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Continuous Production Of Raloxifene Hydrochloride Loaded Nanostructured Lipid Carriers Using Hot-Melt Extrusion Technology

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CONTINUOUS PRODUCTION OF RALOXIFENE HYDROCHLORIDE LOADED NANOSTRUCTURED LIPID CARRIERS USING HOT-MELT EXTRUSION TECHNOLOGY

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ABSTRACT

The aim of this study was to utilize a continuous process for the production of Raloxifene Hydrochloride (RX-HCl) loaded NLC formulations for extended drug release using hot-melt extrusion technology coupled with probe sonication, and also to evaluate the *in vitro* characteristics of the prepared NLCs by particle size, PDI, zeta potential, entrapment efficiency, drug loading, and *in vitro* drug release profile. Preparation of the NLCs using HME technology involved two main steps, first formation of a pre-emulsion after extrusion and then size reduction of the pre-emulsion using probe sonication to obtain the NLCs. Process parameters for the extrusion process like feeding rates for the volumetric feeder and peristaltic pumps, were optimized. A screw speed of 100 rpm and a barrel temperature of 85 °C, were used in the extrusion process. Characterization of the NLCs involved assessment of particle size, PDI, zeta potential, entrapment efficiency, and drug loading. NLCs prepared by HME technology generally showed a lower particle size compared to those prepared by the conventional method. The prepared NLCs had high entrapment efficiency values (>90 %). *In vitro* drug release was evaluated using dialysis bag diffusion technique and USP apparatus 1 (rotating basket). The pure drug showed a faster rate of drug release compared to the NLCs which showed an extended release of the drug. NLCs prepared by HME technology generally showed a higher rate of drug release compared to those prepared by the conventional method. Particle size of the prepared NLCs remained relatively stable over the storage period and all PDI and zeta potential values were ≤ 0.523 and in the range of -15 to -30 mV, respectively, indicating good physical stability of the formulations. In summary, HME

technology and probe sonication were successfully used to prepare RX-HCl loaded NLC formulations as a continuous manufacturing process with shorter processing times as compared to the conventional method, which makes this technique a more industry friendly method.

DEDICATION

To my family, for their unwavering support, for their kindness and understanding, and for their love.

LIST OF ABBREVIATIONS OR SYMBOLS

HME:	Hot-Melt Extrusion
MWCO:	Molecular Weight Cut Off
NLC:	Nanostructured Lipid Carriers
PDI:	Polydispersity Index
RX-HCl:	Raloxifene Hydrochloride
SLN:	Solid Lipid Nanoparticles
T_g:	Glass transition temperature
T_m:	Melting temperature
v/v:	Volume by volume
w/v:	Weight by volume
w/w:	Weight by weight

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CHAPTER I: INTRODUCTION

Nanostructured lipid carriers (NLCs) are a second generation of lipid nanoparticles which offer some advantages over the Solid Lipid Nanoparticles (SLNs) [1]. Advantages offered by the NLCs over the SLNs include higher drug incorporation capacity, improved drug release properties, easier manufacturing process, and less amount of stabilizers required compared to the SLNs [2]. NLCs are produced using a combination of solid lipids and liquid lipids which are organized in nanocompartments inside the solid lipid matrix [3]. The Active Pharmaceutical Ingredient (API) in the NLC is incorporated in the liquid lipid and is encapsulated by the solid lipid [3]. This configuration gives the drug some degree of mobility and offers stability to some extent [3]. NLCs are safe and compatible colloidal drug carriers which are able to provide controlled delivery of the API and have various drug delivery applications via the oral, dermal, pulmonary, and ocular routes [4].

Various methods have been used to produce NLCs and most of these methods have been reported in literature and they include high-pressure homogenization, micro-emulsification, solvent displacement, supercritical fluid technology, high-shear homogenization, and ultrasonication [4]. However, these methods have some disadvantages that include multistep processing, poor energy efficiency, potential for dilution of particle dispersion, and frequent failures due to batch-to-batch variations [3]. These disadvantages make the conventional methods used for NLC production less industry friendly.

Hot-melt extrusion (HME) processing was established in the early 1930s and during that time it rapidly became the most widely applied processing technology in the plastic, rubber, and food industries [5]. HME is a continuous pharmaceutical process that involves pumping polymeric materials with a rotating screw at temperatures above their glass transition temperature (T_g) and sometimes above the melting temperature (T_m) to achieve molecular level mixing of the active compounds and thermoplastic binders, polymers, or other excipients [5]. This molecular level mixing converts the components into an amorphous product with a uniform shape and density, thereby increasing the dissolution profile of the poorly water soluble drug [5]. HME has gained increased interest in the pharmaceutical industry due to advantages that include: HME provides faster and more efficient time to achieve the final product, environmental advantages due to the elimination of solvent use, increased efficiency of drug delivery to the patient, formulations with high drug loading can be manufactured by HME with the desired release profile, good content uniformity can be achieved for very low drug loading due to intimate mixing of components, HME allows conversion of API to amorphous form which can result in enhanced bioavailability, HME is a scalable continuous process and product quality can be monitored online, inline, or offline, and desired dosage forms can be easily manufactured by HME process [5], [6]. HME technology gained a lot of attention and importance in the recent decade after the US Food and Drug Administration encouraged the use of continuous manufacturing processes [3].

