IJPSR (2018), Volume 9, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 January, 2018; received in revised form, 13 June, 2018; accepted, 20 September, 2018; published 01 October, 2018

CHARACTERIZATION OF VERAPAMIL HYDROCHLORIDE ENTRAPPED IN POLY (LACTIDE-CO-GLYCOLIDE) (PLGA) PARTICLES

P. M. L. Sabban and B. S. Balotro *

Department of Industrial Pharmacy, University of the Philippines Manila, College of Pharmacy, Valenzuela Hall, Taft Avenue, Ermita, Manila, 1000, Philippines.

Keywords:

Verapamil hydrochloride microparticles entrapped in PLGA, Solvent displacement method and lyophilization

Correspondence to Author:
Bienvenido S. Balotro

Assistant Professor,
Department of Industrial Pharmacy, University of the Philippines Manila, College of Pharmacy, Valenzuela Hall, Taft Avenue, Ermita, Manila, 1000, Philippines.

E-mail: bsbalotro@up.edu.ph

ABSTRACT: The optimized microparticles of Verapamil hydrochloride entrapped in Poly(lactide-co-glycolide) (Verapamil HCl-PLGA) was prepared through solvent displacement method and lyophilization. The optimization parameters for the formulation include particle size, polydispersity index, zeta potential, and entrapment efficiency. The final product was further characterized based on percent particle recovery, redispersibility, percent drug loading, drug release kinetics, and morphology. Increasing the PLGA 75:25 concentrations resulted to an increase in the particles size, polydispersity index and entrapment efficiency, and a decrease in zeta potential. The increase in poloxamer 188 concentration led to a decrease in zeta potential and an increase in the entrapment of the drug. Lastly, the increase in the pH of the non-solvent phase resulted to an increase in particle size. The addition of sucrose led to an unfavorable increase in the particle size and polydispersity index and a decrease in zeta potential and entrapment efficiency after lyophilization. The final product of the process was a heterogeneous sized (<10µm) irregularly shaped particles with an acceptable particle recovery, redispersibility, and percentage drug loading, but with poor release kinetic property. The Verapamil HCl-PLGA microparticles prepared through solvent displacement method and lyophilization were able to meet the conditions for lymphatic transport: entrapment in a lipophilic polymer in terms of particle size requirement (<10µm). Thus, it is possible that through polymeric drug formulation, the low bioavailability of Verapamil HCl due to hepatic first-pass effect may be addressed.

INTRODUCTION: Verapamil hydrochloride is a commonly prescribed drug in the management of hypertension, angina, and cluster headache prophylaxis. Verapamil hydrochloride suffers from the disadvantage of low bioavailability because of extensive hepatic metabolism (only 10% to 20% becomes bioavailable) and short half-life (2 to 4 h). As a result, it requires frequent dosing leading to the problem of noncompliance in patients and alternating over and under doses of the drug.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(10).4472-80

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(10).4472-80

A method of circumventing hepatic first pass effect is by making the drug particle microsized (<10µm) and lipophilic. This research aims to address this need by preparing an optimized formulation of Verapamil HCl entrapped in PLGA particles by solvent displacement method and lyophilization and the characterization of its properties.

Literature Review: The formulation of small polymeric drug particles is a promising research area in the pharmaceutical industry. Polymeric drug delivery system is designed to provide protection to active substances from degradation in the body and promote penetration across biological barriers [1]. For instance, it was noted that orally administered small polymeric particles (particle size of <10 µm) with hydrophobic biodegradable polymer can be transported through the lymphatic system and

drained into systemic circulation therefore avoiding metabolism by the liver [2]. Polymeric drug formulations allow existing pharmaceutical drugs to be post-processed into various types of patient-friendly dosage forms that provide maximal drug exposure. It is a strategy that provides a means to incorporate an old drug into a new drug-delivery platform, thus opening new avenues for addressing unmet medical needs [3]. With the present application of nanotechnology in drug development different nanosystems have been used. These are vehicles with particle sizes of 10-100 nm, in which drugs can be dissolved in, encapsulated in, or attached to for delivery [4]. Polymeric nanoparticles represent an extension of polymer-based controlled release technology for local and systemic drug delivery [5]. The complexity of nanodrug formulation has been made manageable with the utilization of design of experiments used for screening and optimization of formulations and process-related procedures. Design of experiment is one of the tools of quality by design which enables a risk-based approach to identifying and classifying product attributes and process parameters and ultimately developing a deep understanding of the products, processes, and platform as applied to drug development [5].

Verapamil hydrochloride is the major calcium channel antagonist prescribed in the market for the treatment of several cardiovascular diseases. Although Verapamil HCl is absorbed up to 90% following oral administration, the conventional dosage form suffers from the disadvantages of extensive first pass metabolism (10 to 20% bioavailable) and short half-life (2 to 4 h). This leads to the frequent dosing of the drug at intervals of only 6 h (up to four times a day) and a succession of rapid increase and decrease in the drug plasma level. In this case, the patient is subjected to non-compliance and alternating overdoses and under doses of the drug [6, 7, 8].

