nanoparticle preparations [14&15].

The main aim of the present work was to investigate the effect of different stabilizers and homogenization parameters on the various properties of budesonide loaded nanoparticles meant for ocular purposes which were prepared by a high- pressure emulsification-solvent evaporation method. It has been observed that large particles may irritate the eye. Consequently smaller particles are preferred for ophthalmic delivery systems [16]. Additionally, Calvo et al., have reported that poly ("-caprolactone) nanoparticles (0.20–0.25 μm) improve the ocular bioavailability of indomethacin rather than poly ("-caprolactone) microparticles (6 μm) [17]. Thus, one of the most important characteristics of nanoparticles is their size. For this reason, the influence of homogenization parameters, like pressure and number of cycles applied, on the nanoparticle properties were investigated in detail. The effects of the nature of different stabilizers like polyvinylalcohol (PVA), carbomer (Carbopol 980) and poloxamer (Lutrol F-68) on the nanoparticle size were also studied. PVA is often used as a stabilizing agent for the emulsification-solvent evaporation method. Carbopol and poloxamer were chosen as stabilizers because of their mucoadhesive properties. It is well known that the ocular bioavailability can be improved using mucoadhesive particulate drug delivery systems [18].

Materials and Methods

Materials

Budesonide was obtained from Ranbaxy (Noida, India). Poly (dl-lactide-co-glycolide) (PLGA, Resomer RG 503, lactic:glycolic ratio 52:48, Mw 40 000) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Polyvinylalcohol (PVA) (Average Mw 30 000–70 000) was supplied by Sigma Chemical Co. (St. Louis, USA). Methanol and acetonitrile (HPLC grade) were from Across Organics (New Jersey, USA) and Methylene Chloride from Aldrich (Gillingham, UK). Carbopol 980 NF was obtained from BF Goodrich (Cleveland, USA) and Poloxamer 188 (Lutrol F-68) was from BASF (Ludwig- shafen, Germany).

Preparation of Nanoparticles

Budesonide loaded PLGA nanoparticles were prepared by the combination of double emulsification and homogenization procedure. PLGA (1g) was dissolved in 10 ml of methylene chloride and dispersed in an aqueous solution of budesonide (2.5% w/v). The emulsification was carried out by sonication for 1 min at 80 W (Lark India-02, India) [19]. The resulting emulsion was poured into 50 ml of an aqueous stabilizer solution and was sonicated for 30 s. PVA (1% w/v), carbopol (0.012% w/v) and poloxamer (2.2% w/v) were used as stabilizers. The W/O/W emulsions were then subjected to a high pressure homogenization using a microfluidizer (M-110L, Microfluidics, Newton, USA) and were treated with one or three cycles at an operating pressure of 100 and 500 bar. Finally, the homogenized emulsions were added to aqueous solutions of stabilizer to allow the simultaneous evaporation of the methylene chloride. The concentrations of PVA, carbopol and poloxamer in these water solutions were 0.33, 0.004 and 0.73% (w/v),

respectively. The evaporation was carried out at room temperature under stirring at 700 rpm (Remi, Munchen, Germany). The resulting suspensions were cooled down at -18°C and then freeze dried. For comparison, nanoparticles of the W/O/W emulsion were prepared without high-pressure treatment.

Evaluation of Nanoparticles

Particle size

Particle sizes of nanoparticles were determined by photon correlation spectroscopy with a Zetasizer 3000 (Malvern Instruments, Malvern, UK). The freeze dried samples were diluted 25 times with distilled water before measurements. Each sample was determined four times and average values were calculated.

Drug loading and Encapsulation efficiency

Accurately weighed freeze dried nanoparticles (20 mg) were dispersed in 10 ml distilled water by sonication for 10 min. The samples were centrifuged at 3000 rpm for 3 h and the drug content in the supernatant fluid was determined by an HPLC method. The HPLC system was a Gilson 321 pump (Jasco, Japan). The mobile phase consisted of a water/methanol mixture (97:3, v/v) and potassium dihydrogen phosphate (5%, w/v). Determinations were performed using a column Bondapak C18 (Waters) at a flow rate of 2 ml/min and sensitivity 0.005%, respectively. Budesonide was detected at 216 nm and its concentration was calculated according to the calibration curve prepared under the same conditions. The measurements were made in duplicate. The encapsulation efficiency (EE) for all samples was estimated using the equation:

Encapsulation efficiency (%) =
¼ (actual drug loading/theoretical drug loading) x100

In vitro release studies

The in vitro drug release studies were carried out in duplicate using diffusion cells. The acceptor and donor compartments of the cells were separated by a dialysis membrane (Mw cut off 12,000–14,000 D, Medicell, UK). The membranes were washed with distilled water for 30 min before diffusion experiments. The nanoparticles (20 mg) were placed as an aqueous suspension in the donor compartments of the cells. The acceptor compartments were filled with 18 ml distilled water and stirred magnetically at 200 rpm. At suitable time intervals aliquots of 0.8 ml were withdrawn from the acceptor compartments and replaced by the same volumes of fresh distilled water. The concentrations of samples were determined following the above described HPLC method.