RX-HCl is a selective estrogen receptor modulator (SERM) with a chemical structure as depicted in Figure 1. RX-HCl acts as an estrogen agonist on bone and on the liver and therefore increases bone mineral density and decreases fracture incidence [7]. It is used in the prevention of osteoporosis and prevention of invasive breast cancer in postmenopausal women, although, its

therapeutic efficacy is limited by its low solubility in physiological pH conditions and also undergoes extensive first pass metabolism [7].

In the current study, a continuous production process will be used for the production of RX-HCl loaded NLCs for extended drug release using hot-melt extrusion technology coupled with probe sonication. Physicochemical characteristics of the prepared NLC formulations will be evaluated by particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, drug loading, and *in vitro* drug release. Process parameters for the extrusion process like feeding rates for the volumetric feeder and peristaltic pumps, were optimized. The continuous production process used in this study will overcome most of the limitations presented by the conventional methods.

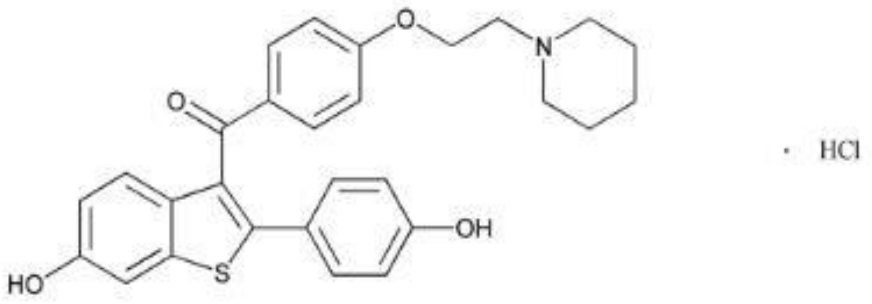

Figure 1. Chemical Structure of RX-HCl

CHAPTER II: MATERIALS AND METHODS

2.1. MATERIALS

RX-HCl, liquid lipids (capmul MCM C8, castor oil, and labrafil M 1944 CS), and surfactants (polysorbate 80, polysorbate 20, and cremophor EL) were purchased from Fisher Scientific (Hanover Park, IL USA). Solid lipids, glyceryl monostearate, compritol 888ATO, precirol ATO5, and dynasan 118 were gifted from Gattefossé (Paramus, NJ USA). All other chemicals and solvents (methanol, acetonitrile) used in the study were of HPLC grade and de-ionized water was used throughout the study.

2.2. METHODS

2.2.1. Selection of Solid Lipids, Liquid Lipids, and Surfactants

Solid lipids were selected by checking the solubility of RX-HCl in melted solid lipid by means of visual observation with the naked eyes under normal light. This was further characterized with HPLC analysis. Solid lipids, glyceryl monostearate, compritol 888ATO, precirol ATO5, and dynasan 118 were screened for their potential to solubilize RX-HCl. Liquid lipids, capmul MCM C8, castor Oil, and labrafil M 1944 CS, were screened for their potential to solubilize RX-HCl. RX-HCl compatibility with various 1 % surfactant solutions, polysorbate 80, polysorbate 20, and cremophor EL was assessed. After the screening studies, two solid lipids, two liquid lipids, and

two surfactants were selected for further studies. The solid and liquid lipids used in this study were reported to be safe, biocompatible, and biodegradable. All experiments were conducted in triplicate.

2.2.2. Preparation of NLC Formulations

Two batches consisting of 24 blank NLC formulations in each batch, were prepared by the conventional probe sonication method. The 1st batch consisted of only one primary surfactant while the 2nd batch consisted of a primary surfactant and a co-surfactant. Composition of the blank NLC formulations is as shown in Table 1. All prepared formulations were stored at 25 °C/ 60 % RH to check for stability. Based on the stability results, formulations F4-2 and F4-3 from the 2nd batch were selected for the preparation of RX-HCl loaded NLC formulations using conventional probe sonication and HME methods. The concentration (% w/v) of the solid and liquid lipids, surfactants, and co-surfactant (Poloxamer 188) in the drug-loaded NLC formulations, is as shown in Table 1 and RX-HCl concentration was kept constant at 1.2 % w/v in all the formulations. Preparation of RX-HCL loaded NLCs using HME technology involved two main steps. The first step involved the formation of a pre-emulsion by extruding a combination of solid lipid, RX-HCl, liquid lipid, and the aqueous phase through the HME barrel, and the second step involved size reduction of the pre-emulsion utilizing probe sonication to obtain the NLC formulation. Extrusion was carried out on an 11-mm co-rotating twin screw extruder (11-mm Process 11™, Thermo Fisher Scientific, Karlsruhe, Germany). Schematic representation of preparation of the NLC formulations using HME and probe sonication is as shown in Figure 2. The solid lipid was passed through a sieve (Standard Mesh No. 16) for size reduction and to improve its flowability. RX-HCl

was uniformly mixed with the solid lipid, and introduced in the barrel using a volumetric feeder. Liquid lipid and aqueous phase (solution of surfactants and water) were heated to 85 °C and then respectively, injected in zone 2 and zone 4 of the barrel using peristaltic pumps. A screw speed of 100 rpm, barrel temperature of 85 °C, and sonication time of 10 minutes, were used in this study. Volumetric feeder and peristaltic pump feeding rates were optimized in this study. Figure 3 shows the screw configuration [3], with a barrel temperature of 85 °C that was used for the extrusion process. The pre-emulsion obtained was subjected to probe sonication (SONICS Vibracell™, Sonics and Material, Inc., Newton, CT) with an amplitude of 40 %, to obtain the RX-HCl loaded NLCs.

