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Formulation hydrophobicity on *in vitro* drug release of crizotinib-loaded PLGA nanoparticles

Hsi-Wei Yeh [1], Jing He [2], Da-Ren Chen [1,*]

[1]Particle laboratory, Department of Mechanical and Nuclear Engineering, Virginia Commonwealth University, 401 W. Main Street, Richmond, VA 23284, USA

[2]Department of Energy, Environmental and Chemical Engineering, Washington University in St. Louis, One Brooking Drive, St. Louis MO 63130, USA

*corresponding author e-mail address:***dchen3@vcu.edu**

ABSTRACT

The effect of formulation hydrophobicity on the drug release profiles of drug-loaded nanoparticles was investigated in this study. Single capillary electrospray was used to prepare drug-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles. Crizotinib was selected as the model drug because of its hydrophobicity to the spray solvent used in this study. The sizes of as-produced particles were characterized by the Scanning Electron Microscope (SEM). Prepared nanoparticles were found to be around 50 and 100 nm. The *in vitro* drug release profiles were measured by high-performance liquid chromatography (HPLC). The drug release profiles of particles prepared in various formulations were compared to investigate the effect of particle hydrophobicity on the drug release. This work provides a new perspective to control the drug release rate of PLGA nanoparticles for the applications of controlled drug release.

Keywords: *anticancer, drug delivery, electrospray, nanoparticles, PLGA.*

1. INTRODUCTION

Sufficient drug concentration at the sites of action is the key to provide desired therapeutic responses against a diseasefor a pharmaceutical product. To augment the amount of drug molecules being delivered to the action sites, researchers have applied the formulation technology to facilitate both the drug distribution to active sites and the absorption into human bodies, especially for drug molecules with unfavorable biopharmaceutical properties such as the hydrophobic nature of the majority of anticancer therapeutics from the viewpoint of pharmacokinetic principles. Crizotinib, an inhibitor of anaplastic lymphoma kinase (ALK)-positive tumors, is currently considered a superior treatment indicated to treat non-small cell lung cancer (NSCLC) patients. The efficacy of Crizotinib however reduces after several administrations because of the drug resistance [1]. A safe and effective sustained drug delivery of Crizotinib is thus expected to maintain the effectiveness of Crizotinib against lung cancers.Poly (lactic-co-glycolic acid) (PLGA)-based biodegradable drug nano-carriers (in the particle form) have attracted great attention for the anticancer drug administration [2]. The advantage of such formulations compared with the conventional ones includes the maintenance of plasma drug concentration within the therapeutic window for a longer duration [3], reduction in the dosing frequency, drug release at/near the intended site of action, better capability to cross biological membranes [2], and the protection of drug molecules from binding to plasma lipoproteins for providing the greater efficacy and less systemic side effect.Among all existing particle formulation techniques, electrospray (ES) is promising for producing monodisperse PLGA nanoparticles with high drug encapsulation efficiency [4]. Previous research has been performed to study the *in vitro* drug release from drug-loaded PLGA particles, prepared by electrospray, as the function of particle size [4,5,6]. Limited research has investigated the additive factors that may affect the drug release of loaded PLGA nanoparticles prepared by an ES process.This study demonstrates the importance of formulation hydrophobicity on the drug release of loaded PLGA nanoparticles and provides a general particle design guideline for the application of PLGA nanoparticles in the controlled release of anticancer drugs.

2. EXPERIMENTAL SECTION

2.1.Particle Formulation.

Drug-loadedPLGA nanoparticles were produced by a single capillary electrospray system. Drug molecules (0.3-1.2 g/l) and PLGA molecules (0.45-2 g/l) with/without certain amounts of polyethylene glycol-poly (lactic-co-glycolic acid) (PEG-PLGA, PEG/PLGA: 5000/10000, LA/GA: 50/50, Mw: 15,000 g/mol) (0.4 g/l) or surfactants (0.05 g/l) were dissolved in the solvent mixture of acetonitrile and dimethyl sulfoxide (DMSO) at the volumetric ratio of 1:1 to form spray solutions. The electric conductivity of spray solutions were measured by the Oakton PC 300 Hand-held Conductivity Meter and ranged from 50 to 400 μS/cm. To produce drug-loaded particles via ES, spray solutions were pumped through a capillary, which was maintained at a positive high voltage (by a programmable syringe pump: Harvard PHD 200 Infusion). By varying the ES operational parameters, such as the voltage applied and the feeding flowrate of spray solutions, monodisperse drug-loaded PLGA particles were produced when operated at the cone-jet mode in which a liquid conical meniscus was established at the capillary tip and a jet was emitted from the cone apex [7].

