Development of poly-ε-caprolactone nanoparticles for lung cancer treatment

Faisalina Ahmad Fisol [1,2], Habibah Abd. Wahab [1]

[1] School of Science Pharmacy, Universiti Sains Malaysia, Penang, Malaysia
[2] Natural Product and Drug Discovery Centre, Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPharm), National Institute of Biotechnology Malaysia (NIBM), Ministry of Science, Technology and Innovation (MOSTI), Penang, Malaysia
*Email: habibahw@usm.my

Abstract— Nanoparticles (NPs) were developed in this research using poly-ε-caprolactone (PCL) biodegradable polymer as nanocarrier for treatment of lung cancer. The optimized PCL nanoparticles formulation were developed by screening the use of different tensioactives (sorbitan monostearate, polysorbate 80, polyvinyl alcohol, sodium caproyl hyaluronate and sodium oleyl hyaluronate) in modified nanoprecipitation method. PCL nanoparticles were then characterized for their physicochemical characteristics such as size and size distribution, surface charge and morphology. Optimised PCL nanoparticles developed in this study gave the best results with particles size 87.84 nm and low PDI of 0.116. The surface charge of the nanoparticles were -24.1 mV. PCL nanoparticles were found stabled up to one month stability in different storage conditions. These results suggested that PCL nanoparticles in this study could be successful nanocarrier for lung cancer hydrophilic drug such as docetaxcel for lung cancer treatment.

Keywords: Poly-ε-caprolactone nanoparticles, nanocarrier, pulmonary delivery system, lung cancer.

I. INTRODUCTION

The therapy of many solid tumours is characterized by a multimodal approach in which surgery, radiation therapy and chemotherapy are combined to obtain the best therapeutic outcome for patients [1]. Despite the advances in this multimodal approach and combination regimens of various anticancer drugs, survival rates for a number of tumours such as lung, ovarian and brain cancers, have not improved as expected [2, 3, 4]. In many malignancies, surgery alone is not curative as the tumour cells infiltrate surrounding tissues and cause relapses.

Futhermore low drug concentrations at the tumour site appear to be one of the major challenges in systemic chemotherapy, leading to drug-resistance and recurrence of disease [5]. Recently, pharmaceutical nanotechnologies have shown to be able to improve the biopharmaceutical characteristics of drugs. Nanoformulation had been demonstrated that it can modify the drug pharmacokinetics by controlling the release and distribution of the active compound. Generally the formulation of drugs in nanoparticles is expected to increase the efficacy and decrease the side effects of the drug. These properties have been exploited clinically in the first generation of nanomedicines, including liposomes and albumin-based nanoparticles, which had been approved for the treatment of life threatening diseases such as tumours resistant through conventional chemotherapy.

Poly-ε-caprolactone (PCL) is United States Food Drug Administration (US FDA) approved biodegradable and biocompatible polyester. This polymer has been widely used to produce nanoparticles and micelles for the encapsulation of hydrophobic anticancer drugs, such as taxanes [6]. Docetaxel-loaded PLGA nanoparticles were produced by solvent displacement method, also referred to as nanoprecipitation [7].

The formulation of polyester biodegradable nanoparticles will help the loading of poorly water-soluble anti-cancer drugs into nanocarrier system. The anticancer drug-loaded polymeric nanoparticles will be released in a controlled and prolonged manner by meditating the interaction with the tumour cells. This nanoparticle production technique will be characterized by simplicity of the process, high reproducibility, low cost and ease to scale-up. Furthermore, the use of well-characterized and medical grade preformed polymers can substitute the use of polymerization steps and toxic solvents.

In the nanoprecipitation method, the organic solvent (generally acetone) is miscible with water and the precipitation of the polymer occurs directly upon mixing the organic solution of polymer and drug with the aqueous phase. The organic solvent can also be rapidly and efficiently removed by rotary

evaporation under vacuum. In order to optimize the formulation, several parameters identified as critical for determining the size and surface charge of particles [8].

The nanoparticles produced will be characterized for its physicochemical characteristics such as mean particle size and size distribution (dynamic light scattering), surface charge (phase analysis light scattering) and morphology (scanning electron microscopy, atomic force microscopy). The physico-chemical properties of the nanoparticles are directly linked to stability, cellular uptake, distribution and drug release [9, 10].

Prior to the final production of the drug delivery system, the chemical and physical stability of the nanoparticles will be studied under accelerated stability conditions (40°C, 75% relative humidity (RH)) to highlight any potential stability issues such as drug degradation or size increase/agglomeration of the nanoparticles.