Presently, there are several ways to prepare hydrophobic polymeric particles with a size of <10 μm. One simple technique is the solvent displacement method. In this method, a drug and polymer is dissolved into an organic solution and then added drop-wise to an aqueous solution (with or without stabilizer). The polymer serves as a drug delivery carrier while the stabilizer prevents Ostwald ripening of the particles. Since the formed particles are suspended in aqueous medium, it becomes highly susceptible to hydrolytic cleavage or chemical degradation, thus, lyophilization is employed to produce a dry powder of polymeric drug particles [9]. The resulting particle size from solvent displacement method can affect the optical, electrical, and magnetic behavior of the material. Because of the size and complexity of the resulting particles, several parameters are used to evaluate its properties. These parameters include mean particle size and distribution, surface charge (Zeta potential), drug entrapment analysis, particle recovery, redispersibility, drug release rate, and morphology.

Objectives of the Study: The main objective of the study was to prepare and characterize the optimized formulation of Verapamil HCl entrapped in Poly (lactide-co-glycolide) through solvent displacement method followed by lyophilization.

Specifically, the goals of this study were as follows:

1. To prepare a Verapamil HCl entrapped in PLGA suspension by solvent displacement method;

2. To determine the optimized polymeric drug suspension through particle size, polydispersity index, zeta potential, and percent entrapment efficiency analysis;

3. To lyophilize the optimized Verapamil HCl entrapped in PLGA suspension and to collect Verapamil HCl-PLGA dried particles;

4. To determine the sucrose concentration (2%, and 20% w/v) that can better preserve the particle size, polydispersity index, zeta potential, and percent entrapment efficiency of the lyophilized Verapamil HCl entrapped in PLGA particles;

5. To determine the physical properties of Verapamil HCl entrapped in PLGA particles after lyophilization in terms of:

- **a.** Percent Particle Recovery
- **b.** Redispersability
- **c.** Percent Drug Loading
- **d.** Drug Release Rate
- **e.** Morphology (under Scanning Electron Microscope).

MATERIALS AND METHODS:

Materials: The following materials were used in the study:

Verapamil hydrochloride was used as the active ingredient. Poly(lactide co-glycolides) (PLGA) is used as a synthetic polymer. It is frequently used in solvent displacement method with acetone (organic solvent) and poloxamer 188 (surfactant). The chemicals used for the study were sourced out from Sigma-Aldrich.

The organic solvent in solvent displacement method is used to dissolve the drug and polymer. In the selection of an organic solvent, the miscibility to water, toxicity potential and ease of removal were highly considered. Solvent displacement method also entails the use of a non-solvent which in this case is distilled water.

Surfactants are added in solvent displacement method to prevent the phenomena of Ostwald ripening which results to aggregation of formed polymeric drug particles as the size decreases [10].

Sucrose 20% is added as a protectant to stabilize PLGA particles during lyophilization [11].

Study Design: This research work focused on the preparation of polymeric drug particles using solvent displacement method and lyophilization and the determination of the physical properties of the selected optimized Verapamil HCl-PLGA dried particles (Refer to **Fig. 1**). The study was divided into 3 stages: 1) solvent displacement method; 2) lyophilization; and 3) characterization of the final product (optimized Verapamil HCl-PLGA dried particles). Two study designs were used for this research work. Factorial design was employed in order to evaluate the main effects and interactions of the independent variables during solvent displacement method while the evaluation of the properties of the polymeric drug particles before and after lyophilization made use of the pre-test and post-test group experimental design (pairwise t-test).

In this study, a 2^3 factorial design for three factors at two levels each was selected to optimize the varied response variables. The three factors were polymer concentration (X1), surfactant concentration (X2), and pH of the non-solvent phase (X3) (see **Table 1**). The particle size, polydispersity index, zeta potential, and entrapment efficiency were selected as the response optimizers. Response optimizers were used as a basis to identify the combination of variable settings that mutually improve a set of responses. Experimental trials were performed in 8 possible combinations. All other formulation variables and processing variables were kept constant throughout the study.

TABLE 1: A 2^3 FORMULATION DESIGN ADOPTED FOR SOLVENT DISPLACEMENT METHOD

Independent Variable	Levels		Dependent Variables
	Low (-1)	High (+1)	
Polymer concentration (X_1)	32	800	Particle Size Polydispersity Index Zeta Potential % Entrapment Efficiency
Surfactant concentration (X_2)	320	3200	
pH of the non-solvent phase (X_3)	7.4	10.2	

Using pre-test and post-test design (pairwise t-test), the dependent variables also called response optimizers (particle size, polydispersity index, zeta potential, and % entrapment efficiency) were measured before and after lyophilization (see **Table 2**). The study design allowed the collection of data that was used to determine whether sucrose effectively protected the Verapamil HCl-PLGA particles from the stress of lyophilization [16].