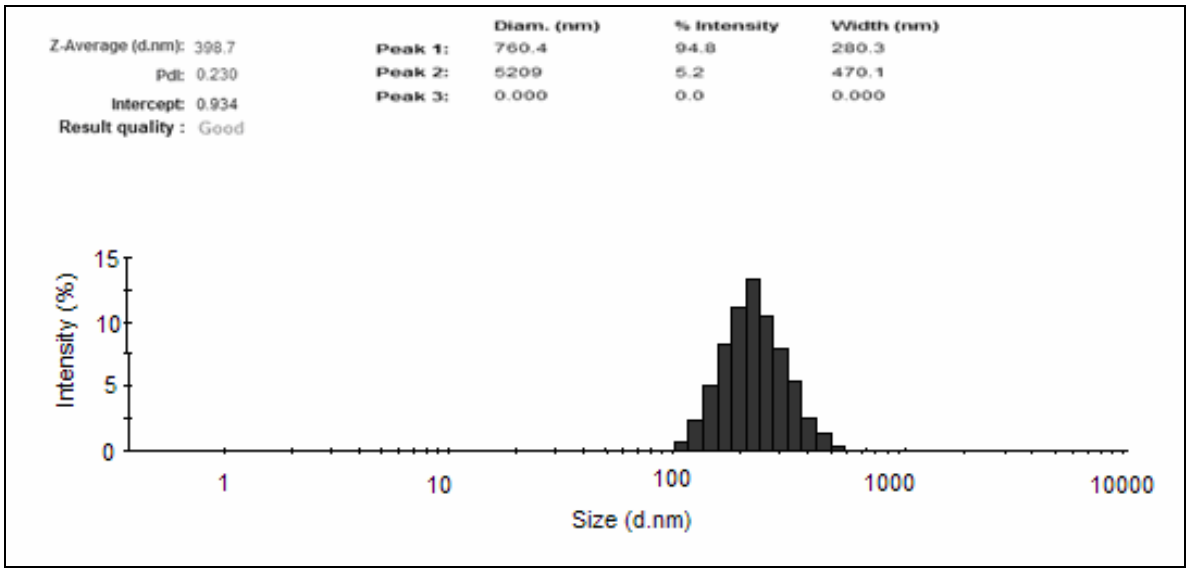

Figure. 1. Influence of PVA on particle size of budesonide loaded nanoparticles.

