Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 8, 2014

Original Article

CONTROLLED RELEASE IBUPROFEN NANOPARTICLES: PHYSICO-CHEMICAL CHARACTERIZATION AND DRUG RELEASE

VALERIE SASTRE[1] AND EVONE S. GHALY[1,2]

School of Pharmacy, University of Puerto Rico, P.O. Box 365067, San Juan, P.R 00936
Email: evone.ghaly@upr.ed

Received: 16 Jun 2014 Revised and Accepted: 20 Jul 2014

ABSTRACT

Objectives: The effect of lipid phase composition, concentration of lipid and surfactant on the entrapment, morphology, particle size and release profiles of ibuprofen-loaded lipid particles was evaluated.

Methods: Formulations containing only a solid lipid (Compritol® 888 ATO) and formulations containing mixture of solid and liquid lipids (Compritol® 888 ATO: Labrafil® M 1944 CS) were prepared at various lipid level (5%, 10% and 15% w/v) and surfactant (1.35% and 3%w/v) concentrations using the hot homogenization method.

Results: The particle size distribution was found to be polydisperse with a high concentration of microparticles. The particles were spherical in shape. The preparation method was effective in producing particles with high drug entrapment efficiencies (65% – 100%). Drug release studies showed a controlled release profile that follow diffusion kinetic model for all formulations. High lipid concentrations (10% and 15%) increased drug entrapment capacity and showed low initial burst of the drug during early time of testing dissolution. X-Ray diffraction and NMR studies showed coexistence of both amorphous and crystalline forms of the ibuprofen within the lipid matrix. Addition of Labrafil® led to a more amorphous internal structure by modifying the crystallinity of both ibuprofen and the lipid Compritol®.

Conclusions: This investigation solved problems associated with nanoparticles

Keywords: Ibuprofen, Lipid matrix, Solid state, Kinetic release model, Lipid-surfactant nanoparticles, Controlled release.

INTRODUCTION

Drug nanoparticles can be defined as drug-containing particles having size smaller than 1 um. In the last years nanoparticles have been introduced as an interesting alternative to the traditional carriers. Over the past decade, lipid matrices became very popular in controlling release of drugs and during the beginning of the 1990s solid lipid nanoparticles (SLN) were developed. SLNs are in the submicron range (50-1000 nm) and are composed of lipid component that are physiologically tolerated, as opposed to the traditional polymeric nanoparticles that have potential to acute and chronic toxicity. The SLNs systems can be used to provide targeted delivery of drugs, to improve oral availability, to sustain drug effect on target tissue, to solubilize drug for intravascular delivery, and to improve the stability of therapeutic agents against enzyme degradation [1-2].

However depending on the nature of the drug and due to the crystal-forming nature of the solid, SLNs may present some challenges such as limited drug loading capacity and drug leakage during storage. To overcome these limitations, a second type of lipid base nanoparticles composed of a mixture of solid and a liquid lipid was developed [3]. This system is known as nanostructured lipid carriers (NLC). According to previous research, NLCs led to special nanostructured with improved properties for drug loading, modulation of drug release and stable drug incorporation during storage [4-8].

Physical characterization of nanoparticles are not fully developed and the mechanistic consideration as to why a specific formulation or process produces such result is not fully explored. On the other hand, drug loading, narrow particle size distribution and stability of drug release are common problems in nanoparticles.

In this research ibuprofen nanostructured lipid carriers are prepared by using a mixture of solid lipid (Compritol), liquid lipid (Labrifil) and surfactant (pluoronic F 127) using hot melt homogenization technology. The effect of the composition of lipid, surfactant concentration and ratio of drug to lipid on the physic-chemical properties of the nanoparticles and the solid state phase transformation were investigated.

Our hypothesis are:1) The incorporation of liquid lipid with the solid lipid may change the structure of the lipid matrix, increase drug loading and affect drug release, 2) formulation components, surfactant concentration, and ratio of lipids to drug may affect particle size of the nanoparticles, prevent burst effect during early time of dissolution, increase drug stability during storage and affect physicochemical properties of the nanoparticles and 3) interaction between drug and lipid may affect the solid state and crystallinity of both drug and lipid. Therefore the specific aims of this research are: 1) Design and develop nanoparticles containing high load of drug, 2) test the physical properties such as: particle size, polydispersibility index, drug dissolution and leakage of the drug from the nanoparticles, 3) Investigate the possibility of the interaction between ibuprofen and the lipid, crystallinity and phase transformation of both drug and lipid.

Experimental design

Lipid-based nanoparticle formulations were prepared at different surfactant and lipid concentrations. Two types of lipids were evaluated as well. The amount of drug remained constant. Table 1 describes the experimental design. Additionally, a control without drug (blank) batch was prepared and measured by U.V spectrophotometer to assure lipids selected did not interfere with drug absorbance.

MATERIALS AND METHODS

Materials

Ibuprofen(+)-2-(4-Isobutyl phenyl) propionic acid, Lot No. C130558, PCCA, USA; Compritol® 888 ATO (solid), Lot No. 134916, Gattefosé, Canada; Labrafil® M 1944 CS (oil), Lot No. 26690, Gattefossé, Canada; Pluronic F68, Lot No. 046K00431, Sigma, USA. All other ingredients are USP grade.

