Design and formulation of nano-sized spray dried Efavirenz-Part I: Influence of formulation parameters

Lebogang Katata [a*], Lesego Tshweu [a], Saloshnee Naidoo [a], Lonji Kalombo [a], Hulda Swai [a], Rui Krause [b]

[a] Council for Scientific and Industrial Research
Materials Science and Manufacturing
Centre of Polymers and Composites
PO Box 395
Meiring Naude Road
Brummeria-Pretoria 0001
South Africa

[b] University of Johannesburg
Faculty of Science
Department of Applied Chemistry
Doornfontein Campus
PO Box 524
Auckland Park 2006
South Africa

*Corresponding author: Lebogang Katata,
E-mail address: lebzakate@yahoo.com,

Abstract

Efavirenz (EFV) is one of the first-line antiretroviral drugs recommended by the World Health Organisation for treating HIV. It is a hydrophobic drug that suffers from low aqueous solubility (4 µg/ml), which leads to a limited oral absorption and low bioavailability. In order to improve its oral bioavailability, nano-sized polymeric delivery systems are suggested. Spray dried polycaprolactone-efavirenz (PCL-EFV) nanoparticles were prepared by the double emulsion method. The Taguchi method, a statistical design with an L_8 orthogonal array, was implemented to optimise the formulation parameters of PCL-EFV nanoparticles. The type of sugar (lactose or trehalose), surfactant concentration and solvent (dichloromethane and ethyl acetate) were chosen as significant parameters affecting the particle size and polydispersity index (PDI). Small nanoparticles with an average particle size of less than 254 ± 0.95 nm in the case of ethyl acetate as organic solvent were obtained as compared to more than 360 ± 19.96 nm for dichloromethane. In this study, the type of solvent and sugar were the most influencing parameters of the particle size and PDI. Taguchi method proved to be a quick, valuable tool in optimising the particle size and PDI of PCL-EFV nanoparticles. The optimised experimental values for the nanoparticle size and PDI were 217±2.48 nm and 0.093±0.02.

Keywords: Spray dried, polycaprolactone, efavirenz, nanoparticles, Taguchi method.

1. Introduction

Highly active antiretroviral therapy (HAART) has managed to successfully enhance the quality of life for human immunodeficiency virus (HIV) infected patients. However, the key challenge facing the current therapy is that many antiretroviral (ARV) drugs undergo extensive first pass metabolism and gastrointestinal degradation leading to low bioavailability (Govender et al. 2008). Furthermore, several drugs such as indinavir (800 mg taken three times a day) requiring frequent administration of large doses owing to their short half-life, lead to patient non-compliance. In the case of efavirenz (EFV), 600 mg (dose for adults) is taken once daily before bedtime because of its adverse side effects (Marzolini et al. 2001). The most common symptomatic side effect of EFV is reported to be associated with a sense of altered mental state, described as "spacey," "high," or "confused," which usually resolves within the first month of treatment (Marzolini et al. 2001). However severe depression, suicidality, or delusions have also been infrequently reported. EFV (also known as Sustiva®) is one of the recommended first-line anti-HIV drugs in the WHO guidelines and used mostly in the developing countries (WHO HIV report 2010). The drug suffers from low aqueous solubility (4 µg/ml) because of its hydrophobic nature, which leads to a limited oral absorption and low bioavailability (40-45%) (Pediatric Guidelines 2011).

Nanotechnology-based drug delivery has shown to hold promise in addressing challenges such as poor bioavailability, toxicity, lengthy treatment for HIV or to overcome drawbacks associated with ARVs (Pediatric Guidelines 2011; Santos-Magalhães and Mosqueira 2010; Neves et al. 2010) and has been successfully employed in the case of cancer (Kateb et al. 2011). For example, Doxil® and Abraxane® are two nanotechnology-based anticancer drug formulations that are approved by the United States Food and Drug Administration (FDA) for ovarian and metastatic breast cancer, respectively. Polymeric materials such as polylactic acid (PLA), polybutylcyanoacrylate (PBCA), poly(lactic-*co*-glycolic acid) (PLGA) and polycaprolactone (PCL) have generated a remarkable interest for encapsulating a variety of therapeutic agents. Nanoparticles prepared from these and other polymers have been explored and utilised as delivery systems for ARVs (Sharma et al. 2010; Shah and Amiji 2006; Kovochick et al. 2011). Several researchers have investigated various ways of improving the current drawbacks associated with EFV (Chiappetta et al. 2010; Chiappetta et al. 2011; Destache et al. 2010; Destache et al. 2009; Nowacek et a.l 2011). The research group of Dr. Sosnik have dedicated some efforts to investigate the encapsulation of EFV by means of