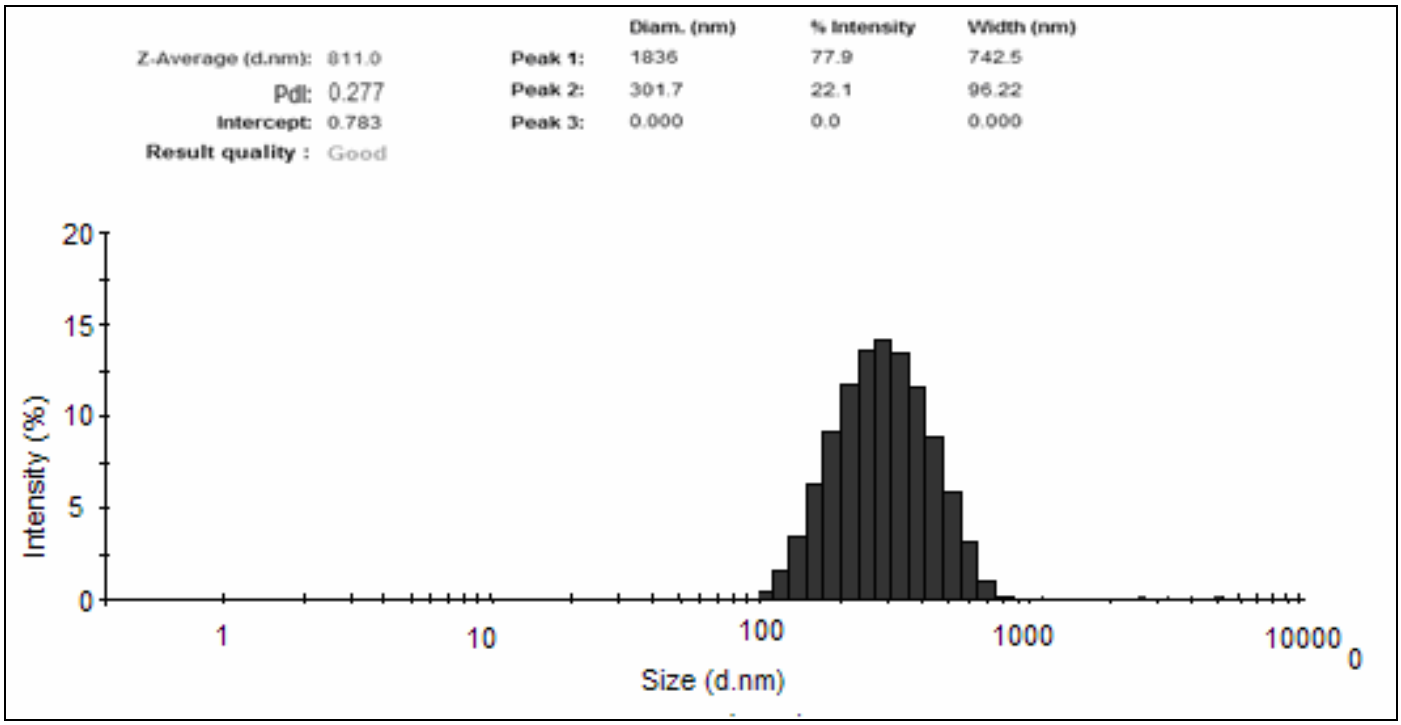

Figure. 2. Influence of Carbopol on particle size of budesonide loaded nanoparticles.

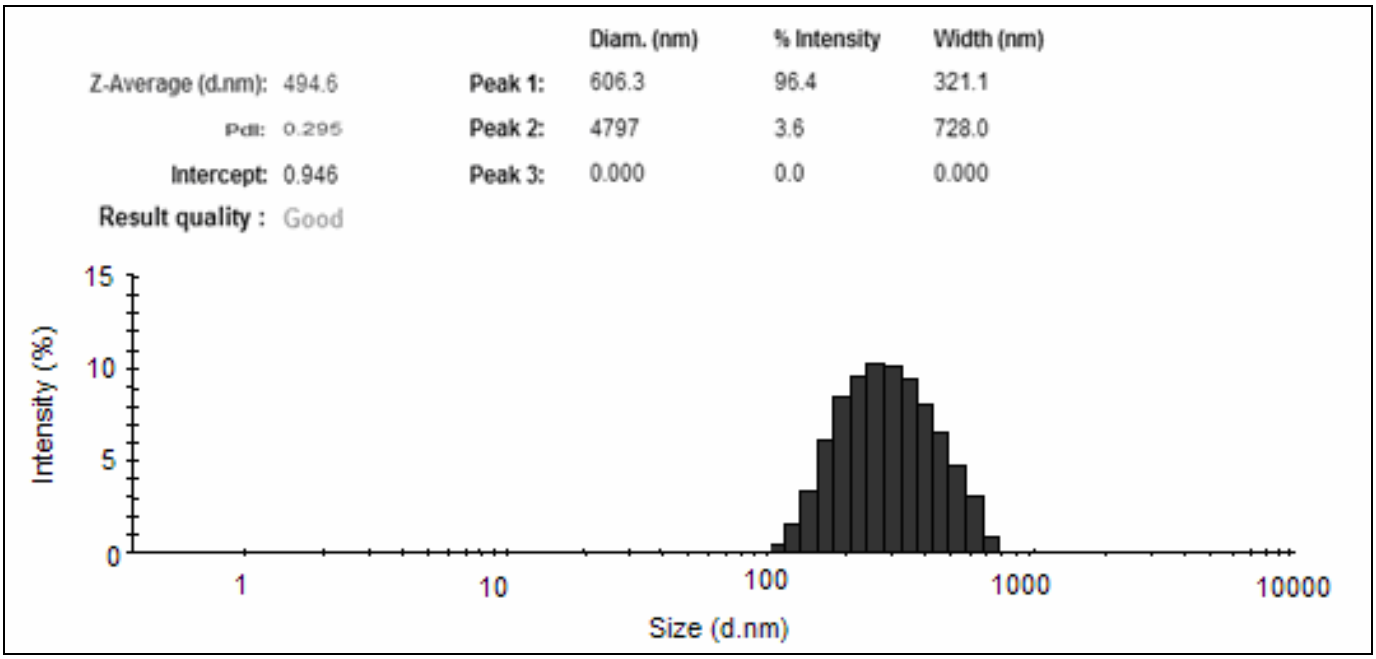

Figure. 3. Influence of Poloxamer on particle size of budesonide loaded nanoparticles.

Results and Discussion

Nanoparticle size

The results of determination of particle size and polydispersity index of various nanoparticles are summarized in Table. 1. The data showed that the nanoparticle size and polydispersity index depend strongly on the homogenization pressure and number of cycles (Figures.1-3). The higher the pressure applied resulting in smaller the size of the nanoparticles. In addition, the increase of

number of cycles even from one cycle to three cycles, contributed to particle size reduction. These observations were valid for all the formulations investigated. In comparison, the nanoparticles formulated without homogenization pressure showed larger in size and high values of polydispersity index (Table 1). However, the nanoparticles prepared without homogenization pressure using poloxamer as stabilizer possessed comparatively smaller particle size and the nanoparticles prepared without pressure using PVA as stabilizer maintained a low value of polydispersity index. The studies performed with nanoparticles prepared using PVA as a stabilizer showed a small dependence of the particle size on the pressure and the number of cycles. Contrarily the pressures as well as number of cycles have significant impact on the mean particle size of nanoparticles prepared with carbopol and poloxamer.