2.2. Particle Characterization.

To characterize the size and morphology of produced particles, samples were collected on the electrically-grounded

substrate and then analyzed by the Scanning Electron Microscope (SEM) (FEI, Nova 2300).

2.3.Determination of Drug Release Profile.

Particles were placed in a centrifuge tube containing 10 ml buffer (1X PBS solution). The tube was continuously shaken by using a nutating mixer (set at 24 rpm) at room temperature. At pre-determined time intervals, the top 9-ml supernatant solution was taken for the drug release measurement [2, 8, 9, 10, 36] after it had been centrifuged at 10,000 rpm for 15 minutes [8]. Crizotinib in the 9-ml supernatant solution was extracted and mixed with a 1-ml methanol solvent by flowing through the Empore C18 standard density solid phase extraction cartridge [2, 11]. The resultant solutions were analyzed by the high-performance liquid chromatography (HPLC).

2.4. HPLC Analytical Method for Drug Detection.

Agilent 1100 Series HPLC system with a LC column (4.6×150 mm, 3.5 μm) was employed to quantify the amount of released drug in the release media at pre-determined time intervals. The mobile phase composed of 28% acetonitrile and 72% Na_2HPO_4 (0.015 M) with 0.01% (v/v) Triethylamine at pH=7.4. The flowrate of the mobile phase was fixed at 1 ml/min. The injection volume of each sample was 60 μl. The UV/VIS detector with the wavelength set at 265 nm was employed to measure the elute exiting the column [16].

Table 1. Summary of studied cases for the drug release profile of drug-loaded PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol and L/G: 85/15, Mw: 50,000-75,000 g/mol) nanoparticles (the particle size: 100 nm) with or without PEG-PLGA (PEG/PLGA: 5000/10000, L/G: 50:50). The electrical conductivity and feeding flowrate of spray solutions was 100 μS/cm and 0.4 μS/cm, respectively.

Spray conditions	Formulation ingredients		
	Concentration of Crizotinib	Concentration of PLGA	Concentration of PEG-PLGA
Case #1	1.2 g/l	1.6 g/l PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol)	0.4 g/l PEG-PLGA (PEG/PLGA: 5000/10000, L/G: 50:50)
Case #2	1.2 g/l	2 g/l PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol)	0 g/l
Case #3	1.2 g/l	1.6 g/l PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol)	0.4 g/l PEG-PLGA (PEG/PLGA: 5000/10000, L/G: 50:50)
Case #4	1.2 g/l	2 g/l PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol)	0 g/l

3. RESULTS SECTION

Poly (ethylene glycol) (PEG) is a hydrophilic stealth material widely proposed in the literature to modify the surface property of drug delivery vehicles for extending their half-lives in the systemic circulation [12, 13]. With the consideration of water infiltration into drug-loaded polymer particles, it is important to investigate the effect of formulation hydrophobicity on the drug release profile. In this part of the study, the effect of PEG presence in the formulation on the drug release profile was investigated by comparing the drug release of sample nanoparticles prepared in four different formulations (summarized in Table 1). The sizes of particles in all these studied cases were kept at approximately 100 nm as measured by the SEM images (shown in Fig. 1). Fig. 2 gives the drug release profiles of drug-loaded PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol) nanoparticles prepared in Cases #1 and #2. It was observed that the drug release rate was enhanced by replacing 20% mass percentage of the total PLGA amount in the formulation with PEG-PLGA (PEG/PLGA: 5000/10000, L/G: 50:50). The reason for this observed phenomenon can be attributed to the decreased hydrophobicity of polymer matrix after adding PEG-PLGA. The buffer solution thus had the greater potential to penetrate into the nanoparticles with 20% PEG-PLGA for dissolving the embedded drug molecules and enhancing the drug concentration gradient required for driving the drug molecules out from the matrix (by the diffusion process), resulting in the higher drug release rate. The similar finding was also observed in the drug release profiles of PLGA 85/15 particles prepared in Cases #3 and #4 (shown in Fig. 3). Further, because of stronger affinity between the hydrophobic Crizotinib and PLGA with the L/G ratio of 85/15 [14], the release rate of drug-loaded PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol) particles were less than that of PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol) particles. Dipalmitoylphosphatidylcholine (DPPC) and d-Alpha Tocopheryl Polyethylene Glycol 1000 Succinate (TPGS) are the surfactants widely used in the pharmaceutical formulations for the enhancement of drug bioavailability.