The optimized formulation was selected based on the combination of good particle size (smallest measured size or preferably <10 µm) with a low polydispersity index (preferably <0.05), high zeta potential (preferably >±30), and the highest % drug entrapment efficiency (>50%). Basic statistical calculations were performed using the SPSS version 22. On the other hand, statistical program Minitab 17.0 was used in factorial design and the pairwise t-test.

TABLE 2: PRE AND POST-TEST DESIGN AFTER LYOPHILIZATION

Time Period 1		Time Period 2
Particle Size, polydispersity index, zeta potential, and % entrapment efficiency before lyophilization (X)	Lyophilization →	Particle Size, polydispersity index, zeta potential, and % entrapment efficiency after lyophilization (Y)
Treatment Effect =	(X-Y)	

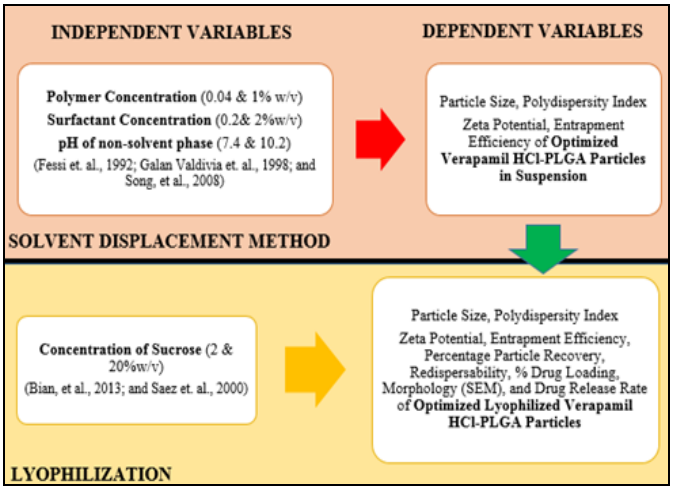

FIG. 1: INDEPENDENT AND DEPENDENT VARIABLES IN THE STUDY

RESULTS AND DISCUSSION:

I. Interpretation of Data of Solvent Displacement Method:

A. Visual Observation of the Verapamil HCl-PLGA Suspension: The different polymeric drug preparations occur as uniform white suspensions: Clustered large aggregates at the sides of the beaker and magnetic stirrer were observed in formulations F2, F4, F6, and F8. These formulations contain high concentrations of the polymer (PLGA) at 3200 mg. Thus, it is possible that supersaturation of PLGA occurred and encouraged multiple formation of hydrophobic nuclei that caused the presence of the clustered aggregates [17].

It was also observed that early formation of aggregates occurred in formulations with a low polymer-surfactant concentration ratio, a consequence of Ostwald ripening phenomenon. F1, F3, F5, and F7 have high polymer and surfactant concentration ratio ranging from 1:10 and 1:100, respectively. Unlike the polymer-surfactant concentration ratio in F4, F8, F2 and F6 with 1:4 and 1:0.4, respectively. Aside from the polymer-surfactant concentration ratio, the pH also seemed to have an influence in encouraging Ostwald ripening since F6 and F8 were formulated at a high pH (10.2) while low pH (7.4) for F2 and F4.

TABLE 3: SUMMARY OF RESULTS OF RESPONSE VARIABLES FOR SOLVENT DISPLACEMENT METHOD

Batch code	PC	SC	pH	Visual Observation	PS (nm)	PI	ZP (mV)	% EE
F1	-1	-1	-1	White solution; (-); (--)	785.1	0.404	-22.02	34.81
F2	+1	-1	-1	White solution; (+); (++3rd)	380.3	0.320	-18.78	27.21
F3	-1	+1	-1	White solution; (-); (--)	710.9	0.312	-23.63	42.37
F4	+1	+1	-1	White solution; (+);(++6th)	469.5	0.312	-7.77	92.88
F5	-1	-1	+1	White solution; (-); (--)	465.1	0.228	-18.86	27.03
F6	+1	-1	+1	White solution; (+); (++1st)	2219.0	0.623	-25.41	48.93
F7	-1	+1	+1	White solution; (-); (--)	406	0.187	-22.72	53.57
F8	+1	+1	+1	White solution; (+); (++1st)	2161.3	0.432	-12.39	81.87

Legend: PC=Polymer Concentration; SC=Surfactant Concentration; PS=Particle Size; PI=Polydispersity Index; ZP=Zeta Potential; % EE=Entrapment Efficiency; (-1)=Low Concentration; (+1)=High Concentration; (-)=no clustered aggregates at the magnetic stirrer or sides of beaker; (+)=clustered aggregates at the magnetic stirrer or sides of beaker are present; (--)=no observed formation of aggregates after 7 days of observation; (++day)=presence of aggregates at observed day.