These particles demonstrated a wide size range, from 446.6–1802.7 nm in the case of carbopol, and from 452.3–875.4 nm for poloxamer respectively. However, due to application of the homogenization pressure, their size drastically dropped down around 300 nm when the emulsion containing protoparticles was treated with 500 bar pressure for three cycles. The same effect was observed on the polydispersity index of both the nanoparticle series. The increase in pressure and especially the number of cycles reduced the polydispersity index.

Table. 1. Effect of nature of stabilizers, homogenization pressure and number of cycles on mean diameter and polydispersity index of prepared nanoparticles.

Stabilizer	Homogenization pressure and number of cycles	Mean Diameter (nm)	Polydispersity index
PVA	Without pressure	332.5 ± 2.5	0.083
	100 bar, 1 cycle	283.1 ± 2.2	0.121
	100 bar, 3 cycles	231.8 ± 1.4	0.097
	500 bar, 1 cycle	231.3 ± 0.7	0.082
	500 bar, 3 cycles	203.8 ± 5.5	0.308
Carbopol	Without pressure	1125.3 ± 776.5	0.783
	100 bar, 1 cycle	631.4 ± 1.72	0.635
	100 bar, 3 cycles	365.7 ± 2.61	0.542
	500 bar, 1 cycle	467.0 ± 4.23	0.661
	500 bar, 3 cycles	308.9 ± 10.8	0.053
Poloxamer	Without pressure	572.4 ± 206.6	0.802
	100 bar, 1 cycle	691.7 ± 189.4	0.765
	100 bar, 3 cycles	424.5 ± 13.4	0.528
	500 bar, 1 cycle	467.0 ± 4.21	0.809
	500 bar, 3 cycles	304.3 ± 34.8	0.307

Table. 2. Effect of nature of stabilizer and process parameters on Drug loading and Entrapment efficiency of prepared nanoparticles

	Without pressure		**100 bar, 1 cycle**		**100 bar, 3 cycles**		**500 bar, 1 cycle**		**500 bar, 3 cycles**	
Stabilizer	**Drug Loading (%)**	**EE (%)**	**Drug Loading (%)**	**EE (%)**	**Drug Loading (%)**	**EE (%)**	**Drug Loading (%)**	**EE (%)**	**Drug Loading (%)**	**EE (%)**
PVA	2.93 ± 0.02	61.5	0.79 ± 0.3	16.6	0.66 ± 0.16	13.9	0.21 ± 0.06	4.4	0.95 ± 0.35	20.0
Carbopol	3.34 ± 0.01	70.2	3.82 ± 0.02	80.5	3.62 ± 0.19	76.1	3.01± 0.05	63.2	1.41 ± 0.12	29.6
Poloxamer	1.54 ± 0.04	32.3	1.47 ± 0.01	30.9	1.33 ± 0.05	16.0	1.33 ± 0.05	27.9	0.80 ± 0.04	16.8

Drug loading and encapsulation efficiency

The results of drug loading and encapsulation efficiency of nanoparticles prepared without or under high pressure are presented in Table 2. Generally, drug loading and encapsulation efficiency decreases with the application of pressure.

For particles made with PVA as a stabilizer, the encapsulation efficiency went down from 61.5% for those prepared without pressure to 13.9–20.0% for those obtained under pressure. Similar trend was also observed for particles formulated in presence of carbopol and poloxamer as stabilizers. For these nanoparticles, well pronounced differences in the encapsulation efficiency were found between the formulation obtained at highest pressure (500 bar) and those which were formulated without pressure. For all the formulations drug loading and encapsulation efficiency were increased approximately two times when no pressure was applied. This phenomenon was probably due to the enhanced diffusion of the hydrophilic budesonide molecules out of the emulsion droplets during their size reduction under pressure. Similar results were reported by Soriano et al. (1995) [20]. They found that albumin-loaded PLGA microspheres manufactured under pressure had lower encapsulation efficiency than the samples produced by only sonication.

The properties of the outer aqueous phase of the W/O/W emulsion may also influence drug diffusion out of polymeric droplets. As it could be seen, different drug loading was measured for nanoparticles prepared with PVA, carbopol and poloxamer as stabilizers at similar pressures (Table. 2). However, the concentrations of stabilizers were chosen so as to give an equal viscosity to the water phases. Moreover, the stabilizers also have different surface active properties. Therefore, the differences of interfacial tension probably have influenced drug diffusion from polymeric droplets to the outer water phase.