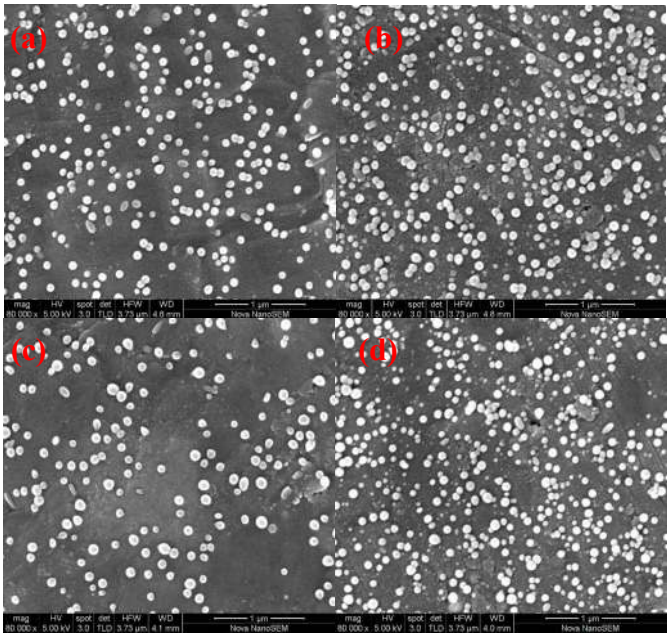

Figure 1. SEM images of drug-loaded PLGA nanoparticles prepared in Cases #1(a); #2 (b); #3(c); and #4(d).

Table 2. Formulation of cases studied for the effects of surfactant in PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol) nanoparticles (particle size: 50 nm), produced by a single nozzle electrospray system, on the drug release profile.

Spray conditions	Formulation ingredients			Process parameters	
	Concentration of Crizotinib	Concentration of PLGA	Concentration of surfactant	Conductivity of spray solution	Feeding flowrate of spray solution
Case #5	0.3 g/l	0.45 g/l PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol)	0.05 g/l TPGS	344 μS/ cm	0.4 μl/ min
Case #6	0.3 g/l	0.45 g/l PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol)	0.05 g/l DPPC	358 μS/ cm	0.4 μl/ min
Case #7	0.4 g/l	0.67 g/l PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol)	0 g/l	136 μS/ cm	0.4 μl/ min

In this study, the effects of DPPC and TPGS on the drug release were also investigated using PLGA (L/G: 85/15, Mw:

50,000-75,000 g/mol) nanoparticles with the sizes of 50 nm (Fig. 4).

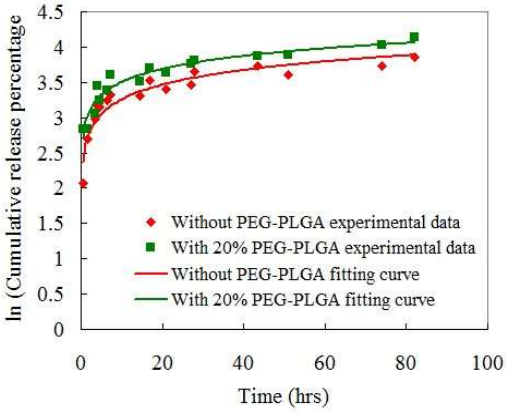

Figure 2. Release of Crizotinib PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol) nanoparticles (particle size: 100 nm) with or without 20% PEG-PLGA of mass percentage of the total PLGA amount in the particles.

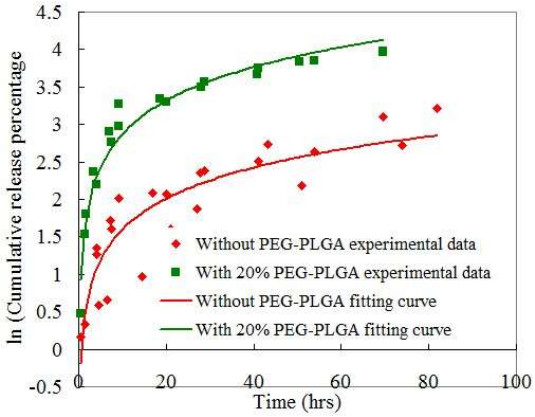

Figure 3. Release of Crizotinib PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol) nanoparticles (particle size: 100 nm) with or without 20% PEG-PLGA of mass percentage of the total PLGA amount in the particles.