TABLE 4: SUMMARY TABLE OF NUMERICAL RANKING OF THE OF MAIN AND INTERACTION EFFECTS ON PS, PI, ZP, & % EE

	Numerical Ranking of the Effect & p Value			
Effect	**PS**	**PI**	**ZP**	**% EE**
PC	3 (p=0.035)	2 (p=0.011)	3 (p=0.031)	2 (p=0.018)
SC	7 (n.s.)	3 (p=0.06)	2 (p=0.047)	1 (p=0..007)
pH	2 (p=0.029)	6 (p=0.802)	7 (p=0.231)	5 (n.s.)
A*B	4 (p=0.218)	7 (n.s.)	1 (p=0.018)	3 (p=0.044)
A*C	1 (p=0.05)	1 (p=0.006)	6 (p=0.061)	7 (n.s.)
B*C	6 (ns)	5 (n.s.)	4 (n.s)	6 (n.s.)
A*B*C	5 (n.s.)	4 (n.s.)	5 (n.s)	4 (n.s.)

A. Particle Size Analysis: The average mean particle size of the different polymeric drug suspension prepared through solvent displacement method ranged from 380.3 nm to 2219.0 nm (as shown in **Table 3**). **Table 4** shows the effect of PC (A), SC (B), and pH (C) and their respective interactions (A*B, B*C, A*C, & A*B*C) to the response variables (PS, PI, ZP, & % EE). The magnitude of the main and interaction effects on the response variables were calculated and ranked

numerically in the table. As shown for particle size, the interaction of polymer concentration and pH had more effect to particle size followed by polymer concentration and pH. Surfactant concentration had the least effect.

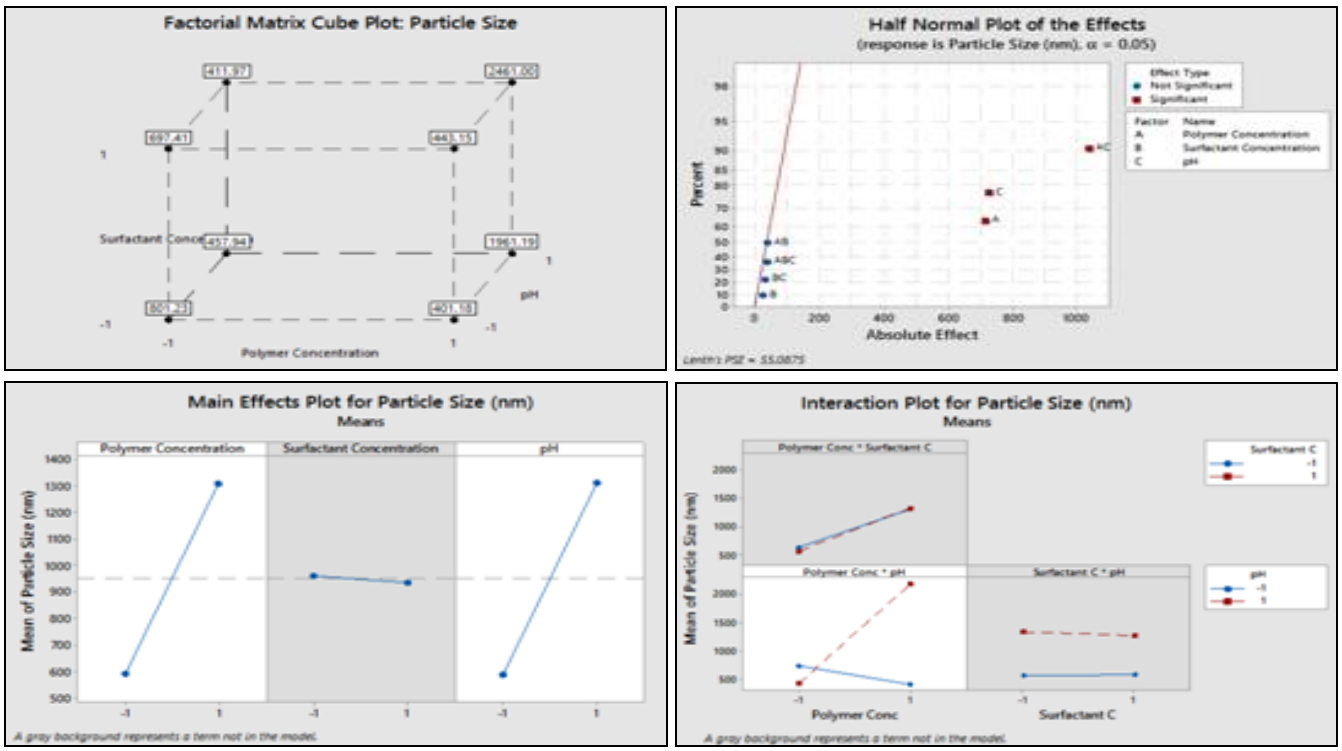

FIG. 2: DIFFERENT PLOTS FOR FACTORIAL DESIGN: FACTORIAL MATRIX CUBE PLOT; HALF NORMAL PLOT OF EFFECTS; MAIN EFFECTS PLOT; AND INTERACTION EFFECTS PLOT FOR PARTICLE SIZE ANALYSIS