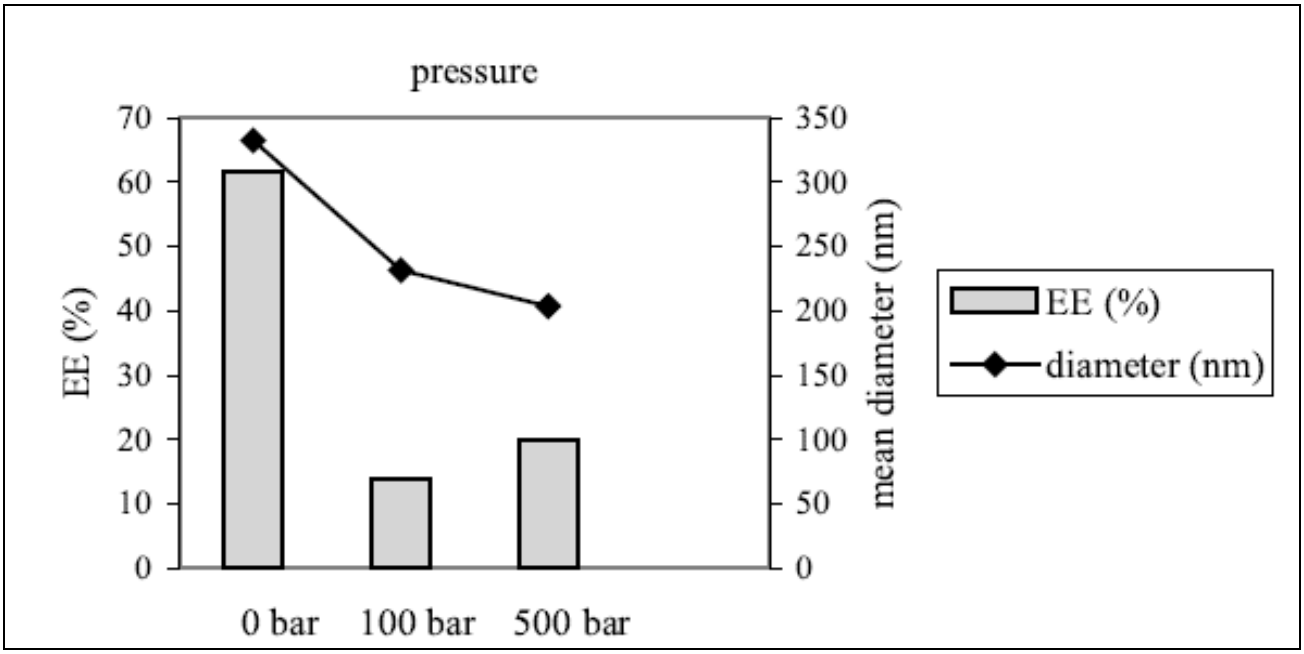

Figure. 4. The effect of pressure (three cycles) on the encapsulation efficiency (EE) and size of nanoparticles prepared with PVA.

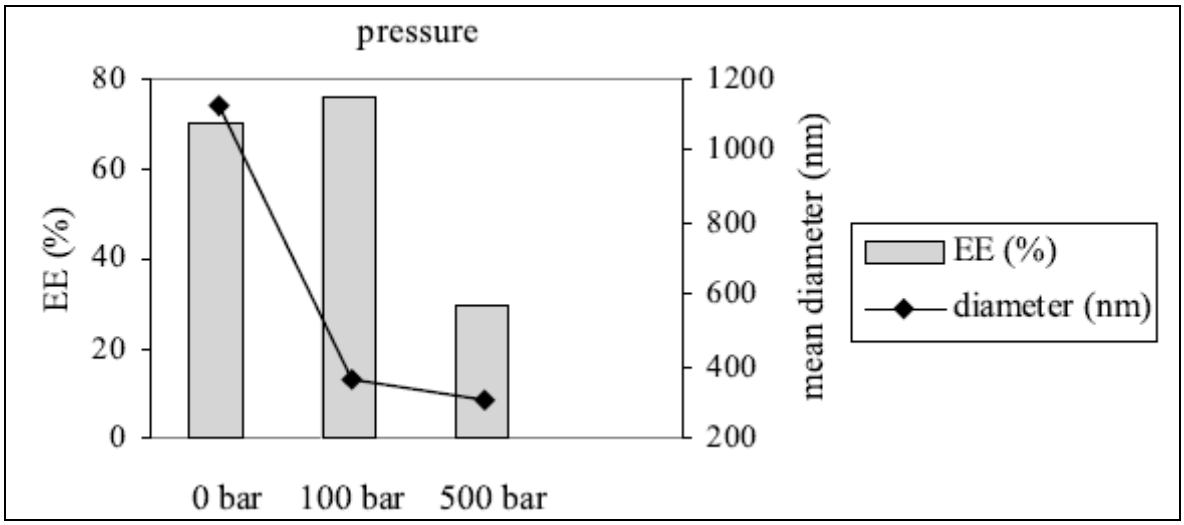

Figure. 5. The effect of pressure (three cycles) on the encapsulation efficiency (EE) and size of nanoparticles prepared with carbopol.