Table 2 summarizes the detail of formulation cases in this part of the study. Fig. 5 shows the drug release profiles of as-prepared PLGA (85/15) nanoparticles. It is evidenced that PLGA nanoparticles with 10% TPGS in mass percentage of the total PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol) in the formulation exhibits a faster drug release rate than those without any surfactant in the formulation. The increased drug release rate of particles with 10% TPGS can be attributed to the decreased hydrophobicity caused by the PEG chains within TPGS molecules. As a result the inclusion of TPGS in PLGA particles expedites the release rate of Crizotinib. On the contrary the effect of DPPC presence in the formulation on the drug release profile was deviated from what expected. Previous works indicated that the DPPC presence in the formulation could reduce the rate of drug release from PLGA microspheres by decreasing their porosity [8]. However, in our study, the release profiles of PLGA (L/G: 85/15, Mw: 50,000-70,000 g/mol) nanoparticles with and without the DPPC in the formulation were in fact in-distinguishable.

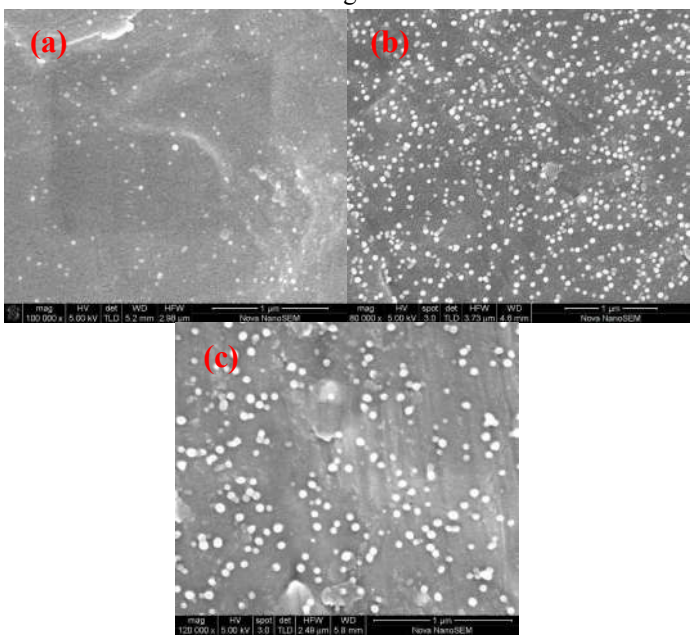

Figure 4. SEM images of drug-loaded PLGA nanoparticles prepared in Case #5 (a) ; #6 (b); and #7 (c).

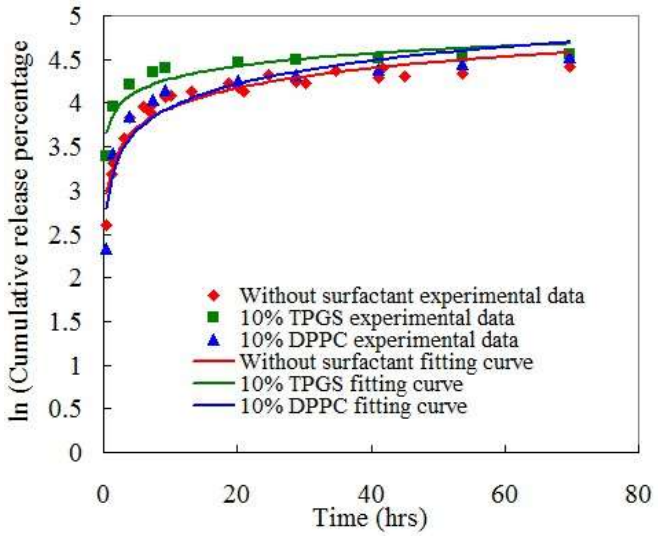

Figure 5. Release profiles of Crizotinib from PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol) nanoparticle carriers (the particle size: 50 nm) with/without 10% surfactant in the total PLGA amount.

The possible reason for the above observation might be on the sizes of particles tested in this study. As the size of particles is reduced to the nanometers the effect of DPPC on reducing the carrier matrix porosity may be insignificant. The expected decrease of the drug release rate was thus not measurable.