Table 1. Composition of blank NLC formulations

Formulation	Composition (%w/v)									
	Solid Lipids		Liquid Lipids		Surfactants					
					1st Batch		2nd Batch			
	Glyceryl Monostearate	Compritol 888ATO	Capmul MCM C8	Castor Oil	Polysorbate 80	Cremophor EL	Polysorbate 80	Co-surfactant	Cremophor EL	Co-surfactant
F1-1	5		1			1			0.75	0.25
F1-2	4.5		1.5			1			0.75	0.25
F1-3	4		2			1			0.75	0.25
F2-1	5		1		1		0.75	0.25		
F2-2	4.5		1.5		1		0.75	0.25		
F2-3	4		2		1		0.75	0.25		
F3-1	5			1		1			0.75	0.25
F3-2	4.5			1.5		1			0.75	0.25
F3-3	4			2		1			0.75	0.25
F4-1	5			1	1		0.75	0.25		
F4-2	4.5			1.5	1		0.75	0.25		
F4-3	4			2	1		0.75	0.25		
F5-1		5	1			1			0.75	0.25
F5-2		4.5	1.5			1			0.75	0.25
F5-3		4	2			1			0.75	0.25
F6-1		5	1		1		0.75	0.25		
F6-2		4.5	1.5		1		0.75	0.25		
F6-3		4	2		1		0.75	0.25		
F7-1		5		1		1			0.75	0.25
F7-2		4.5		1.5		1			0.75	0.25
F7-3		4		2		1			0.75	0.25
F8-1		5		1	1		0.75	0.25		
F8-2		4.5		1.5	1		0.75	0.25		
F8-3		4		2	1		0.75	0.25		

2.2.3. Characterization of NLC Formulations

Particle size (z-average diameter) and polydispersity index (PDI) were measured by dynamic light scattering technique using Malvern Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C [8]. Zeta potential was also measured using the same instrument. Prior to measurements, the formulations were suitably diluted with distilled water (100x dilution factor) and all measurements were carried out at a scattering angle of 90° at 25 °C. Percent entrapment efficiency and drug

loading of the prepared NLC formulations were determined [9]. Entrapment efficiency was determined by calculating the entrapped drug after removal of the unentrapped drug using Amicon filters (100 kDa MWCO) centrifuged at 13,000 rpm for 15 minutes at 20 °C. The filtrate was diluted appropriately with methanol and analyzed using a suitable HPLC method as described in the analytical method section . The following formulas were used to calculate percent entrapment efficiency and drug loading [10];

$$\% \text{ Entrapment efficiency} = \frac{Amount\ of\ RX{-}HCl\ entrapped\ in\ NLCs}{Amount\ of\ RX{-}HCl\ added\ to\ the\ formulation} * 100$$

$$\% \text{ Drug loading} = \frac{Amount\ of\ RX{-}HCl\ entrapped\ in\ NLCs}{Amount\ of\ RX{-}HCl\ and\ lipids\ added\ to\ the\ formulation} * 100$$

2.2.4. HPLC Analysis Method for Raloxifene Hydrochloride

RX-HCl was analyzed using HPLC (Waters Corporation) equipped with a UV-vis detector operating at 288 nm. Samples were chromatographed on a stainless steel RP-C18 column with dimensions of 250 x 4.6 mm, packed with 5 µm particles. The mobile phase consisted of a mixture of acetonitrile (33 % v/v) and monobasic potassium phosphate buffer (67 % v/v). A flow rate of 1 mL/min and an injection volume of 10 µL were used for analysis [11].

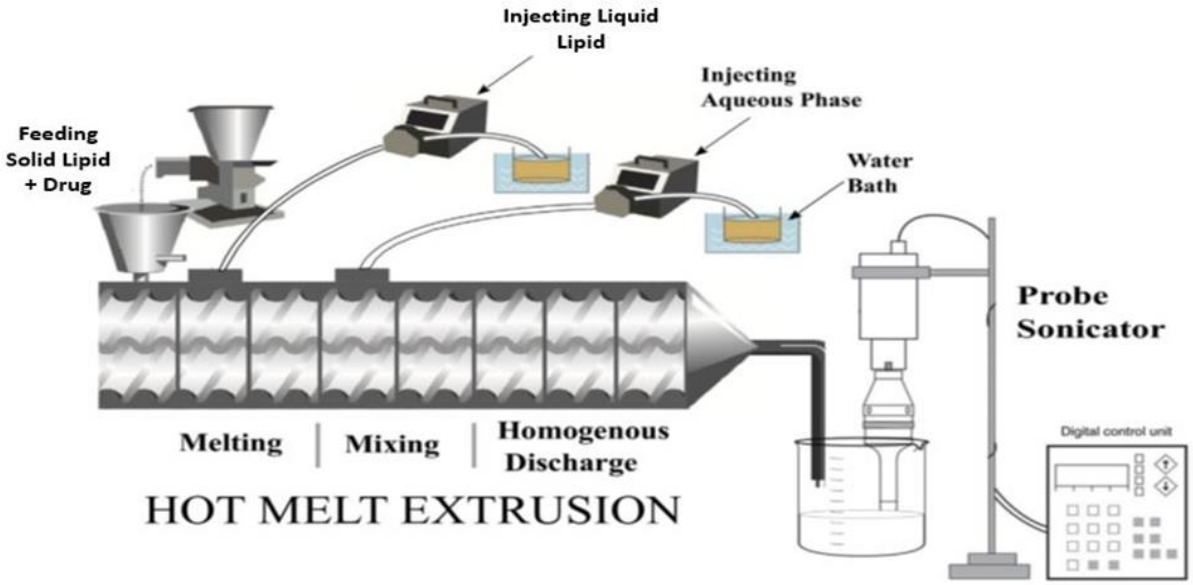

Figure 2. Schematic representation of preparation of RX-HCl loaded NLCs using HME technology coupled with probe sonication [3]