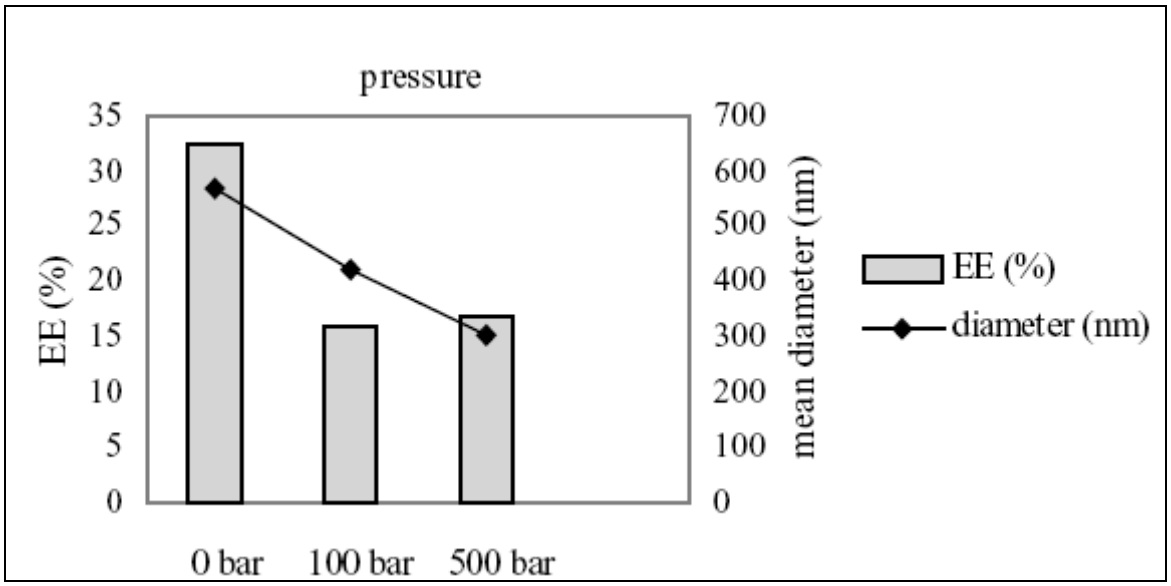

Figure. 6. The effect of pressure (three cycles) on the encapsulation efficiency (EE) and size of nanoparticles prepared with PVA as an emulsifier.

Drug loading of the larger particles produced without or at the lower pressure was higher for all the preparations. This is due to available surface area of the particles, which was depending on their size. In absence of pressure the larger protoparticles provide minimum possibility for drug diffusion out to the external water phase. However, the nanoparticles made with PVA showed a narrow size distribution. Despite their similar sizes, drug loading dramatically dropped when pressure was applied (Figures. 4-6). The results illustrated the fact that particle size was not the only factor responsible for the low drug loading value. The low drug loading could be due to two reasons. First, the high pressure promoted drug diffusion out of protoparticles during emulsification was either by size reduction or by shear forces and second, the characteristic of the outer water phase of the emulsion also might have influenced the drug loading of nanoparticles.

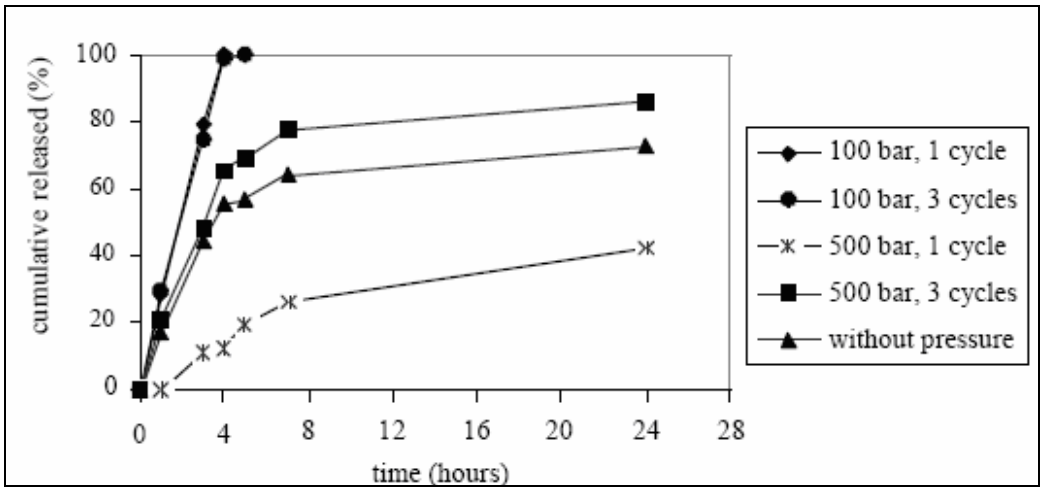

Figure. 7. Influence of manufacturing parameters on in-vitro drug release process from nanoparticles prepared with PVA.