Preparation and characterization of nanoparticles

Ibuprofen loaded lipid nanoparticles were prepared by the hot homogenization method. For the 5%, 10% and 15% (w/v) solid lipid nanoparticle formulations (SLN), 2.5g, 5g or 7.5g of Compritol®

respectively were weighed, placed inside a 50 mL beaker and melted at 80°C in a hot water bath. Simultaneously, 50 mL of a 1.35% or 3% (w/v) surfactant solution was heated at the same temperature. The surfactant solutions were prepared prior to heating of the lipids. These were prepared by placing 1.35g or 3g of Pluronic® inside a volumetric flask and adding ultrapure water until a volume of 100 mL was reached to obtain a solution of 1.35% and 3% concentration respectively.

For the solid:liquid lipid mixture formulations, also referred to as nanostructured lipid carriers (NLC), 30% of the total lipid phase was replaced with the liquid lipid (solid:liquid ratio equal to 70:30). The liquid lipid was added to the solid lipid after it was completely melted. An amount of 1600 mg of Ibuprofen was added to the melted lipid phase. After the drug was completely dissolved in the lipid phase, the surfactant solution was added to the lipid phase to create an oil-in-water (O/W) emulsion. The dispersion was mixed using a high speed homogenizer for 10 minutes at 10000 rpm, maintaining the same temperature (80°C). The homogenous emulsion was then removed from heat and placed in ice water bath at 0-2°C under magnetic stirring (200 rpm) for 20 minutes, allowing the inner oil phase to solidify and form aqueous dispersed particles. The obtained dispersion was stored at 4°C. For further processing and testing, within a week from preparation the emulsions were lyophilized (freeze dried) to remove water and obtain a dry powder. The purpose of freeze drying of the nanoparticles was to stabilize the system and increase shelf life during storage [9]. Samples were freeze-dried in a LabConco freeze dryer system at 20°C under vacuum at 10 microns of pressure for 24 hours. The dry powder samples were stored at 4°C.

Table 1: Experimental design

Factor	Level	Description
Type of lipid	2	Solid Lipid and Solid/Liquid Lipid Mixture (SLN and NLC)
Surfactant Concentration	2	1.35% and 3%
Lipid Phase Concentration	3	5%, 10% and 15%

Drug content determination

Drug content was determined by UV spectrophotometry. An amount of 0.1g of the lyophilized particles was accurately weighed and transferred to a 100 mL volumetric flasks. Volume was brought up to100 mL with phosphate buffer (pH= 7.4). The samples were stirred for six hours and all assay tests were run in triplicate. After stirring, 1mL of the solution was diluted with buffer to specific volume and filtered using 0.22µm syringe with filters. Samples were analyzed in a UV Beckman Coulter spectrophotometer, at a wavelength of 223 nm. Testing was performed in triplicates. Drug content was calculated using the standard curve equation. Drug entrapment percent in the lipid matrix was calculated using the following equation:

$$\text{Drug Entrapment \%} = \frac{\text{actual drug content}}{\text{Theoretical drug content}} \times 100$$

Particle size

Mean particle size was measured by dynamic light scattering (DLS) using a 90 Plus Particle Size Analyzer (Brookhaven Instrument Corporation). Analyses were performed at 25°C using a 90° scattering angle. The samples were prepared by dispersing approximately 20 mg of the dry powder in previously filtered ultrapure water. The dispersion was placed in ultrasonic bath for 10-15 minutes to homogenize the suspension. A small time in the ultrasonic bath is sometimes useful in breaking up loosely-held agglomerates). The suspension was diluted until a suitable concentration was obtained for analysis. This concentration varied from 0.002% to 0.004% depending on the formulation. An aliquot from the suspension (3mL) was withdrawn and placed in disposable acrylic square analysis cells.

Scanning electron microscopy

Morphological evaluation of selected particles was performed by scanning electron microscopy (SEM) using a Phillips SEM 515 microscope. A small amount of the dry powder was placed on aluminum specimen stubs covered with double-sided tape. The samples were then submitted to a gold/palladium coating using a Hummer®6.2 Sputtering System at 10mA for six minutes under Argon atmosphere. Magnifications of 600X and 3100X were used to evaluate the samples at a voltage of 30kV.

Dissolution testing

Dissolution testing was performed in a Branson Research SR6, apparatus 1 using 900 ml phosphate buffer pH 7.4at 50 rpm and temperature of 37^0 c + 0.5^0 C. An amount equivalent of 200 mg of Ibuprofen was weighed and placed in a dissolution basket. The basket was placed inside a flask containing 900 mL of phosphate buffer at pH = 7.4. Aliquots were withdrawn at the following time intervals: 15 minutes, 30,, 45, 60, 120, 180, 240, and 360 minutes. The same amount of volume withdrawn was replaced with buffe at room temperature.