2.2.5. In vitro Drug Release Studies

In vitro release of RX-HCl from NLCs was evaluated using dialysis bag diffusion technique with some modifications [12]. The receptor compartment (drug release media) consisted of 500 mL pH 6.8 phosphate buffer containing 0.5 % v/v polysorbate 80 to maintain sink conditions. The dialysis bags were soaked in the release media overnight prior to the experiment. RX-HCl loaded NLC formulation (equivalent to 18 mg of RX-HCl) was placed in the dialysis bags (12-14 kDa MWCO) and then the bags were tightly sealed at both ends to avoid any leakages. The dialysis bags were immersed in the receptor compartment placed in USP apparatus 1 vessels and maintained at 37 ± 1 °C and 100 rpm. At predetermined time points, aliquots of 1 mL were withdrawn from the release media and filtered through 0.45 µm filter. The concentration of RX-HCl in the filtrate was suitably analyzed using the HPLC method as described in the analytical method section. Equal amounts (1 mL) of fresh release media were added to the receptor compartment immediately after withdrawal

to maintain a constant release media volume. All experiments were conducted in triplicate and the average values were taken. At the end of the experiment, percent drug release was calculated to determine RX-HCl release mechanism from the NLC formulations.

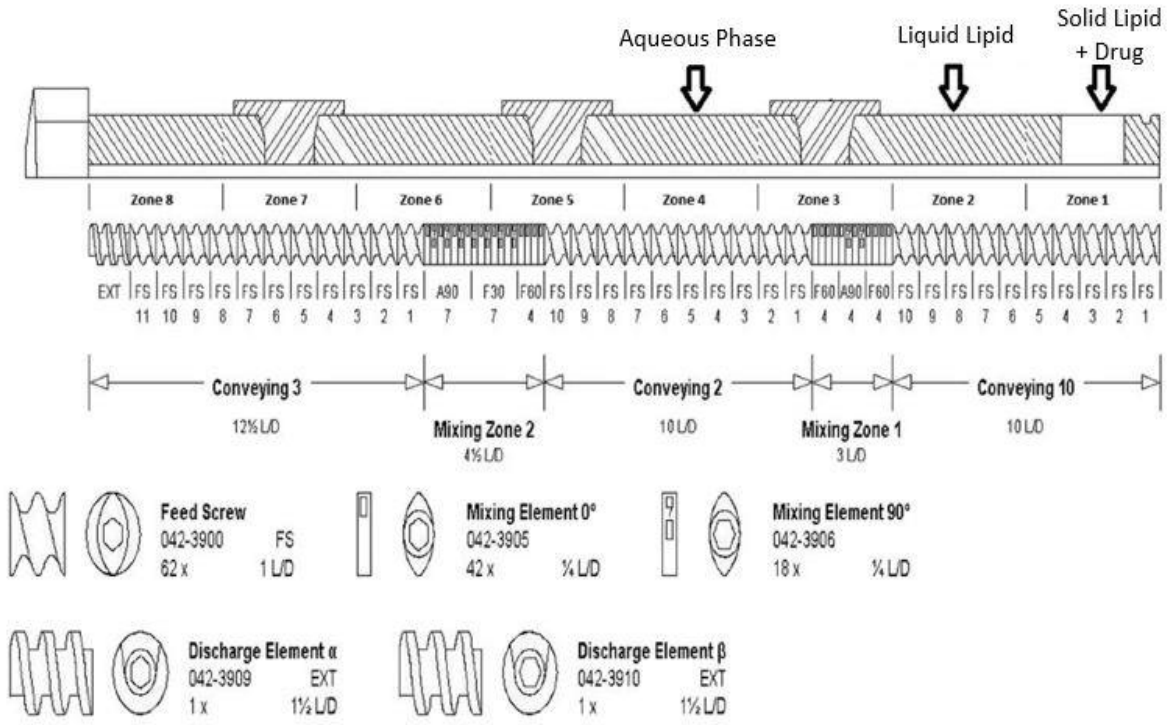

Figure 3. Screw configuration used for the extrusion process

2.2.6. Stability Study

The physicochemical stability of the prepared NLC formulations was assessed based on particle size, polydispersity index, zeta potential, percent entrapment efficiency, and percent drug loading. These parameters were assessed at the beginning and end of the stability period. The NLC formulations were stored at 25 °C/ 60 % RH and 40 °C/ 75 % RH for a period of one month.

CHAPTER III: RESULTS AND DISCUSSION

3.1. SELECTION OF SOLID LIPIDS, LIQUID LIPIDS, AND SURFACTANTS

The first step in the selection of lipids for the preparation of NLC formulations, is the assessment of solubility of drug in the solid and liquid lipids. This is because solubility of the drug in the lipids is one of the most important factors for determining entrapment efficiency of the drug in NLC formulations [13]. Four solid lipids, three liquid lipids, and three surfactants with different physicochemical properties were assessed for RX-HCl solubility and compatibility. With the aid of visual observation with naked eyes under normal light, solubility of RX-HCl in the lipids was based on the amount of precipitated drug at the bottom of the scintillation vials at the end of the solubility studies, and this amount of precipitated drug was inversely proportional to the solubility of RX-HCl in the lipids. Solubilization capacity of the lipids for RX-HCl was in the following order; glyceryl monostearate > compritol 888ATO > precirol ATO5 > dynasan 118 for solid lipids, and capmul MCM C8 > castor oil > labrafil M 1944 CS for the liquid lipids. Based on the results, solid lipids, glyceryl monostearate and compritol 888ATO, and liquid lipids, capmul MCM C8 and castor oil, were selected for further studies since they had a lower amount of precipitated drug compared to the other lipids, and hence had a higher solubilization capacity for RX-HCl. Surfactants, polysorbate 80 and cremophor EL were reported to be compatible with RX-HCl [14], [15].