A graphical representation of the "main effects" and "interaction effects" can be seen in **Fig. 2**. Based on the main effects plot, as the polymer concentration and pH level increases, the size of Verapamil HCl-PLGA particles also increases. On the other hand, the surfactant had an opposite effect to particle size as its concentration increases, though insignificant by ANOVA. The interaction effect plot of the factorial design included polymer and surfactant, polymer and pH, and surfactant and pH interactions. For polymer concentration and pH interactions, at pH 7.4, as the polymer concentration increases, the size of the polymeric drug particles were smaller while at pH 10.2, as the polymer concentration increases, the particle size also becomes larger. In the case of the polymer and surfactant concentration interaction plot, as the polymer concentration increases, particle sizes increases which is more significant when surfactant concentration is high than when it is low. For the surfactant concentration and pH plot under low and high pH conditions, there is no significant change in the particle size as surfactant concentration increases.

B. Polydispersity Index: The average mean polydispersity index of the different Verapamil HCl-PLGA suspension prepared through solvent displacement method ranged from 0.187 (F7) to 0.623 (F6) (as shown in **Table 3**). Again as in particle size, the magnitude of the main and interaction effects were calculated and compared for polydispersity index as presented in **Table 4**. The polymer concentration and pH interaction had the greatest significant effect on PI. Among the main effects, pH had the least impact to polydispersity index while polymer and surfactant concentration had significant effect to the size distribution of the formed polymeric drug particles.

C. Zeta Potential Analysis: A surface charge of >±30mV are considered stable particles in suspension. Based on data of the different sample suspension **Table 3**, the average zeta potential of all prepared polymeric drug suspensions were below -30mV. As shown in **Table 4**, among the main effects, surfactant concentration had the most impact to zeta potential, followed by polymer concentration.

The effect of pH is the least among the main and interaction effects. The combination of polymer concentration and surfactant concentration had the greatest magnitude effect to zeta potential than the rest. A study by Santander-Ortega *et al*., [18] describes that PLGA particles have a negative mobility. By adding Poloxamer 188, the mobility of the particles remained negative. However, as a general rule, the mobility value decreases in absolute value by increasing the surfactant load. It was explained that when surfactant concentration increases, it gives rise to differences in electrokinetic behavior due to structural changes of the adsorbed poloxamer chains. The absorbed non-ionic poloxamer layer partially screens the surface of the PLGA particles, thus decreasing zeta potential. Aside from the surfactant, the increase in polymer concentration cause a shift in the "shear plane" of the particles, thus decreasing zeta potential [19].

D. Entrapment Efficiency: The entrapment efficiency of the polymeric drug particles were measured by direct method. Based on **Table 4**, the effects polymer concentration and surfactant concentration had the highest magnitude of effect to entrapment efficiency. The effect of pH is one of the least. The combination of polymer and surfactant concentration had the greatest impact to entrapment efficiency compared to other interaction effects.

II. Interpretation of Results from Lyophilization: The selected optimized polymeric drug suspension was lyophilized using 2% and 20% sucrose as protectant F7a and F7b respectively. Sugar was used as protectant during lyophilization to shield the submicron polymeric particles from freezing and desiccation stresses. The same response optimizers were used to evaluate the properties of the collected dried particles. The comparison of the pre- and post-lyophilization of the particle size, polydispersity index, zeta potential, and % entrapment efficiency were interpreted and analyzed using the pairwise t-test.

The mean diameter and PI of the polymeric drug particle was influenced by the the sucrose protectant. Increase in particle size and PI due to sucrose can be explained by two mechanisms: (1) the crystallization of the protectant can cause phase separation in the cryo-concentrated portion of the frozen polymeric particles with no opportunity for a stabilization interaction with the submicron particles, and thus, individual polymeric particles in the particle-rich phase can interact and form aggregates; another explaination may be (2) the growing crystals of water and sucrose may exert mechanical forces on the polymeric particles leading to their fusion [9].