X-ray diffraction and Nuclear magnetic resonance

To evaluate the internal behavior and structure of the particles, X-Ray diffraction (XRD) and Nuclear Magnetic Resonance (NMR) studies were applied. A small amount of the freeze dried powder was used. Also, the drug, the lipids and the surfactant used during formulation were separately analyzed. X-Ray diffraction was performed between Bragg angles 5° and 40° 2θ range. Data was plotted in order to analyze the peaks. Nuclear Magnetic Resonance analysis was performed in a Brucker Solid State NMR with a magic angle spinning (MAS) of 4000Hz.

Statistical analysis

Quantitative data was reported as mean ± standard deviation. Statistical analysis was performed in Minitab Software using one-way ANOVA (analysis of variance) in combination with Tukey's test for comparison between two means with statistical significance evaluated at $p < 0.05$, 95% confidence interval.

RESULTS AND DISCUSSION

Ibuprofen entrapment percent in the particles was estimated based on the drug content calculation and the theoretical amount of drug content in 100 mg of the dry powder as described previously. The results obtained for each formulation are summarized in Table 2. Satisfactory drug entrapments percent were obtained for all formulations, ranging from 65% to 102%. The lowest entrapment percent was obtained from formulations containing 5% Compritol (approximately 68% ± 3.63 and 65% ± 0.29 from formulations containing 1.35% and 3% Pluronic respectively), most probably due to the low amount of lipid available to entrap the drug. However, this was not the case for formulations containing 5% Compritol: Labrafil, where the entrapment efficiency was surprisingly high (aproximately 96% ± 1.03 and 102% ± 2.75 for formulations containing 1.35% and 3% Pluronic respectively). The liquid lipid in the lipid phase enhanced for entrapment of the drug in the 5% lipid formulations since the presence of the liquid lipid led to more imperfections in the crystal and higher drug loading capacity [10]. In all SLN formulations, the entrapment efficiency increased with increasing lipid phase concentration. This is related to the fact that more lipid is available to entrap the drug particles. No statistical significant difference was detected between 1.35% Pluronic and 3%

Pluronic formulations in SLNs, therefore, surfactant concentration did not significantly impact the drug entrapment capacity in these formulations. Significant difference, however, was detected in NLCs between formulations with 5% lipid-3% Pluronic and 5% lipid-1.35% Pluronic. On the contrary, the entrapment percent in the formulation containing 10% lipid-1.35% Pluronic showed higher drug entrapment (99.29% ± 2.21) than formulation containing 3% Pluronic (92.58% ± 1.70). Significant difference between SLNs and NLCs formulations was only evident for formulations containing 5% lipid (1.35% and 3% Pluronic). All other formulations were statistically similar. When comparing different lipid concentrations within SLNs (besides the evident difference between 5% lipid and the other formulations) significant differences were also present between 10% and 15% lipid - 3% Pluronic, where the drug entrapment efficiency was higher for formulation containing 15% lipid (approximately 98% ± 6.38 versus 89% ± 0.70 for formulation containing 10% lipid). No such statistical difference was apparent for the 1.35% Pluronic formulations. For NLCs formulation containing 5% lipid – 3% Pluronic is statistically different from both 10% and 15% counterparts. This formulation showed unexpected higher drug entrapment efficiency when compared with formulations with higher lipid concentration. Also, NLC formulations containing 10% and 15% lipid resulted significant difference between both Pluronic concentrations (1.35% and 3%); the entrapment percent was higher with increasing lipid phase concentration, similar to the results obtained with SLNs.

Table 2: Drug entrapment percent in SLN and NLC formulations

Lipid type	Surfactant type Concentration	Lipid phase Concentration	Entrapment %
		5%	67.84
	1.35%	10%	95.87
		15%	101.90
Compritol (SLN)		5%	65.14
	3%	10%	88.79
		15%	98.01
		5%	96.13
	1.35%	10%	93.72
		15%	99.29
Compritol (SLC)+ Labrafil		5%	102.38
	3%	10%	86.10
		15%	92.58

Table 3: Particle size distribution

Nanopaericle batches based on lipid type	Lipid %	Pluronic %	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)	Overall mean (µm)
SLN	5	1.35	0.123	0.142	8.0	4.3
NLC	5	1.35	0.168	7.5	10.0	5.9
SLN	10	1.35	0.44	7.9	9.0	5.4
NLC	10	1.35	0.293	0.4	6.6	3.0
SLN	15	1.35	0.355	0.447	8.9	4.3
NLC	15	1.35	0.144	0.193	8.6	4.5
SLN	5	3	0.331	1.3	1.6	1.1
NLC	5	3	0.135	7.3	9.0	6.9
SLN	10	3	0.129	0.149	8.0	4.4
NLC	10	3	0.142	2.0	2.3	1.6
SLN	15	3	0.145	7.5	9.0	6.5
NLC	15	3	0.074	1.2	1.5	1.1

Particle size

A polydisperse particle size distribution was observed in all formulations with particles in the nano- and micro- range. Due to the high concentration of particles larger than 1µm in some formulations, the overall mean particle size for all SLN formulations was 4.9 µm ± 2.3 and 3.9 µm ± 2.3 for NLCs. Table 3 provides a detailed description of how the entire population in the sample is distributed, where the terms d_{10} d_{50} and d_{90} are the 10%, 50% and 90% percentiles.