II. MATERIALS AND METHODS

A. Materials

Poly-ε-caprolactone M_w 14000 (Sigma-Aldrich, Germany).The tensioactives being used to formulate the nanoparticles were polysorbate 80 (Sigma-Aldrich, Germany), polyvinyl alcohol M_w 9,000-10,000 Da (Sigma-Aldrich, Germany), L-α-phosphatidylcholine from soybean Type II-S (Sigma-Aldrich, Germany), sorbitan monostearate (Sigma-Aldrich, Germany), sodium oleyl hyaluronate 5-20 kDa (Contipro, France), sodium caproyl hyaluronate 10-200 kDa (Contipro, France). The oil core is D-α-Tocopherol polyethylene glycol 1000 succinate BioXtra, water soluble vitamin E conjugate (Sigma-Aldrich, Germany). The general chemicals are acetone (HMBG) and ethanol (HMBG).

B. Methodology

The nanoparticles were prepared using a methodology previously reported for lipid-core nanocapsules stabilized with polysorbate 80 [11]. The preparation of the lipid core were initiated by preparing the organic phase containing Poly-ε-caprolactone (0.1 g), sorbitan monostearate (0.004 g), and vitamin E TPGS (0.12 g) dissolved in acetone (25.0 mL). In parallel, an ethanolic solution (5.0 mL) containing lecithin (0.03 g) will be prepared and poured into the organic phase. This mixture will then be poured into aqueous solution (50.0 mL) containing polysorbate 80 (0.08 g) under moderate magnetic stirring at 40ºC. After 10min, the mixture will be evaporated under reduced pressure to eliminate the acetone and to concentrate the suspension (near 9.0 mL). The final volume will be adjusted in a volumetric flask to 10.0 mL.

C. Optimisation of tensioactives

The effect of surfactant types and amount were studied with polysorbate 80, PVA, sorbitan monostearate, sodium oleyl hyaluronate and sodium caparol hyaluronate.

The different formulations were consisted of 100.0 mg PCL, 25.0 mL acetone, 0.12 g vitamin E TPGS and 0.04 g hydrophobic surfactant for the organic phase and 50.0 mL with 0.08 g hydrophilic surfactant for the aqueous phase. The effect of different tensioactives combination were evaluated.

D. Physicochemical Characterizations

The droplet size of the prepared nanoparticles was measured using Zetasizer (Nano ZS, Malvern Instrument Ltd., UK) at 25 °C using dynamic light scattering technique, scattered at an angle 173 °. The Z-average size were obtained by the cumulants analysis. This mean size is intensity mean and directly calculate from the signal intensity. The sample were prepared and syringed injected into a folded capillary cell. The operating measurements were; material RI 1.59, material absorption 0.01, dispersant: water, dispersant RI 1.330, viscosity 0,8872, temperature 25°C and analysis time 10 minutes per sample. Size measurements were performed in triplicate.

The obtained suspension were determined for polydispersity using the same measurement process as described above. This analysis gave only two values, a mean value for the size and a width parameter known as Polydispersity Index (PDI). PDI measurements were performed in triplicate.

Zeta potential were measured using a ZetaSizer Nano-ZS 90 Malvern Instruments, Worcestershire, UK). The ZetaSizer Nano-ZS 90 calculates the zeta potential by determining the electrophoretic mobility and then applying the Henry equation.

The obtained suspension were determined for zeta potential analysis using the same measurement process as described above. The operating measurements were; dispersant name: water, dispersant RI 1.330, viscosity 0.8871, dispersant dielectric constant 78.5, temperature 25°C and zeta runs 12. Each measurement were done in triplicate.

The shape and surface morphology of the formulated nanoparticles were investigated by the scanning electron microscopy (SEM) (Leo Supra 50VP Field Emission SEM, Carl-Ziess SMT, Oberkochen, Germany) SEM requires prior coating of the sample with gold, which were performed in a gold coated machine (SC515, Polaron). Before observation, the samples were diluted (1:5) and dries in room temperature overnight before they were

fixed on a double-sided sticky tape that were sticked on the standard sample stand.

The suspensions of PCL nanoparticles were evaluated for their stability at room temperature, 4ºC and 35ºC. The average particle diameter and polydispersity index of these suspensions stored at different temperature were determined at 1, 7, 14 and 30 days. All suspensions were analyzed in triplicate.

III. RESULTS AND DISCUSSION

The influence of different tensioactives on particle size, PDI and particles charge

As shown in **Fig. 1**, the particles size of all formulations are below 200 nm. Combination of sorbitant monostearate and PVA in F4 gave the optimal formulation characterization (87.84±1.01 nm, PDI 0.116±0.02 and zeta potential value -24.1±1.44 mV). These tensioactives combination were chosen as the most optimal combination due to their amphiphilic character, ability to mediate the interaction between the polymer thus avoiding the formation of flakes and precipitates and these natural emulsifiers may have little side effects and better performance in preparation of polymeric nanoparticles for clinical administration of anticancer drugs. The present of sorbitan monostearate and PVA influenced the average diameter size and size distribution of PCL nanoparticles and have significant effect on the elimination of flakes/polymeric mass in the suspension.