TABLE 5: PAIR-WISE T-TEST FOR PARTICLE SIZE, POLYDISPERSITY INDEX, ZETA POTENTIAL, AND PERCENTAGE ENTRAPMENT EFFICIENCY OF F7 (PRE-TEST) AGAINST F7A (POST-TEST; W/ 2% SUCROSE), AND F7B (POST-TEST; W/ 20% SUCROSE)

Formulation Code (Mean ± STD)	Particle Size F7 (406±8nm)	Polydispersity Index F7 (0.1867±0.0354)	Zeta Potential F7 (-2.72±1.88mV)	% Entrapment Efficiency F7 (53.577±0.067)
F7a	6041 (95%CI, 5117 to 6965)	0.0610 (95% CI, -0.1643 to 0.2863)	10.848 (95% CI, 9.467 to 12.229)	-45.763 (95% CI, -47.172 to -44.355)
PS(6447±380nm) PI(0.2477±0.1144) ZP(-11.872±1.437mV) EE(7.813±0.616)	Significant	Not Significant	Significant	Significant
F7b	20624 (95% CI, 10183 to 31065)	1.5637 (95% CI, 1.2065 to 1.9209)	11.75 (95% CI, 6.77 to 16.72)	-49.180 (95% CI, -50.316 to -48.044)
PS(21030±4202nm) PI (1.7503±0.1539) ZP(-10.97±8.39mV) EE(4.397±0.422)	Significant	Significant	Significant	Significant

Results of the pairwise sample t-test **Table 5** show that there was a significant difference in the properties of the suspension after lyophilization using either 2% or 20% sugar as protectant.

The particle size and polydispersity index of both 2% and 20% sucrose containing formulations significantly increased except for polydispersity index before and after lyophilization using 2%

sucrose while the zeta potential and entrapment efficiency of the polymeric particles significantly decrease. Changes in these properties may be attributed to the protectant and surfactant used in the formulation. Between the different F7 samples, F7a (w/2% sucrose) was selected for the final characterization analysis. The selection of F7a was based on the results of the selected response optimizers. Although the ideal criteria for zeta potential and entrapment efficiency were not met, F7a was selected because of better results than F7b.

After lyophilization, 67.83% of the formulation was recovered. F7a has good dispersibility property. The drug loading of F7a was 5.581% of Verapamil HCl in 1mg of particulate system or polymeric particle. The calculated % drug load of Verapamil HCl was within the acceptable range of <10% [20]. Based on SEM results, the polymeric drug particles formed have an irregular shape (fragment-like particles) with particle aggregation and wide size distribution. See **Fig. 3**.