Overall, the formulations showing the smallest particle size were 5%SLN-3% Pluronic, 10%NLC-3% Pluronic and 15%NLC-3% Pluronic where no particles above 3µm were detected. In case of SLNs, this result is consistent with previous research [11] where it was found that low concentration of lipid combined with high concentration of surfactant favored a small mean particle size in lipid nanoparticles.

With exception of 15%SLN-1.35% Pluronic, there was tendency for presence of larger particles as the lipid concentration of surfactant was increased in the SLNs formulations. It has been reported previously that mean particle size increased with increasing lipid concentration in solid lipid nanoparticles systems [12]. In the case of NLCs, Labrafil appears to have the opposite effect and the formation of smaller particles was favored with increasing both lipid and surfactant concentrations. This may be related to the co-surfactant properties of Labrafil which further reduces the surface tension and probably promotes higher rate of partition of the particles during preparation. Furthermore, the presence of the oil may promote higher molecular mobility of the matrix and, thus, helps in formation of a small particle population [13]

As the lipid concentration in NLCs formulations increased, the population of particles of smaller size also increased as well. This was true for both 1.35% and 3% surfactant concentrations.

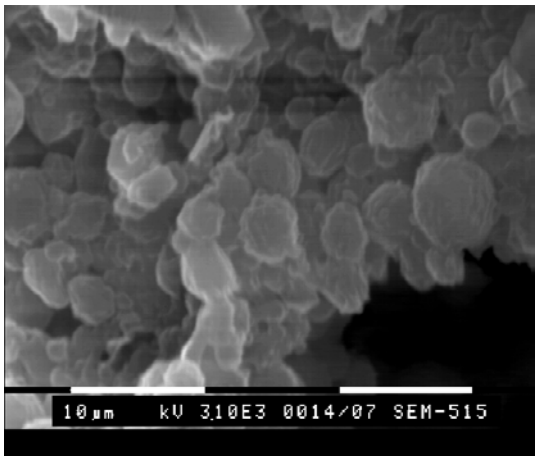
The 3% surfactant concentration seemed not to be enough to consistently produce particles of smaller size in all formulations; However, the three formulations with the lowest overall mean particle size, all contained 3% surfactant.

The broad distribution in particle size may be due to a combination of two factors: preparation method and lyophilization process. Further work is in process using high pressure microfluidizer method for preparing lipid nanoparticles and including in the formulation fructose as cryoprotectent to prevent agglomeration during freeze drying

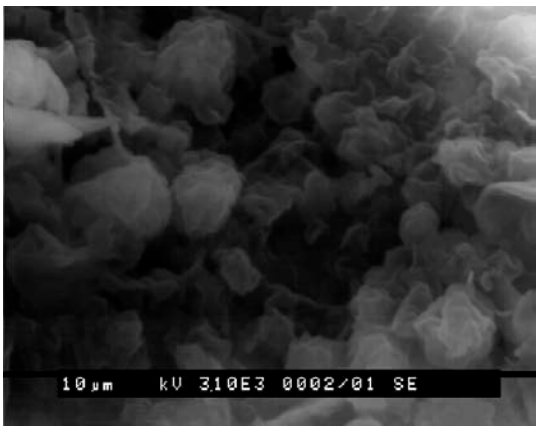
Scanning electron microscopy

SLN and NLC formulations containing 15% lipid with both 1.35% and 3% surfactant concentrations were selected for morphology evaluation. All particles show, predominantly, a spherical shape (Figres 1 and 2). At a lower magnification (600X), the NLC formulation containing 3% surfactant appears to have a higher tendency to form aggregates. It is presumed that the aggregation may have occurred during storage. The different contents of emulsifying monoglycerides and diglycerides (e.g. quantified by hydroxyl -OH- number) might lead to different contents of water in the lipid nanoparticle matrix, which could potentially also destabilize the particles [12] during storage.

This type of aggregation was not observed in the SLNs formulations (prepared the same day as NLCs) which maintained their spherical shape during storage as shown in the micrographs. At a higher magnification (3100X), however, the NLC particles seem to be smaller in size than SLNs at the same magnification. This correlates with the particle size analysis previously discussed. From the micrographs, presence of free drug crystals is not apparent (in the scanning electron micrographs ibuprofen appears as smooth-surface rectangular crystals), suggesting that most of the drug is embedded within the lipid matrix. The effect of surfactant concentration in SLNs was unclear in the scanning electron microscopy. In the NLC formulations, the particles containing 3% surfactant appear smaller than the particles containing 1.35% surfactant, which is also supported by particle size analysis

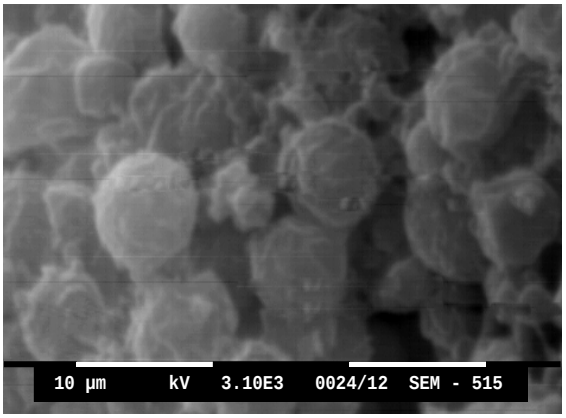


(A)