polymeric micelles (Chiappetta et al. 2010; Chiappetta et al. 2011). In this context, they have developed a micelle-based liquid paediatric formulation of EFV (the first water-based one reported) with promising preclinical results and they are pursuing bioequivalence studies in adult healthy volunteers in Argentina. PLGA nanoparticles loaded with three ARVs namely ritonavir, lopinavir and EFV with average particle size of 331.20 ± 77.20 nm were developed by Destache *et al* 2009. Their experimental results demonstrated that PLGA can be used to fabricate nanoparticles as a drug delivery system that can be used for intravenous administration. Furthermore, their delivery system showed a prolonged release of combination ARVs for 28 days. However they prepared PLGA nanoparticles using a freeze drying technique which is often not scalable.

In this work, encapsulation of EFV in PCL will be investigated using a scalable spray drying technique and a Taguchi experimental design method to obtain the optimum formulation for preparing nanoparticles. Taguchi method was chosen since it is useful for complex studies thus minimising the number of experiments especially in multifactor experiments. In addition it has economic advantage since the method saves time and reduces material costs. The study was designed to assess the influence of formulation parameters on the characteristics of PCL-EFV nanoparticles such as the particle size and polydispersity index (PDI). The type and concentration of sugar (lactose or trehalose), surfactant type and concentration and solvent (dichloromethane and ethyl acetate) were selected as formulation parameters.

2. Materials and methods

2.1 *Materials*

Polycaprolactone (PCL) with average molecular weight number 10 000, was obtained from Sigma-Aldrich (Johannesburg, South Africa). The organic solvent, ethyl acetate (EA) was also purchased from Sigma Aldrich, while dichloromethane (DCM) was purchased from Merck (Johannesburg, South Africa). Polyvinylalcohol, molecular weight 13,000–23,000 (PVA, 87-89 % hydrolyzed) was supplied by Sigma Aldrich. Efavirenz (EFV) was bought from Aspen Pharma (Johannesburg, South Africa). Ultra purified water was used throughout and all other chemicals were of analytical grade.

2.2 *Methods*

2.2.1 *Experimental design and analysis*

The Taguchi Design of Experiment was used to study the influence of formulation parameters in optimising the preparation of spray dried PCL-EFV nanoparticles. This method is a combination of mathematical and statistical techniques incorporated into an empirical study (Kim et al. 2007) and has previously been applied to the development of pharmaceuticals (Hang et al. 2009; Palmieri and Wehrle 1997; Varshosaz et al. 2009). Furthermore, Taguchi offers an easy, efficient and systematic approach to determine few optimum well defined experimental sets (Jahanshahi et al. 2009; Lee et al. 2011). In this study, a standard orthogonal array L_8 was used to examine a seven factor system at two levels, as given in Table 1. L and subscript 8 denote the Latin square and the number of experiments to be carried out, respectively. Surfactant concentration, the type of sugar (lactose or trehalose) and organic solvent (DCM and EA) were selected as important factors affecting the particle size and PDI of PCL-EFV nanoparticles. All experiments were performed in triplicate. The average particle size and PDI were considered to be the responses. Statistical analysis of the results were determined by analysis of variance (ANOVA) which was performed by using software for the design and analysis of Taguchi experiments (Qualitek-4 Version 4.75) to determine which factors had significant effect on the response parameters, and the optimum conditions.

Table 1 Taguchi L_8 experimental parameters and levels for preparation of PCL-EFV nanoparticles

2.2.2 *Preparation of PCL-EFV nanoparticles*

Nanoparticles were prepared by a modified double emulsion solvent evaporation technique discussed in our previous work (Semete et al. 2010). Briefly, an aqueous phosphate buffer solution (PBS) pH 7.4 or PVA (1 % or 2 %) was emulsified for a short period with a solution of 100 mg PCL and 100 mg EFV dissolved in 8 ml of solvent (EA or DCM), by means of a high speed homogeniser (Silverson L4R GX-10 model, Silverson Machines Limited, Buckinghamshire, United Kingdom) with a speed varying between 3000 and 5000 rpm. The resulting water-in-oil (w_1/o) emulsion was then transferred into a specific volume of an aqueous solution (w_2) of (1 % or 2 % wt/vol) PVA used as an emulsion stabilizer, (0.5 % or 1 %) PEG and (3% or 5 %) sugar. The double emulsion ($w_1/o/w_2$) obtained was directly fed into a bench top Buchi mini-spray dryer (Model B-290, BÜCHI Labortechnik AG, Flawil, Switzerland) and spray dried at 95 degrees Celsius, with an atomizing pressure varying between 6 and 7 bars. The solid PCL-EFV nanoparticles were collected and kept in the dessicator at room temperature. All experiments were prepared according to the suggested Taguchi design experiments trials as shown in Table 1.