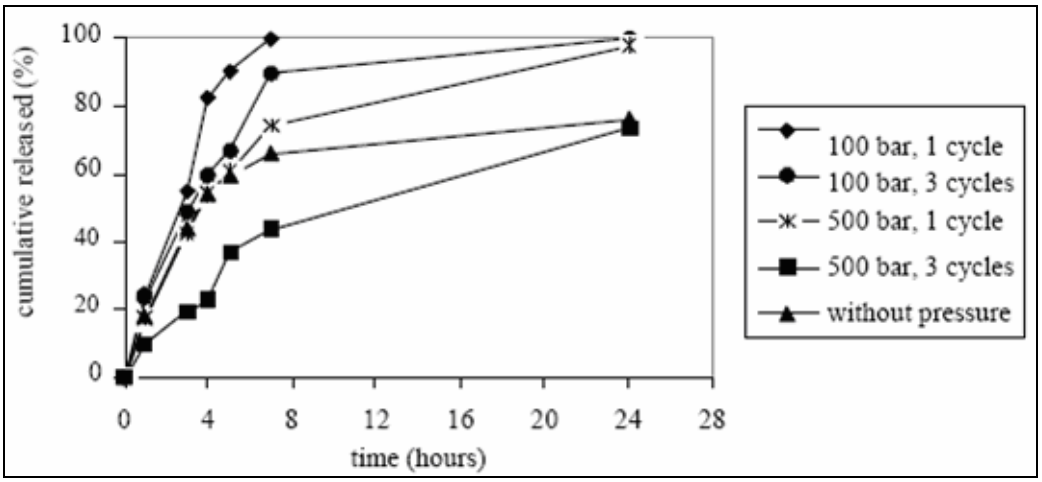

Figure. 8. Influence of manufacturing parameters on in-vitro drug release process from nanoparticles prepared with carbopol.

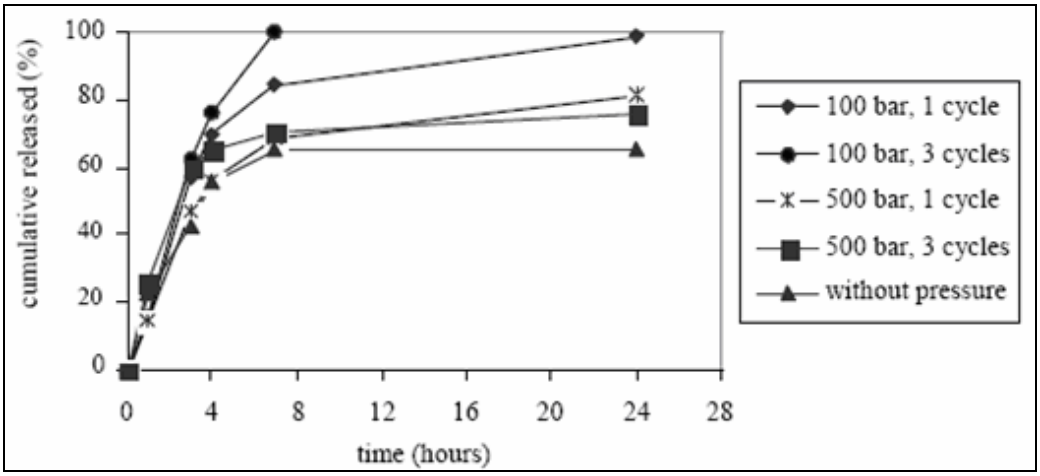

Figure. 9. Influence of manufacturing parameters on in-vitro drug release process from nanoparticles prepared with poloxamer.

In vitro drug release

Comparatively faster drug release was detected from nanoparticles obtained after homogenization of the W/O/W emulsion than those prepared without homogenization (Figures. 7-9). It was more pronounced for the batches formulated at

the lower pressure. The fastest release of budesonide occurred from all nanoparticles treated at 100 bar pressure which was independent of either the number of cycles applied or the nature of the stabilizer used. These release profiles were unexpected because of small sizes of nanoparticles formulated at the higher pressure (500 bar). Consequently, their ultimate surface area became larger favouring a faster release of the drug. Taking into account of different values of homogenization pressure applied, the present study revealed a difference possibly occurred between density and structure of the nanoparticles. This explanation could be deduced from the comparison between the drug releases of two batches of formulation of similar size. Both nanoparticle samples made with PVA as stabilizer had similar size [Table 1]. However, the drug release was faster from the low-pressure homogenized particles (100 bar, 3 cycles) compared to the higher-pressure homogenized particles (500 bar, 1 cycle). Therefore, the observations revealed that the main factor influencing drug release property of nanoparticles was the pressure applied during homogenization of protoparticles.

Conclusion

Budesonide loaded PLGA nanoparticles were prepared by high-pressure emulsification-solvent evaporation technique. The effects of three different stabilizers on the various properties of budesonide loaded nanoparticles were studied. The higher the pressure as well as the number of cycles applied, the smaller the sizes of nanoparticles were produced. Simultaneously increase in homogenization pressure and number of cycles resulted in reduction of polydispersity index. The lower drug loading and encapsulation efficiency of nanoparticles were observed due to high pressure promoted drug diffusion from the protoparticles and the characteristics of outer aqueous phase of the emulsion. The in-vitro drug release profile of nanoparticles was influenced by the pressure applied during homogenization of protoparticles. Amongst three stabilizers used in the study PVA found to be comparatively better in all aspects. The present study may prove the way for formulation of budesonide loaded PLGA nanoparticles meant for various therapeutic uses through the ocular delivery system.