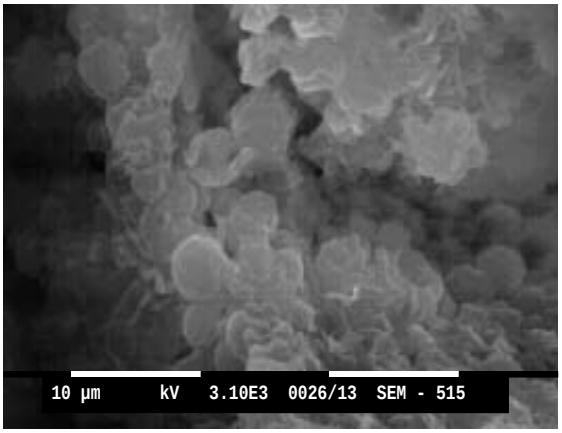


(B)

Fig. 1: SEM of (A) formulation containing 15% Compritol and 3% Pluoronic; (B) formulation containing 15% Compritol:Labrifil and 3% Pluoronic. (3100 X)



(A)



(B)

Fig. 2: SEM of (A) formulation containing Compritol and 1.5% Pluoronic; (B) formulation containing Compritol:Labrifil amd 1.5% Pluoronic (3100 X)

Dissolution study

Dissolution testing was performed to evaluate ibuprofen release from the lipid particles over a 6 hours period in phosphate buffer, pH: 7.4. Statistical differences were evaluated between surfactant concentrations (1.35% and 3%), lipid phase concentrations (5%, 10% and 15%) and between SLN (solid lipid) and NCL (solid/liquid lipid blend) at the 1st, 2nd, 3rd, 4th and 6th hour (5 intervals). Formulations with significant differences ($p < 0.05$) in three or more intervals were considered statistically different.

The mean percent of drug dissolved at 1 hour and 6 hours intervals for all formulations is shown in Table 4.

Both SLN and NLC formulations showed similar drug release profiles upon comparison except for the 5% lipid-1.35% Pluronic and 10% lipid-1.35% Pluronic combinations, where the release rate for NLC was higher than for SLN (approximately 75% and 78% drug released vs. 56% and 70% respectively after 6-hr interval). No statistical difference was observed between SLNs and NLCs when the lipid concentration was 15%. No difference was observed either between SLNs and NLCs when the surfactant concentration was increased to 3%. (Figure 3).

The highest initial burst observed was 39% of ibuprofen released at 15 min. of dissolution from NLC formulation containing 5% lipid-1.35% Pluronic. This formulation was also one of the formulations showing an overall higher release rate, with approximately 76% of drug dissolved at the 6 hours interval. The high initial burst may be attributed to the presence of drug precipitates on the surface of the particles. Since ibuprofen solubility in aqueous media at 37°C is higher than at room temperature (24°C), drug particles located at

the surface would dissolved first. The other formulation showing a high release rate was NLC formulation containing 10% lipid -1.35% Pluronic, approximately 79% of ibuprofen was released at the 6 hours interval. This formulation also showed a high initial burst (approximately 29% of drug was released at 15 min.). This data suggests that the addition of Labrafil in the Compritol lipid matrix did not play a significant role in retarding ibuprofen drug release from the particles in these formulations. The combination of low lipid concentrations with low surfactant concentration in the NLC system led to a more rapid release perhaps due to increased water penetration. As the lipid concentration increased to 15% in the NLC system containing 1.35% surfactant, the concentration of drug released at 15 min. also decreased.

Another formulation showing a high initial burst was the SLN formulation containing 15% lipid-1.35% Pluronic (approximately 30% drug released at 15 minutes) However, the drug concentration at the end of the 6 hours interval was approximately 56%.

Table 4: Mean percent of drug dissolved at 1-hr. and 6-hr. intervals

Formulation	Percentage drug released at 1 hr. and 6 hr. intervals			
	1 hr.		6 hr.	
	Mean %	SD %	Mean %	SD %
5% Comperitol 1.35% Pluronic	37.4	1.99	56.0	4.59
5% Comperitol 3% Pluronic	33.5	2.70	68.4	4.21
10% Comperitol 1.35% Pluronic	37.1	3.00	69.2	2.87
10% Comperitol 3% Pluronic	30.7	3.53	54.3	2.01
15% Comperitol 1.35% Pluronic	37.2	3.32	56.4	4.47
15% Comperitol 3% Pluronic	34.5	5.47	71.0	3.88
5% Comperitol/Labrafil 1.35% Pluronic	48.9	2.34	75.6	4.88
5% Comperitol / Labrafil 3% Pluronic	37.3	4.92	66.2	3.37
10% Comperitol/ Labrafil- 1.35% Pluronic	48.2	3.01	77.8	3.31
10% Comperitol/ Labrafil- 3% Pluronic	27.5	1.54	59.3	4.17
15% Comperitol/ Labrafil -1.35% Pluronic	31.6	2.72	60.11	2.01
15% Comperitol / Labrafil- 3% Pluronic	34.3	1.22	64.7	1.38