2.2.3 *Particle size and particle size distribution*
The average particle size and particle size distribution of the nanoparticles were determined by dynamic laser scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom). For each sample, 1–3 mg of nanoparticles were suspended in deionised water, then vortexed and/or sonicated for a few minutes. Each sample was measured in triplicate.

2.2.4 *Morphology of nanoparticles*
The morphology of the optimised nanoparticles was analysed using a transmission electron microscopy (TEM, JEOL JEM-2100, Japan). The nanoparticles were stained with uranyl acetate solution before observation.

3. Results and discussion

3.1 Effect of formulation parameters on average particle size and PDI

An orthogonal array L_8 Taguchi design was used to explore seven control factors selected in the optimisation study of spray dried polycaprolactone-efavirenz (PCL-EFV) nanoparticles. Generally, in the case of seven factors with two levels, $2^7 = 128$ experiments should be conducted. However, Taguchi design reduces them to 8 experiments. Table 2 shows the results obtained from the study and the smallest average particle size was found in formulation 2, 4, 5 and 7 when EA was used as solvent. The particle size plays an important role in drug delivery and has significant pharmacological effects like degradation, uptake and clearance (Illum et al. 1982; Semete et al. 2010). EA is considered as one of the pharmaceutically acceptable less hazardous partially water miscible solvents over the conventional hazardous solvents, such as dichloromethane (Prasanna et al. 2010).

Table 2 Taguchi L_8 experimental conditions and measured average particle size and polydispersity index of PCL-EFV nanoparticles

Optimal formulation conditions and parameters having the most principal influence on the average particle size and the size distribution of PCL-EFV nanoparticles were identified using Taguchi design. The terms, “signal” and “noise” (S/N) in the Taguchi method signify the desirable and undesirable values for the output characteristic, respectively. The quality of characteristic deviating from the desired value is measured by S/N in the Taguchi method. It has been reported that there are three signals-to-noise ratios of common interest for the optimization of static problem (Bendell et al. 1989), namely N, S and B. In our case, to achieve optimal conditions, the-smaller-the-better quality characteristic was used as given in Eq. 1 for average particle size and the size distribution of nanoparticles (Bendell et al. 1989).

$$\frac{S}{N_s} = -10\log_{10}\frac{1}{n}\left(\sum y_i^2\right) \quad (1)$$

where y_i is the characteristic property that is "average particle size" and n is the number of measurements in each experiment.

The summarised results given in Table 3 illustrate the mean S/N ratio for each level of the parameters and the highest maximum-minimum values were seen for the organic solvent. As a result, it was found that the organic solvent is the significant parameter for affecting particle size and PDI on the formulation preparation of the nanoparticles. Since a double emulsion technique was used for the preparation of nanoparticles, organic solvent has been shown to be an important factor. Schubert and Müller 2005 illustrated that the particle size and size distribution are dependent on the emulsification process kinetics which are affected mostly on the miscibility of the organic solvent in water. In addition, Figure 1 showed the S/N graph response for particle size and this reaffirms Eq. 1 that the greater the S/N ratio, the smaller is the variance of the particle size around the desired value. Therefore the optimum condition for particle size is A1B1C2D1E2F2G1 as given in Table 5. In the case of PDI the following conditions were found as optimal. The results are clearly shown by the S/N response Table 4 and Fig. 1b. This implies that these parameters have most significant influence over PDI. Consequently the use of organic solvent played a major role in the particle size and PDI of PCL-EFV nanoparticles. The particle size decreased from 689 nm to 237 nm when EA was used compared to DCM.

Table 3 Average S/N ratio for each level of the parameters for particle size of PCL-EFV nanoparticles

Table 4 Average S/N ratio for each level of the parameters for PDI for PCL-EFV nanoparticles

Fig.1 S/N graph for a) particle size and b) PDI

Table 5 Taguchi L_8 proposed optimum conditions for formulating PCL-EFV nanoparticles

There are two types of statistical analysis of the experimental results in Taguchi design namely, analysis of variance (ANOVA) which is used to identify contribution of each experimental parameter into the results and analysis of means (ANOM) that determine the optimal parametric settings (Bendell et al. 1989). ANOVA was used in this study to further validate Taguchi results and about 68 % of the organic solvent showed the largest effect on reducing average particle size compared to other factors as illustrated in Table 6.