4. CONCLUSIONS

Crizotinib-loaded PLGA nanoparticles were prepared by the single capillary electrospray when operated at the cone-jet mode for monodisperse particle generation. Measured by the SEM, the sizes of as-prepared particles were around 50 and 100 nm. The effects of additives in different formulations of PLGA nanoparticles on the drug release profiles were investigated. Two categories of additives, i.e., stealthy substances and surface active substances, were evaluated. For the stealthy substance, the existence of PEG-PLGA (PEG/PLGA: 5000/10000, L/G: 50:50) in both PLGA (L/G: 50/50, Mw: 24,000-38,000 g/mol) and PLGA (L/G: 85/15, Mw: 50,000-75,000 g/mol) nanoparticles increased the drug release rate by decreasing the hydrophobicity of particles, thus increasing the penetration of buffers into the polymer matrix. As for the surface active substances, the incremental increase of drug release rate was observed in the cases of 50-nm PLGA (L/G: 85:15, Mw: 50,000-75,000 g/mol) particle carriers with the TPGS presence in the formulation (due to the PEG chains in the TPGS molecules). However, the presence of DPPC in the formulation

was unable to slow the drug release rate of these 50-nm PLGA particles. This study demonstrates that the drug release rate of loaded PLGA nanoparticles can be controlled by varying the formulation hydrophobicity via including selected additives. The obtained result can serve as the general direction in designing drug-loaded nanoparticles for the application of controlled drug release.

5. REFERENCES

[1] D. Melo-Diogo, V.M. Gaspar, E.C. Costa, A.F. Moreira, D. Oppolzer, E. Gallardo, I.J. Correia, Combinatorial delivery of Crizotinib–Palbociclib–Sildenafil using TPGS-PLA micelles for improved cancer treatment, *European Journal of Pharmaceutics and Biopharmaceutics*, 88, 3, 718–729, **2014**.

[2] Y. Dong, S.S. Feng, Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs, *Biomaterials*, 25, 14, 2843–2849, **2004**.

[3] L.S. Nair, C.T. Laurencin, Polymers as biomaterials for tissue engineering and controlled drug delivery, *Advances in Biochemical Engineering / Biotechnology*, 102, 47–90, **2006**.

[4] Y.H. Lee, M.Y. Bai, D.R. Chen, Multidrug encapsulation by coaxial tri-capillary electrospray, *Colloids and Surfaces B: Biointerfaces*, 82, 1, 104–110, **2011**.

[5] S. Hao, Y. Wang, B. Wang, J. Deng, L. Zhu, Y. Cao, Formulation of porous poly(lactic-co-glycolic acid) microparticles by electrospray deposition method for controlled drug release, *Materials Science and Engineering C*, 39, 113–119, **2014**.

[6] Y.H. Lee, F. Mei, M.Y. Bai, S. Zhao, D.R. Chen, Release profile characteristics of biodegradable-polymer-coated drug particles fabricated by dual-capillary electrospray, *Journal of Controlled Release*, 145, 1, 58–65, **2010**.

[7] J. Xie, L.K. Lim, Y. Phua, J. Hua, C.H. Wang, Electrohydrodynamic atomization for biodegradable polymeric particle production, *Journal of Colloid and Interface Science*, 302, 1, 103–112, **2006**.

[8] L. Mu, S.S. Feng, Fabrication, characterization and in vitro release of paclitaxel (Taxol) loaded poly (lactic-co-glycolic acid) microspheres prepared by spray drying technique with lipid / cholesterol emulsifiers, *Journal of Controlled Release*, 76, 3, 239–254, **2001**.

[9] Y. W. Cho, J. Lee, S.C. Lee, K.M. Huh, K. Park, Hydrotropic agents for study of in vitro paclitaxel release from polymeric micelles, *Journal of Controlled Release*, 97, 2, 249– 257, **2004**.

[10] C. Fonseca, S. Simoes, R. Gaspar, Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity, *Journal of Controlled Release*, 83, 2, 273–286, **2002**.

[11] M. Suno, T. Ono, S. Iida, N. Umetsu, K. Ohtaki, T. Yamada, T. Awaya, M. Satomi, Y. Tasaki, K. Shimizu, K. Matsubara, Improved high-performance liquid chromatographic detection of paclitaxel in patient's plasma using solid-phase extraction, and semi-micro-bore C18 separation and UV detection, *Journal of Chromatography B*, 860, 1, 141–144, **2007**.

[12] L. Zhang, J.M. Chan, F.X. Gu, J.W. Rhee, A.Z. Wang, A.F. Radovic-Moreno, F. Alexis, R. Langer, O.C. Farokhzad, Self-assembled lipid_polymer hybrid nanoparticles: a robust drug delivery platform, *ACS Nano*, 2, 8, 1696-1702, **2008**.

[13] S. Jiang, Z. Cao, Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications, *Advanced Materials*, 22, 9, 920–932, **2010**.

[14] B. Almería, T.M. Fahmy, A. Gomez, A multiplexed electrospray process for single-step synthesis of stabilized polymer particles for drug delivery, *Journal of Controlled Release*, 154, 2, 203–210, **2011**.

6. ACKNOWLEDGEMENTS

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