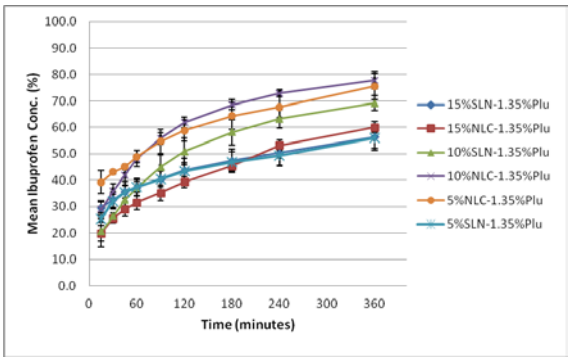


Fig. 3: Dissolution profiles of formulations containing 1.35% Pluronic

When comparing the release profiles of formulations containing 1.35% of surfactant versus formulations containing 3% surfactant, significant differences were observed for the formulations containing 10% lipid (both SLN and NLC), where the release rate was higher for the 10% lipid - 1.35% Pluronic combination than for the 10% lipid – 3% Pluronic combination. In contrast, the SLN formulation containing 15% lipid – 3% Pluronic combination Showed significant difference from the SLN containing 15% lipid - 1.35% Pluronic formulation that gave higher release rate was higher for. Even though the two formulations followed similar release rates during the first 2 hours of dissolution. However the initial burst for 15% lipid - 1.35% was higher (approximately 30% of drug was released at 15 minutes) the release rate for the 15% lipid – 3% Pluronic formulation increased after the third hour and gave 71% drug released after 6 hours vs. 56% for the 1.35% Pluronic formulation. As shown in Figure 3.

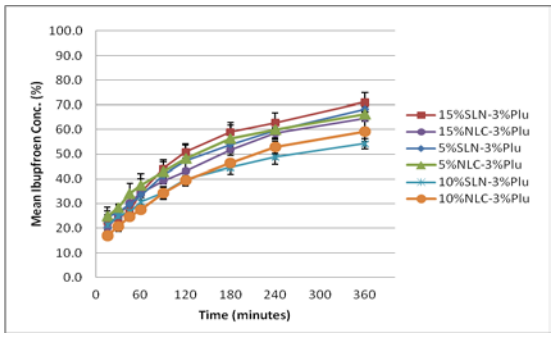


Fig. 4: Dissolution profiles of formulations containing 3% Pluronic

Comparing the release profiles of the different lipid concentrations in the SLNs formulations, it was observed that the release rate was higher for the 10% lipid formulations when the surfactant concentration was 1.35%. Therefore, 5% and 15% lipid formulations containing 1.35% showed similar release profiles. The opposite occurred when the surfactant concentration was increased to 3%, where the 10% lipid formulation showed a lower release rate than the 5% and 15% formulations. In the case of NLC formulations, the formulations containing 1.35% surfactant showed lower release

profile with the highest lipid concentration. Therefore, 5% and 10% lipid formulations were not significantly different from each other (76% and 78% drug released at 6 hours interval respectively) but both were different from formulation containing 15% lipid, which showed lower release rate (60% drug released at 6 hours). However, when the surfactant was increased to 3%, all lipid concentrations showed similar release rate and no significant difference was detected between them.

As mentioned previously, at 3% surfactant concentration both SLN and NLC of same lipid concentration showed similar release rates and no significant difference was detected. It appears that increasing surfactant concentration stabilizes the system in a certain way during dissolution and tends to retard the release more than a lower surfactant concentration. The surfactant-lipid interactions play a significant role in the release rate as the type of surfactant and its concentration can affect the chemical stability of the lipid matrix (e.g. different surfactant incorporation in the outer shell of the particles, will have different solubilizing capacities for water in the lipid phase) (11) and this is why the formulation behavior depends on specific combinations of lipid concentration and surfactant concentration used. Low surfactant concentration with high lipid concentration had a different effect than high surfactant concentration and low lipid level on the release profile for the solid lipid formulations. In summary, all formulations showed prolonged drug release over 6 hours without exceeding approximately 75% of drug released at 6 hours. Formulations containing higher surfactant concentration showed lower concentration of drug released at 15 minutes (decreased burst effect). With a few exceptions, formulations containing higher lipid content (10% and 15%) also showed the lowest initial bursts, which is a desirable attribute when developing controlled release dosage forms. These data suggests that formulation composition is the determinant factor on drug release profile. No correlation was identified between mean particle size and drug dissolution rate,

Controlled release kinetics

The dissolution data was evaluated against the most commonly used kinetic models to understand the release mechanism from the lipid matrices developed in this research. The dissolution data were analyzed using first order, zero order and Higuchi diffusion model to determine the kinetic model for the drug release from the nanoparticles.