Table 6 Analysis of variance for particle size of PCL-EFV nanoparticles

3.2 Optimised formulation of PCL-EFV nanoparticles

The optimal level of the selected design parameters (Table 5) were used as the final Taguchi design step to calculate the predicted S/N ratio by the following equation 2:

$$\left[\frac{S}{N}\right]_{\text{predicted}} = \left[\frac{S}{N}\right]_m + \sum_{i=1}^{n}\left(\left[\frac{S}{N}\right]_i - \left[\frac{S}{N}\right]_m\right) \quad (2)$$

where $[S/N]_m$ is the total mean S/N ratio, $[S/N]_i$ is the mean S/N ratio at the optimal level, and n is the number of the main design parameters that affect the quality characteristic. The $[S/N]_m$ value of -50.27 was calculated from Table 2 for the particle size. $[S/N]_i$ values for the optimised method i.e. A1 = -49.53, B1 = -49.75, C2 = -49.71, D1 = -49.50, E2 = -47.86, F2 =

-49.62, G1 = -49.56 were obtained from Table 3, respectively. Equation 2 was utilised to determine the predicted S/N ratio by substituting the latter values as follows $[S/N]_{predicted}$ = -50.39 + [(-49.53 + 50.39) + (-49.75+ 50.39) + (-49.71+ 50.39) + (-49.50+ 50.39) + (-47.86+ 50.39) + (-49.62+ 50.39) + (-49.56 + 50.39)]. Thus, the obtained predicted S/N ratio for particle size was -43.90 and an estimated particle size was then calculated by substituting the predicted S/N ratio into Eq.(1) i.e. -43.90 = -10 log (y^2) and resulted in a particle size value of 157 nm. A similar method was used to calculate the predicted PDI value of 0.112 as shown in Table 7.

Table 7 Estimated and experimental results for particle size and PDI for PCL-EFV nanoparticles

The experimental results using the predicted optimal conditions for preparing PCL-EFV nanoparticles showed a good agreement as given in Table 7. There was as slight difference between the predicted and experimental particle size. Figure 2 shows the intensity average distribution graph of the optimised PCL-EFV nanoparticles giving a monomodal distribution with a mean diameter of 217 nm and PDI of 0.09, respectively obtained from Zetasizer. A spherical morphology of the prepared nanoparticles was shown in the TEM images (Fig. 3).

Fig.2 Particle size distributions by intensity as a function of particle size for PCL-EFV nanoparticles in water.

Fig.3 Transmission electron microscopy image of PCL-EFV nanoparticles, reported at 200× magnifications.

4. Conclusion

A Taguchi L_8 method showed to be a fast, simple and valuable tool in optimising the various parameters for the preparation of spray dried PCL-EFV nanoparticles. The organic solvent was found to be main parameter having significant effects on the particle size and polydispersity index. A good agreement was achieved between the predicted optimised Taguchi method and the experimental work done in the study. Further experiments on the physicochemical characteristics, stability studies, assessment of the *in vitro* and *in vivo* properties of the spray dried PCL-EFV nanoparticles to determine their suitability for oral delivery will be described in Part II of this paper.

Acknowledgements

The authors would like to thank CSIR Young Researcher's Fund and South African Department of Science and Technology for providing financial support for the work presented in this paper. Dr James Wesley-Smith is acknowledged for the TEM analysis of the nanoparticles.

References

Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach– 2010 revision http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf (Accessed 25 May 2012)

Bendell A, Disney J, Pridmore W A (1989), Taguchi Methods: Applications in World Industry, IFS Publications, UK,

Chiappetta D A, Hocht C, Taira C, Sosnik A (2010). Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailability. Nanomedicine; 5 11-23.

Chiappetta DA, Hocht C, Taira C, Sosnik A (2011) Oral pharmacokinetics of the anti-HIV efavirenz encapsulated within polymeric micelles. Biomaterials; 32 2379-87.

Destache CJ, Belgum T, Goede M, Shibata A, Belshan MA (2010) Antiretroviral release from poly(DL-lactide-co-glycolide) nanoparticles in mice. Journal of Antimicrobial Chemotherapy;.65 2183-2187.

Destache C J, Belgum T, Christensen K, Shibata A, Sharma A, Dash A (2009), Combination antiretroviral drugs in PLGA nanoparticle for HIV-1, BMC Infections. Diseases; 9 198.

Govender T, Ojewole E, Naidoo P, Mackraj I (2008) Polymeric Nanoparticles for Enhancing Antiretroviral Drug Therapy. Drug Delivery;15 493-501.