3.2. PREPARATION OF NLC FORMULATIONS

HME technology is a continuous process of pumping raw materials at high temperatures and pressures resulting in a product of uniform shape and density [16], [17], [18]. Preparation of RX-HCL loaded NLCs involved the formation of a pre-emulsion by extruding a combination of solid lipid, RX-HCl, liquid lipid, and the aqueous phase through the HME barrel, and finally size reduction of the pre-emulsion utilizing probe sonication to obtain NLC formulations. The extruder's screw consisted of a conveying system and mixing zones which helped in transport and efficient mixing, a die at the end of the barrel, and downstream equipment that was used to collect the pre-emulsion for further processing [3]. The screw configuration is a very important aspect of HME and has a significant contribution to the characteristics of the final product [19]–[21]. The screw configuration as shown in Figure 3, had two mixing zones. The first mixing zone served the purpose of mixing the melted solid lipid and RX-HCl with the liquid lipid and the second mixing zone served the purpose of efficiently mixing the oil and aqueous phase to form a pre-emulsion that was collected in a beaker at the end of the barrel for further processing. It took about 2 hours to prepare 10 mL of the NLC formulation using the conventional method, while with the HME technique, 400 mL of the NLC formulation was produced in less than one hour. This makes the HME technique used in this study a faster and industry friendly method for the preparation of NLC formulations. Production of the NLC formulations by the conventional method is a batch process which is time consuming and may result in variability of end-product quality attributes. The HME technique used in this study is a continuous manufacturing process and is able to overcome challenges in variability of end-product quality attributes.

3.3. CHARACTERIZATION OF THE NLC FORMULATIONS

As part of the preliminary studies, two batches consisting of 48 blank NLC formulations, were prepared by the conventional probe sonication method. Composition of the blank NLC formulations is as shown in Table 1. Assessment of formulation variables of the blank NLC formulations from the 1st and 2nd batches was based on particle size, PDI, and zeta potential measurements after a 14-day storage stability period. Particle size, PDI, and zeta potential are key factors for evaluating the stability of colloidal dispersions [22], [23]. Selection of formulations for further studies was based on stability results and the physical properties (e.g. gelling) of the formulations. Based on the stability results as shown in Figure 4, the 2nd batch showed a relatively more stable particle size compared to the 1st batch. On day 14, particle size was within a range of 242.9 ± 6.76 to 721.2 ± 188.20 for the 1st batch and 192.9 ± 3.28 to 416.9 ± 7.90 for the 2nd batch, and thus F4-2 and F4-3 from the 2nd batch were selected for further studies for preparation of RX-HCl loaded NLC formulations using the conventional probe sonication and HME methods. This could be explained by the tendency of the primary surfactant/co-surfactant combination of the 2nd batch to stabilize the system [24]. RX-HCl loaded NLC formulations prepared by the HME technique generally showed a lower particle size compared to the NLCs prepared by the conventional method. On Day 0, particle size of the NLCs prepared by HME was 229.6 ± 6.35 and 199.5 ± 1.23 for F4-2 and F4-3 respectively compared to 327.4 ± 9.79 and 284.2 ± 45.08 for F4-2 and F4-3 respectively for NLCs prepared by the conventional method. The reduced particle size can be explained by the additional shearing effect of the screws in the HME extrusion process that could cause a reduction in particle size of the extruded pre-emulsion material.

3.4. ENTRAPMENT EFFICIENCY AND DRUG LOADING

Percent entrapment efficiency and drug loading results for F4-2 and F4-3 RX-HCl loaded NLC formulations are as shown in Figure 5. The high entrapment efficiency values (> 90 %) can be explained by the high solubilization capacity of the lipids and a relatively high surfactant concentration that leads to increased solubility of the drug in the lipid [25]. F4-3 NLC formulations generally had relatively higher entrapment efficiency values (97.65 % ± 0.029 for the conventional method) compared to F4-2 (96.10 % ± 0.069 for the conventional method) and this can be explained by the effect of increasing the liquid lipid concentration from 1.5 % w/v in F4-2 to 2 % w/v in F4-3. High proportion of liquid lipid may help increase drug solubility in lipid matrix which leads to high entrapment efficiency [26]. NLC formulations prepared by HME showed a relatively higher entrapment efficiency and this could be explained by the high shear generated by the screws inside the barrel which may cause more interaction of drug, lipids, and surfactant resulting in a homogeneous emulsion and an increase in entrapment efficiency [16].