Accknowledgement

The authors are very much thankful to the School of Pharmaceutical Sciences and Siksha ‘O’ Anusandhan University and Jazan University for extending the financial assistance and other supports.

References

1. Coombes AGA, Rafati H, Adler J, Holland J, Davis SS. Proteinloaded poly (d, l-lactide –co -glycolide) microparticles for oral administration: formulation, structural and release characteristics. Journal of Controlled Release, 1997;43:89–102.
2. Gurny R, Ibrahim H, Aebi A, Buri P, Wilson CG, Washington N, Edman P, Camber O. Design and evaluation of controlled release system for the eye. Journal of Controlled Release, 1987;6:367–373.
3. Tice TR, Mason DW, Gilley RM,. Clinical use and future of parenteral microsphere delivery systems. In Novel Drug Delivery, edited by G. F. Prescott and W. S. Nimmo (Chichester: Wiley), 1989;24:223–235.
4. Bodmeier R, McGinity JW. The preparation and evaluation of drug containing poly(dl-lactide) microspheres formed by the solvent evaporation method. Pharmaceutical Research, 1987;4:465–471.
5. Ogawa Y, Yamamoto M, Okada H, Yashiki T, Shimamoto T. Nanoparticle preparation by high-pressure emulsification: A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acid. Chemical and

Pharmaceutical Bulletin, 1988;36:1095–1103.
6. Conti B, Genta I, Modena T, Pavanetto F. Investigation on process parameters involved in polylactic-co-glycolic microspheres preparation. Drug Development and Industrial Pharmacy, 1995;21:615–622.
7. Jalil R, Nixon JR. Biodegradable poly (lactic acid) and poly (lactide-coglycolide) microcapsules: problems associated withpreparative techniques and release properties. Journal of Microencapsulation, 1990;7:297–325.
8. Schwarz C, Mehnert W, Lucks JS, Muller RH. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. Journal of Controlled Release, 1994;30:83–96.
9. Jumaa M, Muller BW. The effect of oil components and homogenization conditions on the physicochemical properties and stability of parenteral fat emulsions. International Journal of Pharmaceutics, 1998;163:81–89.
10. Teixeira H, Dubernet C, Puisieux F, Benita S, Couvreur P. Submicron cationic emulsions as a new delivery system for oligonucleotides. Pharmaceutical Research, 1999;16:30–36.
11. Washington C, Davis SS. The production of parenteral feeding emulsions by Microfluidizer. International Journal of Pharmaceutics, 1988;44:169–176.
12. Bachmann D, Brandl M, Gregoriadis G. Preparation of liposomes using a Mini-Lab 8.30 H high-pressure homogenizer. International Journal of Pharmaceutics, 1993;91:69–74.
13. Chen RH, Win HP, Fang HJ. Vesicle size, size distribution, stability, and rheological properties of liposomes coated with water-soluble chitosans of different molecular weights and concentrations. Journal of liposome research, 2001;11:211–228.
14. Lamprecht A, Ubrich N, Hombreiro Perez M, Lehr CM, HoFFman M, Maincent P. Influences of process parameters on nanoparticle preparation performed by a double emulsion pressure homogenization technique. International Journal of Pharmaceutics, 2000;196:177–182.
15. Ueda M, Kreuter J. Optimization of the preparation of loperamide-loaded poly(l-lactide) nanoparticles by high pressure emulsification-solvent evaporation. Journal of Microencapsulation, 1997;14:593–605.
16. Zimmer A, Kreuter J. Microspheres and nanoparticles used in ocular delivery systems. Advanced Drug Delivery Reviews, 1995;16:61–73.
17. Calvo P, Alonso MJ, Vita-Jato JL, Robinson JR. Improved ocular bioavailability of indomethacin by novel ocular drug carriers. Journal of Pharmacy and Pharmacology, 1996;48:1147–1152.
18. Zimmer A, Chetoni P, Saettone M, Zerbe H, Kreuter J. Evaluation of budesonide-loaded albumin particles as controlled drug delivery systems for the eye. II. Co-administration with bioadhesive and viscous polymers. Journal of Controlled Release, 1995;33:31–46.
19. Dinda I, Biswal S, Kumar Si S, Barik BB. Budesonide Loaded PLGA-Nanoparticles: Effect of Stabilizers and Process Parameters. International Journal of Drug Formulation & Research, 2011;2(1):206–217.
20. Soriano I, Delgado A, Diaz RV, Evora C. Use of surfactants in polylactic acid protein microspheres. Drug Development and Industrial Pharmacy, 1995;21:549–558.