Heng, K. Ogawa, D.J. Cutler, H.K. Chan, J.A. Raper and L. Ye *et al* (2009)., Pure drug nanoparticles in tablets: what are the dissolution limitations?. Journal of Nanoparticle Research; 12 1743–1754.

Ilium L,Davis S S, Wilson C G (1982) Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape. International Journal of Pharmaceutics; 12 135–146

Jahanshahi M, Sanatiand M H , Babaei Z (2008) Optimization of parameters for the fabrication of gelatin nanoparticles by the Taguchi robust design method, Journal of Applied Statistics; 35 1345–1353.

Kateb B, Chiu K, Black K L, Yamamoto V, Khalsa B, Ljubimova J Y, Ding H, Patil R, Portilla-Arias J A, Modo M, Moore D F, Farahani K, Okun M S, Prakash N, Neman J, Ahdoot D, Grundfest W, Nikzad S, Heiss J D (2011) Nanoplatforms for constructing new approaches to cancer treatment, imaging, and drug delivery: What should be the policy? .NeuroImag*e*; 54 S106-S124

Kim K D, Choi D W, Choa Y H, Kim H T (2007) Optimization of parameters for the synthesis of zinc oxide nanoparticles by Taguchi robust design method.Colloids and Surfaces A: Physicochemical and Engineering Aspects ;311 170–173.

Kovochich M, Marsden MD, Zack JA (2011), Activation of Latent HIV Using Drug-Loaded Nanoparticles PLoS ONE 6(4): e18270. doi:10.1371/ journal.pone.0018270

Lee S H, Heng D, Ng W K, Chan H, Tan, R B H (2011) Nano spray drying: A novel method for preparing protein nanoparticles for protein therapy, International Journal of Pharmaceutics; 403 192-200.

Marzolini C, Telenti A, Decosterd L A, Greub G, Biollaz J, Buclin T (2001) Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS 15; 71-75

Neves J D, Amiji MM, Bahia M F,SARMANTO B (2010) Nanotechnology-based systems for the treatment and prevention of HIV/AIDS. Advanced Drug Delivery Reviews; 62 458-477.

Nowacek A S, Balkundi S, McMillan J, Roy U, Martinez-Skinner A, Mosley R L, Kanmogne G,Kabanov A V, Bronich T,Gendelman H E (2011) Analyses of nanoformulated antiretroviral drug charge, size, shape and content for uptake, drug release and antiviral activities in human monocyte-derived macrophages. Journal of Controlled Release; 150 204–211.

Palmieri G F, Wehrle P, (1997) Evaluation of ethylcellulose-coated pellets optimized using the approach of Taguchi. Drug Development and Industrial Pharmacy*;* 23 1069–1077.

Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. August 11, 2011; pp 1-268. Available at http://aidsinfo.nih.gov/ContentFiles/lvguidelines/PediatricGuidelines.pdf.(Accessed 25 May 2012)

Prasanna L, Giddam A K (2010) Nano-suspension technology: A review. International Journal of Pharmacy and Pharmaceutical Sciences; 2:35-40

Santos-Magalhães N S, Mosqueira V C (2010) Nanotechnology applied to the treatment of malaria. Advanced Drug Delivery Reviews; 62 560–575

Schubert MA, Goymann-Müller CC (2005). Characterisation of surface-modified solid lipid

nanoparticles (SLN): Influence of lec-ithin and non-ionic emulsifier. European Journal of Pharmaceutics and Biopharmaceutics; 61:77–86.

Semete B, Booysen L, Lemmer Y., Kalombo L, Katata L., Verschoor J, Swai H. (2010) In vivo evaluation of the biodistribution and safety of PLGA nanoparticles as drug delivery systems. Nanomedicine: Nanotechnology, Biology, and Medicine; 6 662–671.

Semete B, Kalombo L, Katata L, Swai H (2010) Nano-drug delivery systems: Advances in TB, HIV and Malaria treatment. Smart Biomol. Medicine book In press, Chapter 2

Shah L K, Amiji M M (2006) Intracellular Delivery of Saquinavir in Biodegradable Polymeric Nanoparticles for HIV/AIDS. Pharmaceutical Research;23 11

Sharma P. Gaeg S. (2010) Pure drug and polymer based nanotechnologies for the improved solubility, stability, bioavailability and targeting of anti- HIV drugs. Advanced Drug Delivery Reviews; 62 491-502.

Varshosaz J, Tavakoli N, Minayian M, Rahdari N (2009), Applying the Taguchi Design for Optimized Formulation of Sustained Release Gliclazide Chitosan Beads: An In Vitro/In Vivo Study,AAPS PharmSciTech; 10 DOI: 10.1208/s12249-009-9191-8