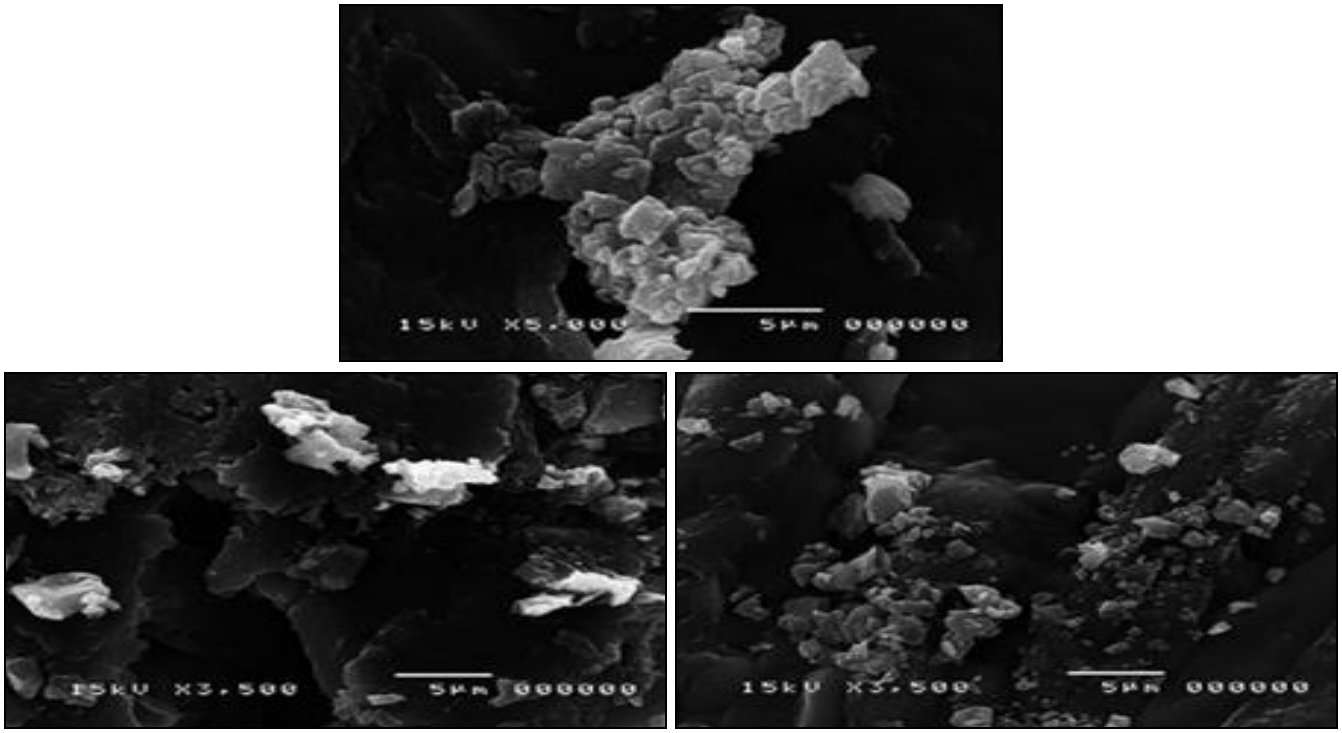

FIG. 3: IMAGES OF VERAPAMIL HCL-PLGA PARTICLES TAKEN USING SCANNING ELECTRON MICROSCOPE

The kinetic release of Verapamil HCl from PLGA particles was non-linear with the drug concentration decrease over time (see **Fig. 4**). According to Stefana-Oana *et al.*, [21], a release kinetic curve should be present or visible in order to fit the different kinetic models to the collected data. However, in this case, the release kinetic curve is not visible, therefore, the different kinetic models cannot be used to determine the drug release mechanism of Verapamil HCl from the polymeric drug carrier. Nevertheless, the observed release kinetics of the polymeric particle was very similar to that of the polymeric amorphous solid dispersion (PASD). According to the mechanism of drug release of PASD, the drug molecule attains supersaturation quickly "spring" before crashing in solution due to increased precipitation [22].

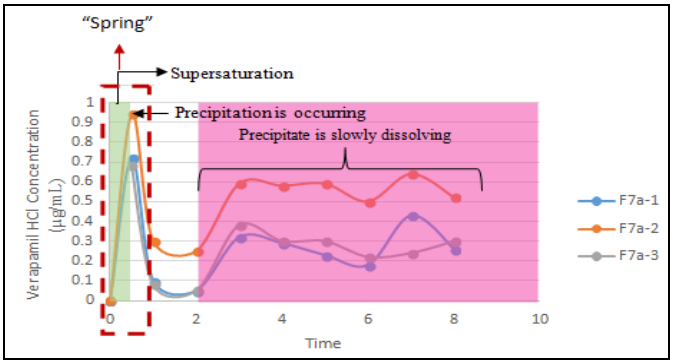

FIG. 4: RELEASE KINETICS OF VERAPAMIL HCL FROM PLGA POLYMER

CONCLUSION: Optimized formulation of micro-particles of Verapamil hydrochloride entrapped in Poly(lactide-co-glycolide) (PLGA) (Verapamil HCl-PLGA) was prepared through solvent displacement method followed by lyophilization.

The effect of polymer concentration, surfactant concentration, and pH on the initial formulation was determined in relation to the optimization parameters of the formulation. The final product was further characterized based on percent (%) particle recovery, redispersibility, percent (%) drug loading, drug release kinetics, and morphology. Increasing the PLGA 75:25 concentration resulted to an increase in the particles size, polydispersity index and entrapment efficiency, and a decrease in zeta potential. The increase in Poloxamer 188 concentration led to a decrease in zeta potential and an increase in the entrapment of the drug. Lastly, the increase in the pH of the non-solvent phase resulted to an increase in particle size. The addition of sucrose as protectant during lyophilization, led to an unfavorable increase in the particle size and polydispersity index, and a decrease in zeta potential and entrapment efficiency. The final product of the process was a heterogeneous sized (<10 µm) irregularly shaped particles (fragment-like), with an acceptable particle recovery, redispersibility, and percentage (%) drug loading, but with poor release kinetic property (non-linear and decreasing concentration over time).

The study shows that solvent displacement method and lyophilization is suitable in the preparation of Verapamil HCl-PLGA microparticles. Application of the method resulted in the formulation of a hydrophobic polymeric drug carrier system with a particle size of <10 µm (10,000 nm). The formulated Verapamil HCl-PLGA microparticles are able to meet the conditions noted by Chu and Lui (2008) for lymphatic transport: lipophilicity and a small particle size of <10µm, and therefore, could be useful in overcoming its difficulties due to its low bioavailability caused by extensive hepatic metabolism.

ACKNOWLEDGEMENT: The researchers are thankful to the Industrial Pharmacy Department, University of the Philippines Manila, College of Pharmacy; the Orthopedic Department, Philippine General Hospital, University of the Philippines Manila; the Department of Mining, Metallurgical, and Materials Engineering, University of the Philippines Diliman, College of Engineering; and the Physics Department, De La Salle University, Manila.

CONFLICT OF INTEREST: The researchers declare no conflict of interest with any parties in relation to this research.