The release profiles of all the formulations tested could be best explained by the Higuchi model, as the plots show high linearity, with correlation coefficient (R^2) higher than 0.95. Fair linearity to the first order model was observed. The diffusion mechanism of drug release was further confirmed by Korsmeyer-Peppas plots with R^2 values between 0.96 and 0.99, and slope values (n) less than 0.45, indicating that ibuprofen release mechanism from the particles followed non-Fickian diffusion transport (14, 15) as shown in

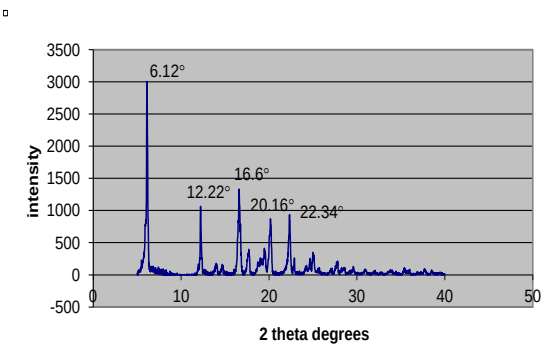
Table 5: Correlation coefficients for different kinetic models

Formulation	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas	
				R^2	N
5%Compritol-1.35%Pluronic	0.8845	0.9317	0.9681	0.9876	0.2337
5%Compritol-3%Pluronic	0.9388	0.9826	0.9893	0.9751	0.3564
10%Compritol-1.35%Pluronic	0.8733	0.944	0.9701	0.9888	0.4001
10%Compritol-3%Pluronic	0.9222	0.9561	0.9879	0.9892	0.3141
15%Compritol-1.35%Pluronic	0.9405	0.9692	0.9962	0.9921	0.2042
15%Compritol-3%Pluronic	0.8902	0.955	0.966	0.9581	0.413
5%Compritol/Labrafil-1.35%Pluronic	0.9312	0.9804	0.9912	0.9834	0.2149
5%Compritol/Labrafil-3%Plu	0.903	0.9557	0.9821	0.9912	0.3308
10%Compritol/Labrafil-1.35%Pluronic	0.8372	0.9307	0.9504	0.9845	0.3272
10%Compritol/Labrafil-3%Pluronic	0.9324	0.9716	0.9922	0.9939	0.4196
15%Compritol/Labrafil-1.35%Pluronic	0.9525	0.9841	0.9959	0.9950	0.3433
15%Compritol/Labrafil-3%Pluronic	0.9252	0.9715	0.991	0.9973	0.3781

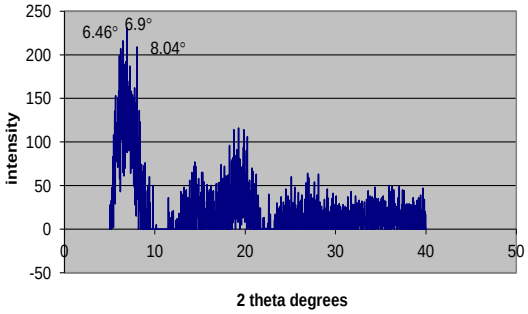
X-ray diffraction

Formulations containing 15% lipid-3% surfactant were selected and analyzed using XRD to evaluate drug-lipid interactions. Each component was analyzed separately as shown in Figures 5 to 6 to evaluate changes after incorporation into the lipid nanoparticle system. The diffractogram of pure ibuprofen powder showed high-intensity peaks at Bragg angles 6.12°, 12.22°, 16.6°, 20.16° and 22.34° as shown in Figure 5. The diffractogram for pure Compritol shows a high intensity peak at 21.26°, denoting its crystalline nature, and another less intense at 21.34° (Fig. 5). Pure Pluronic showed two peaks also low in intensity when compared with the drug and the solid lipid: one at 19.42° and another at 23.44° (Fig. 5). Pure Labrafil showed multiple agglomerated peaks due to its complex structure, nonetheless, they are low in intensity as Labrafil is not a crystalline component (Fig. 6).

The most notable peaks in this diffractogram are at 6.46° and 8.04 The SLN diffractogram (Figure 7) showed intensity peaks that correspond to ibuprofen (at 6.1° and 16.8°) with significantly reduced intensity and a very slight shift of the third peak. The disappearance of several ibuprofen peaks represents the coexistence of amorphous and crystalline drug within the lipid matrix (10). The highest intensity peak in this diffractogram (21.4°) corresponds to the lipid (Compritol). It is observed that this peak is also less intense and slightly shifted when compared with the pure lipid. The reduced intensity may be attributed to the presence of ibuprofen in the crystal lattice which changes the crystallinity of the SLN. A less intense peak detected at 23.52° may be attributed to the surfactant (Fig. 14). In the NLC diffractogram (Fig. 7) all peaks are less intense, similar to the pure Labrafil. The addition of the liquid lipid modified the crystallinity of both ibuprofen and Compritol most probably leading to a more amorphous structure. A peak corresponding to ibuprofen can be observed at 16.6° and the most notable peak corresponds to Compritol at 21.46°. Also, a peak corresponding to Labrafil can be identified at 6.4° and another to Pluronic at 23.44°.