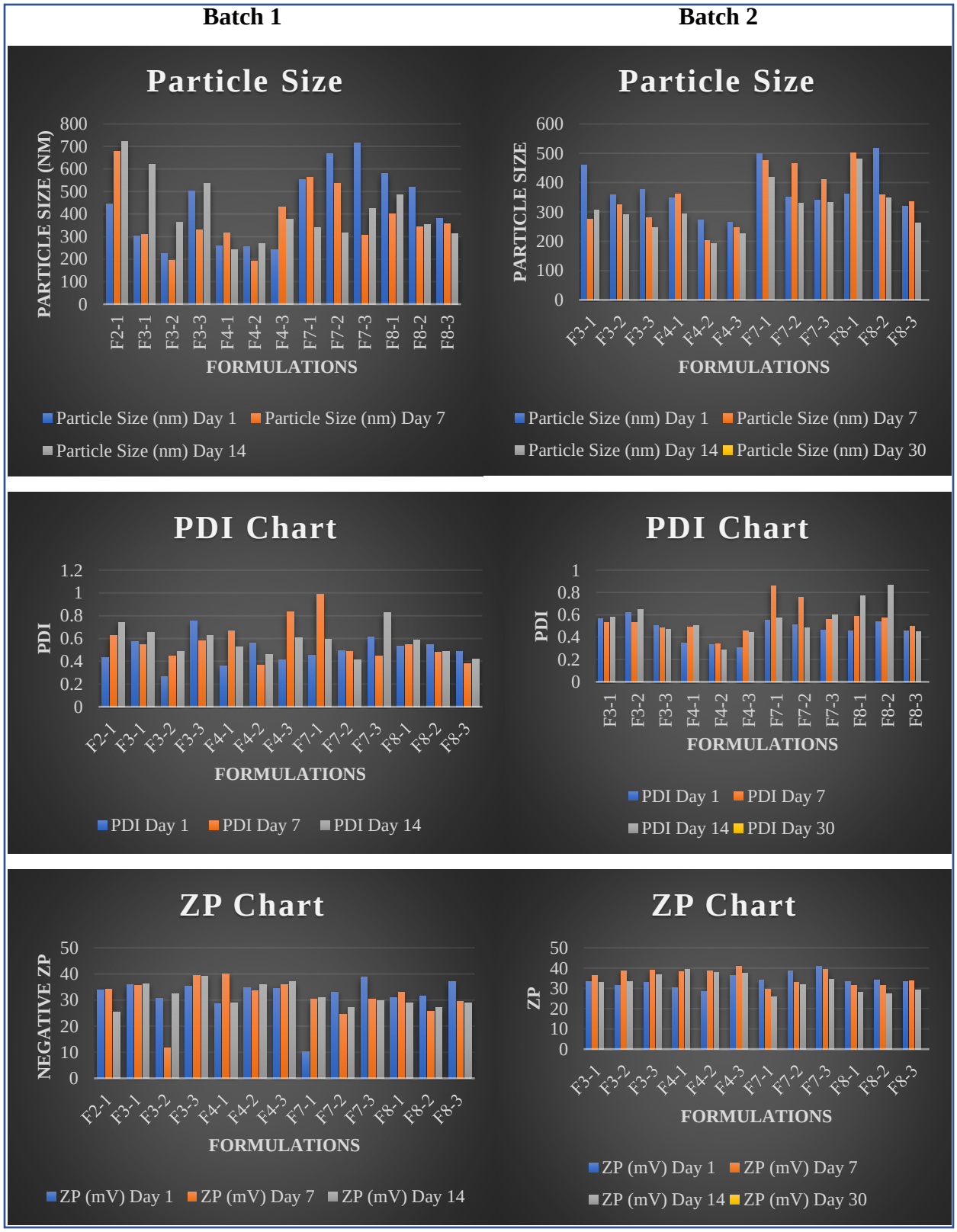

Figure 4. Particle size, PDI, and zeta potential for the blank NLC formulations

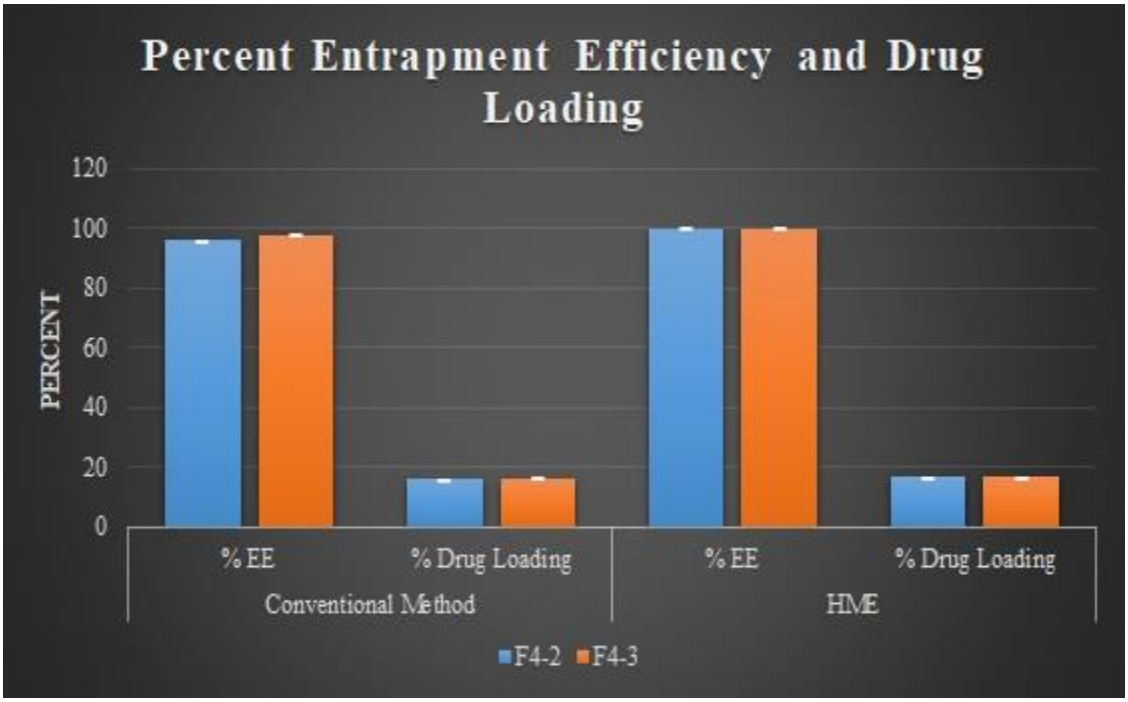

Figure 5. Percent entrapment efficiency and drug loading for the prepared NLC formulations