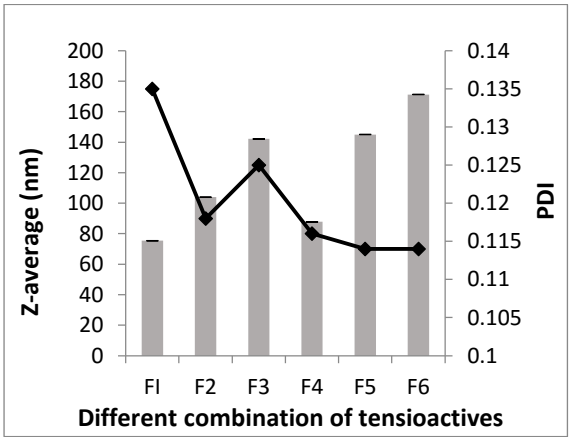

Fig. 1. The influence of different combination of tensioactives on particle size and PDI.
F1: sorbitan monostearate and polysorbate 80
F2: sodium caproyl hyaluronate and polysorbate 80
F3: sodium oleyl hyaluronate and polysorbate 80
F4: sorbitan monostearate and PVA
F5: sodiym caproyl hyaluronate and PVA
F6: sodium oleyl hyaluronate and PVA

The polydispersity index range 0 to 1, values between 0 and 0.2 indicate a narrow and homogenous nanoparticles size distribution while values larger than 0.2 indicated a more broadly dispersed nanoparticles size distribution or even the presence of different particles populations. PDI values from all formulations showed homogeneous nanoparticles with PDI value below 0.2.

The value of zeta potential depends on the charges present on the particle surface (Nernst potential). An increase or decrease in the surface charged groups will result in a higher or lower zeta potential[19]. In PCL nanoparticles produced with different tensioactives, a change in the number of surface charges would be due to the incorporation of ionic surfactant and/or its adsorption on the surface. **Fig 2.** showed the zeta potential of PCL nanoparticles obtained with different tensioactives. Values of zeta potential for PCL nanoparticles were measured in the range of -9.5 mV to -24.1 mV.

However, formulation F4 showed highest zeta potential value. The higher value of particles surface charge showed greater stability of the formulation.

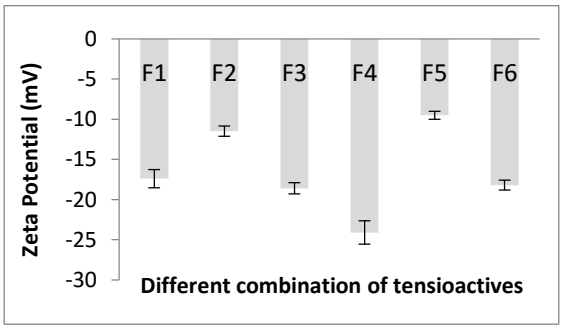

Fig. 2. The influence of different combination of tensioactives on particle charge.
F1: sorbitan monostearate and polysorbate 80
F2: sodium caproyl hyaluronate and polysorbate 80
F3: sodium oleyl hyaluronate and polysorbate 80
F4: sorbitan monostearate and PVA
F5: sodiym caproyl hyaluronate and PVA
F6: sodium oleyl hyaluronate and PVA

Morphology of PCL nanoparticles

A representative SEM image of PCL nanoparticles prepared was shown in **Fig. 3.** The nanoparticles were found by SEM to be spherical in shape. As expected, the average size of the nanoparticles derived from the micrograph was apparently smaller than that determined using particle sizer (Zeta sizer Nano Series, Nano ZS, Malvern, UK). This could be explained by the particle size from the Zetasizer measurement. The size of the particles plus an aqueous layer that surrounds the particles moved together with the particles determining the hydrodynamic diameter as what is measured by

dynamic light scattering. The NPs showed smooth surface within the SEM resolution level.

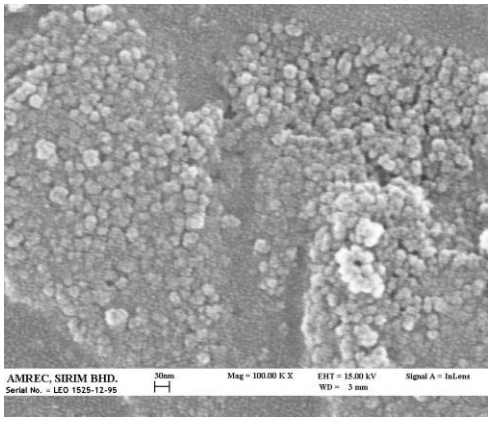

Fig. 3. SEM image of PCL nanoparticles.