REFERENCES:

1. Kadam RS, Bourne DW and Kompella UB: Nano-advantage in enhanced drug delivery with biodegradable nanoparticles: contribution of reduced clearance. The American Society for Pharmacology and Experimental Therapeutics 2012; 40(7): 1380-1388. Retrieved from http://dx.doi.org/10.1124/dmd.112.044925
2. Chu PK and Liu X: Biomaterials Fabrication and Processing Handbook. New York: CRC Press 2008:171-193.
3. Merisko-Liversidge E and Liversidge G: Drug Nanoparticles: formulating poorly water-soluble compounds. Toxicologic Pathology 2008; 36: 43-48. doi: 10.1177/0192623307310946
4. Jeevanandam J, Chan YS and Danquah MK: Nano-formulations of drugs: recent developments, impact and challenges. Biochimie 2016; 128-129: 99-112.
5. Troiano G, Nolan J, Parson D, Hoven VGC and Zale S: A Quality by design approach developing and manufacturing polymeric nanoparticle drug products. The AAPS Journal 2016; 18(6): 1354-1365. doi: 10.1208/s12248-016-9969-z.
6. Baudier P, De Boeck A and Fossion J: United States of Americ Patent No. 4,832,958, 1989. Retrieved from http://www.google.com.au/patents/US4832958
7. Patel VM, Prajapati BG and Pate AK: Controlled release gastroretentive dosage form of verapamil hydrochloride. International Journal of PharmTech Research 2009; 1(2): 215-221.
8. Yassin AE, Alsarra IA and Al-Mohizea AM: Chitosan beads as a new gastroretentive system of verapamil. Scientia Pharmaceutica 2009; 175-188. doi: 10.3797/scipharm.2006.74.175
9. Abdelwahed W, Degobert G, Stainmesse S and Fessi H: Freeze-drying of nanoparticles: formulation, process, and storage considerations. Advanced Drug Delivery Reviews 2009; 58(15): 1688-1713. doi: 10.1016/j.addr.2006.09.017.
10. De PK, Chakraborty S and Das S: Nanosuspensions: potent vehicles for drug delivery and bioavailability enhancement of lipophilic drugs. Journal of Pharmacy Research 2006; 5(3): 1548-1554.
11. Saez A, Guzmán M, Molpeceres J and Aberturas MR: Freeze-drying of polycaprolactone and Poly(d,l-lactic-glycolic) nanoparticles induce minor particle size changes affecting the oral pharmacokinetics of loaded drugs. European Journal of Pharmaceutics and Biopharmaceutics, 2000; 50(3): 379-387. doi: 10.1016/S0939-6411(00)00125-9.
12. Fessi HC, Devissaguet JP, Puisieux F and Thies C: France Patent No. 5118528. 2009.
13. Valdivia GFJ, Mas VJA and Bergamini MV: United States Patent No. 5705196. 1998
14. Song X, Zhao Y, Wu W, Bi Y, Cai Z, Chen Q and Li Y: PLGA nanoparticles simultaneously loaded with vincristine sulfate and verapamil hydrochloride: systematic study of particle size and drug entrapment efficiency. International Journal of Pharmaceutics 2009; 350: 320-329. doi:10.1016/j.ijpharm.2007.08.034
15. Bian X, Liang S, John J, Hsiao CH, Wei X, Liang D and Xie H: Development of PLGA-based itraconazole injectable nanospheres for sustained release. International

Journal of Medicine 2009; 8: 4521-4531. doi: 10.2147/ IJN.S54040
16. Kothari CR: Research methodology: Methods & techniques. New Delhi: New Age International (P) Ltd., Edition 2, 2009.
17. Zauner R: Scale-up of Precipitation Process. London, England. 2009. Retrieved from http://discovery.ucl.ac.uk/ 1317927/1/300960.pdf
18. Santander-Ortega M, Jodar-Reyes A, Csaba N, Bastos-Gonzalez D and Ortega-Vinuesa J: Colloidal stability of pluronic F68-coated PLGA nanoparticles: a variety of stabilisation mechanism. Journal of Colloid and Interface Science 2006; 522-529. doi: 10.1016/j.jcis.2006.07.031.
19. Tadros T: Applies surfactants: principles and application. morlenbach: Wiley-VCH Verlag GmbH&Co. KGaA. 2006. doi: 10.1002/ange.200585309.
20. Chu KS, Schorzman AN, Finniss MC, Bowerman CJ, Peng L, Luft JC and DeSimone JM: Nanoparticle drug loading as a design parameter to improve docetaxel pharmacokinetics and efficacy. Biomaterials 2013; 1-6. doi: 10.1016/j.biomaterials.2013.07.038
21. Stefana- Oanan P, Mihaela I, Raneti C, Anuta V, Mirciol I and Belu I: Study of nimesulide release from solid pharmaceutical formulations in tween 80 solutions. Current Health Sciences Journal 2010; 36(1): 42-47.
22. Tsinman O, Tsinman K and Ali S: Excipient Update - Soluplus®: An understanding of supersaturation from amorphous solid dispersions 2015. Retrieved from Drug Development & Delivery: http://www.drug-dev.com /Main/Back-Issues/EXCIPIENT-UPDATE-Soluplus-An-Understanding-of-Supe-824.aspx.

How to cite this article:
Sabban PML and Balotro BS: Characterization of verapamil hydrochloride entrapped in poly (lactide-co-glycolide) (PLGA) particles. Int J Pharm Sci & Res 2018; 9(10): 4472-80. doi: 10.13040/IJPSR.0975-8232.9(10).4472-80.