3.5. IN VITRO DRUG RELEASE STUDIES

RX-HCl is very slightly soluble in water and so, 0.5% v/v Polysorbate 80 was added to the release media in order to increase its saturation solubility, as well as to maintain the sink condition. *In vitro* release of RX-HCl from the F4-2 and F4-3 optimized formulations prepared by the conventional method and HME technology, was compared to the release of the pure drug over a period of 30 hours. The release profile of the prepared NLC formulations is as shown in Figure 6. The amount of RX-HCl released from the NLC formulations was determined by an *in vitro* dialysis bag that retained the NLC particles, whereas the drug released from the NLCs diffused through the dialysis membrane into the release media. It can be observed that the pure drug showed a faster rate of drug release in the first 8 hours compared to the RX-HCl loaded NLC formulations which

showed a sustained release of RX-HCl, and from the 8^{th} to the 30^{th} hour, the pure drug showed a much slower rate of drug release compared to the drug loaded NLCs. This indicates that lipid carriers play a predominant role in sustaining the drug release. The sustained release of the drug from the NLC formulations may be explained by the increased diffusional distance and hindering effects of the surrounding solid lipid shell. It has also been reported that a reduced particle size (in the nm range) as seen in the NLC formulations, provides sustained drug delivery [4]. Based on statistical analysis, there was a significant difference in dissolution profile between the drug loaded NLCs and the pure drug as follows: $t = -1.092$, $p < 0.05$ and $t = -0.959$, $p < 0.05$ for F4-2 and F4-3 formulations prepared by the conventional method respectively, and $t = -0.983$, $p < 0.05$ and $t = -0.655$, $p < 0.05$ for F4-2 and F4-3 formulations prepared by HME technology respectively. F4-3 NLC formulations generally showed a higher dissolution rate than the F4-2 NLC formulations and this may be explained by the decrease of the solid lipid amount and increase in liquid lipid amount in F4-3 NLC formulations that could cause a reduction of formulation viscosity and hence lead to an increase in dissolution rate. NLC formulations prepared by HME also showed a higher dissolution rate than those prepared by the conventional method. Based on statistical analysis, there was a significant difference ($t = -2.398$, $p < 0.05$ and $t = -4.309$, $p < 0.05$ for F4-2 and F4-3 respectively) in dissolution profile between formulations prepared by the conventional method and those prepared by HME technology. This effect could have resulted from the further reduction of particle size and increase in surface area of the NLC formulations prepared by the HME method. According to Nernst-Noyes-Whitney equation, which describes the dissolution rate of drug in a diffusion-controlled process, an increase in the surface area could result in an increase in the dissolution rate [27]–[30].