Stability study of PCL nanoparticles

30 days stability study of PCL nanoparticles were performed by placing PCL nanoparticles at 25°C (room temperature), 4°C (refrigerator) and 35°C (incubator). This study was carried out to investigate the storage conditions effect on PCL nanoparticles physicochemical properties such as size, PDI and surface charge. All the formulations showed no marked differences in the mean particle size of the nanoparticles. Average size increased a little with respect to the initial values, probably because of particle aggregation. This type of phenomenon has been reported earlier by Pignatello et al. and Das et al. [12, 13]. However, all batches showed mean sizes below 150 nm which were suitable for pulmonary delivery. PDI result showed very homogeneous nanoparticulate suspensions which were below 0.2. Good zeta potential values were also obtained for most preparations. The physicochemical characteristics indicated that PCL nanoparticles were stable and that the nanoparticles could be stored up to 30 days in different temperature condition from 4°C to 35°C without major effect on their characteristics.

IV. CONCLUSIONS

The present study demonstrated that PCL nanoparticles were developed using sorbitan monostearate and polyvinyl alcohol as tensioactives. The optimal composition of PCL nanoparticles were 0.05% of sorbitan monostearate and 0.10% of polyvinyl alcohol to produce 87.84±1.01 nm particles size, PDI 0.116±0.02 and zeta potential -24.1±1.44 mV optimised PCL nanoparticles. Nanoparticles prepared showed suitability as nanocarrier for efficient pulmonary delivery of lung cancer treatment.

Acknowledgement

This work was supported financially by the grant from NanoMITe Long Term Research Grant Scheme under Malaysia Ministry of Higher Learning.

References

[1] W. Eberhardt and S. Korfee, "New approaches for small-cell lung cancer: local treatments," *Cancer Control*, vol. 10, no. 4, pp. 289-296, 2003.

[2] Sawyer, A. J., Piepmeier, J. M. and Saltzman, W. M., "New methods for direct delivery of chemotherapy for treating brain tumors," *Yale J Biol Med*, vol. 79, pp. 141-152, 2006.

[3] Limsoo P. et al, "Drug release mechanism of paclitaxel from a chitosan–lipid implant system: Effect of swelling, degradation and morphology," *Eur J Pharm Biopharm*, vol. 69, pp. 149-157, 2008.

[4] Zarogoulidis P. et. al, "Inhaled chemotherapy in lung cancer: future concept of nanomedicine," *Int J Nanomedicine*, vol. 7, pp. 1551-1572, 2012.

[5] Minchinton, A. I. and Tannock, I. F. , "Drug penetration in solid tumours," *Nat Rev Cancer*, vol. 6, pp. 583-592, 2006.

[6] Gaucher, G., Marchessault, R. H. and Leroux, J.C., "Polyester-based micelles and nanoparticles for the parenteral delivery of taxanes," *J Control Release*, vol. 143, pp. 2-12, 2010.

[7] C. Vauthier and K. Bouchemal, "Methods for the preparation and manufacture of polymeric nanoparticles," *Pharm Res*, vol. 26, p. 1025–1058, 2008.

[8] C. E. Mora-Huertas, H. Fessi and A. Elaissari, "Influence of process and formulation parameters on the formation of submicron particles by solvent displacement and emulsification-diffusion methods critical comparison. Adv Colloid Interface Sci 163, 90–," *Adv Colloid Interface Sci*, vol. 163, pp. 90-122, 2011.

[9] S. M. Moghimi, A. C. Hunter and T. L. Andresen, "Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective," *Annu. Rev. Pharmacol. Toxicol. 52, 481–503 (2012).*, vol. 52, pp. 481-503, 2012.

[10] G. Mittal, D. K. Sahana, V. Bhardwaj and M. N. V. Ravi Kumar, "Estradiol loaded PLGA nanoparticles for oral administration: effect of polymer molecular weight and copolymer composition on release

behavior in vitro and in vivo," *J Control Release,* vol. 119, pp. 77-85, 2005.

[11] Jager E. et al, "Sustained release from lipid-core nanocapsules by varying the core viscosity and the particle surface area," *J. Biomed. Nanotechnol,* vol. 5, pp. 130-140, 2009.

[12] Pignatello, R, Bucolo, C, Ferrara, P, Maltesa, A, Puleo, A and Puglisi, G, "PIGNATELLO REudragit RS100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen," *Eur J Pharm,* vol. 16, pp. 53-61, 2002.

[13] Das,S, Suresh, P.K and Desmukh, R, "Design of Eudragit RL 100 Nanoparticles by nanoprecipitation method for ocular drug delivery. Nanomedicine," *Nanotechnology, Biology, and Medicine,* vol. 6, pp. 318-323, 2010.