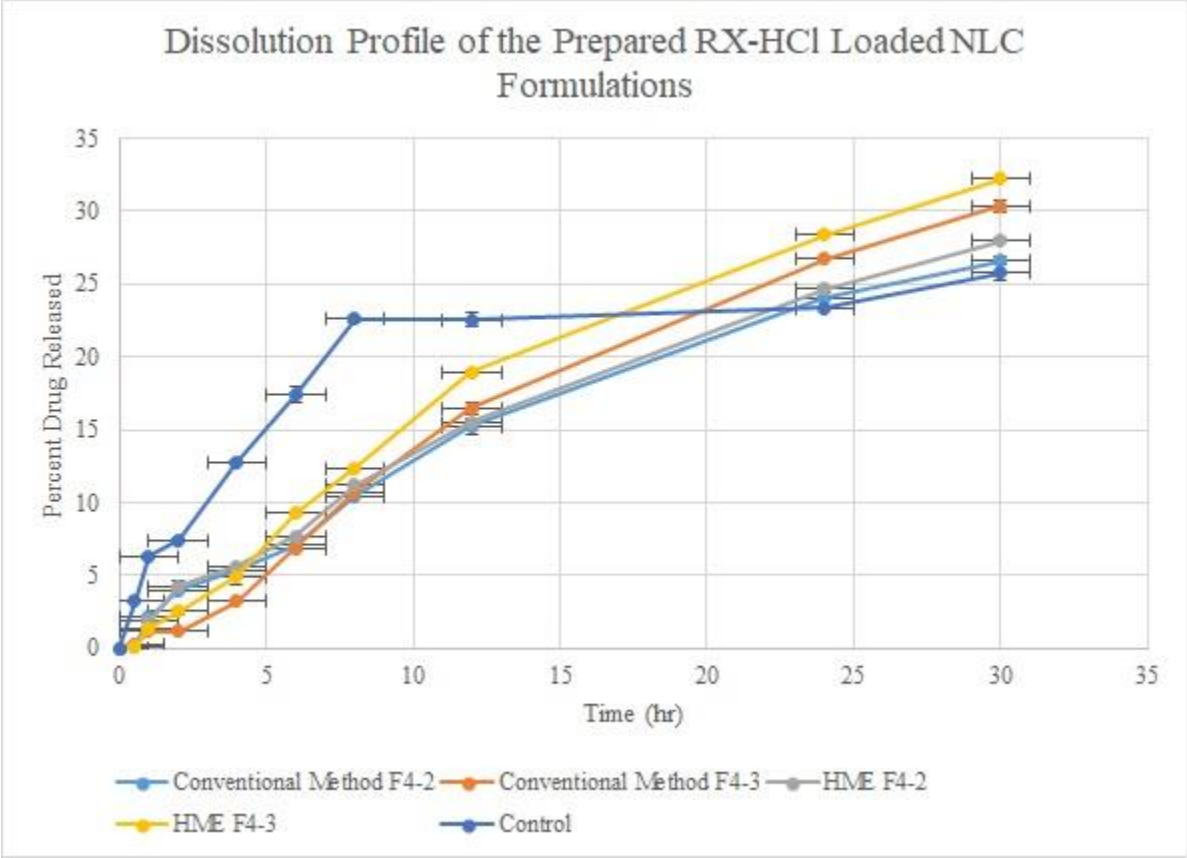

Figure 6. In vitro drug release profile of the prepared RX-HCl loaded NLC formulations

3.6. STABILITY STUDIES

Stability results are as shown in Table 2. Particle size, PDI, and zeta potential are key factors for evaluating the stability of colloidal dispersions [22], [23]. Based on statistical analysis, there was a significant difference in particle size between Day 0 and Day 30 for formulations prepared by the conventional method and stored at 25 °C/ 60 % RH ($t = -20.111$, $p < 0.05$) and 40 °C/ 75 % RH ($t = -1.129$, $p < 0.05$); and also a significant difference for formulations prepared by HME technology and stored at 25 °C/ 60 % RH ($t = -4.901$, $p < 0.05$) and 40 °C/ 75 % RH ($t = -118.5$, $p < 0.05$). Despite these differences, the particle size of the NLC formulations were kept within a

desirable submicron range (less than 300 nm) except for F4-3 prepared by the conventional method that had a particle size of 483.9 ± 8.85 on day 30. The higher particle size of F4-3 could be explained by the low negative zeta potential value (-17.9 ± 2.63) on day 30 that could cause flocculation of the nanoparticles. There was a significant difference ($t = 13.931$, $p < 0.05$) in particle size on day 0 between NLC formulations prepared by the conventional method and HME technology. The low particle size of the NLCs prepared by HME could be explained by the additional shearing effect of the screws in the HME extrusion process that could cause a reduction in particle size. A low PDI value indicates a narrow size distribution, whereas a high PDI value indicates a broad size distribution [31]. Usually a PDI value below 0.5 is acceptable [31]. All PDI values were within an acceptable range (≤ 0.523), and thus the change in PDI was not considered to be significant in the present study. The amount of charge on the particle surface is one of the important characteristics giving information on the tendency of nanoparticles to agglomerate and on their long-term stability [4], [32]. A zeta potential greater than -60 mV is known to require for excellent physical stability of nanoparticles, whereas greater than -30 mV it indicates good electrostatic stabilization and good physical stability [4], [33]. All zeta potential values were within an acceptable range of -15 to -30 mV, and thus the change in zeta potential was not considered to be significant in the present study.

In the present study, all PDI and zeta potential values were kept within an acceptable range, and this indicates good physical stability of the prepared NLC formulations.

Table 2. Particle size, PDI, and zeta potential of the drug loaded NLCs

Preparation Method	Formulation	Particle Size (nm)			PDI			Zeta Potential (mV)		
		Day 0	Day 30		Day 0	Day 30		Day 0	Day 30	
			25 °C/ 60 % RH	40 °C/ 75 % RH		25 °C/ 60 % RH	40 °C/ 75 % RH		25 °C/ 60 % RH	40 °C/ 75 % RH
Conventional Method	F4-2	327.4 ± 9.79	336.9 ± 3.26	483.9 ± 8.85	0.426 ± 0.03	0.513 ± 0.04	0.523 ± 0.05	-18.1 ± 1.88	-19.1 ± 0.21	-17.9 ± 2.63
	F4-3	284.2 ± 45.08	292.8 ± 24.70	293.7 ± 11.84	0.432 ± 0.03	0.417 ± 0.02	0.389 ± 0.01	-25.2 ± 0.23	-24.1 ± 1.37	-20.5 ± 1.21
HME	F4-2	229.6 ± 6.34	259.4 ± 11.25	276.6 ± 8.53	0.347 ± 0.08	0.434 ± 0.03	0.464 ± 0.03	-17.9 ± 0.55	-17.5 ± 1.91	-26.6 ± 1.29
	F4-3	199.5 ± 1.23	219.2 ± 10.25	247.3 ± 3.80	0.376 ± 0.04	0.412 ± 0.06	0.448 ± 0.04	-22.7 ± 0.66	-25.3 ± 0.70	-20.5 ± 1.21

CHAPTER 4: CONCLUSION

HME technology coupled with probe sonication was successfully utilized to prepare RX-HCl loaded NLC formulations as a continuous manufacturing process with shorter processing times as compared to the conventional method. HME coupled with probe sonication is a continuous manufacturing process that is able to overcome challenges in variability of end-product quality attributes that are common with the conventional batch process. Results from this study suggest an efficient method that can be used to prepare RX-HCl loaded NLC formulations with extended drug release and good physical stability, and hence could provide a better regimen for the prevention and treatment of postmenopausal osteoporosis

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