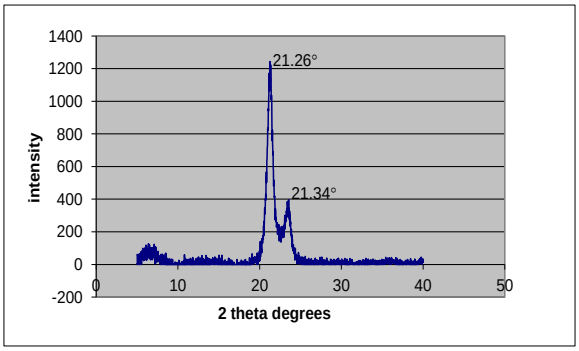


(A)

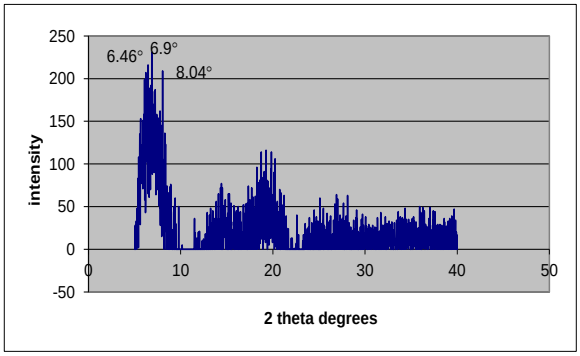


(B)

Fig. 5: XRD for ibuprofen (A) and Pluoronic (B).

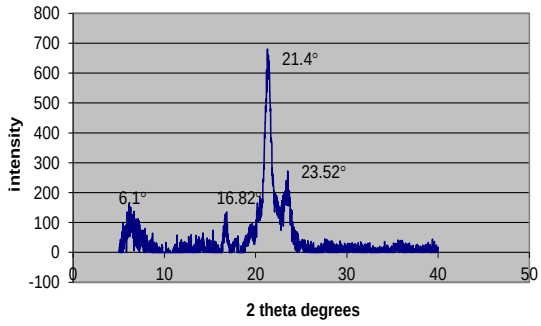


(A)

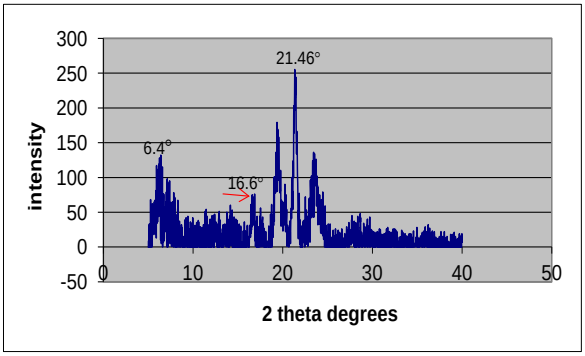


(B)

Fig. 6: XRD for Compritol (A) and Labrafil (B).



(A)

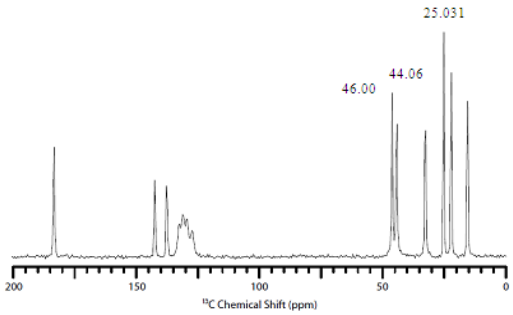


(B)

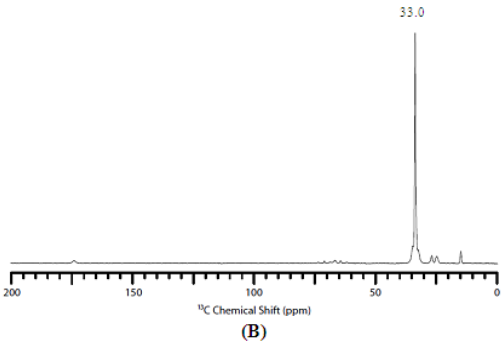
Fig. 7: XRD of SLN, 15^ lipid-1.35% surfactant (A) a NLC, 15^ lipid-1.35% surfactant (B).

Nuclear magnetic resonance

The same approach applied for XRD was utilized for the NMR analysis. The same two formulations were selected and all components were analyzed individually. (Figures 8 to 9) The spectra for ibuprofen, Compritol, Labrafil and Pluronic are shown in Fig. 16, Figures 8 to 9. Some of the strongest signals for pure ibuprofen were detected at 46.00ppm, 44.06ppm and 25.03 ppm. Compritol showed a strong peak at 33.0ppm and two less intense peaks between 27.0ppm and 25.0 ppm. In the SLN formulation (Fig. 10) the signals of Compritol are well visible (33.50 ppm).



(A)



(B)

Fig. 8: NMR of ibuprofen (A) and Compritol (B)

Also, two less intense ibuprofen peaks were detected. The peaks noted in the region between 26 and 14 ppm are less smooth than the ones observed in the pure lipid spectra, which suggests that the drug is well embedded in the lipid matrix and the resulting peaks are a combination of both lipid and drug signals. The NLC formulation (Fig. 10) showed the Compritol peak at 33.09 ppm and a peak at 30.37 ppm which is characteristic of the liquid lipid. Also, the same ibuprofen signals are detectable. The NMR studies confirmed close interaction of the drug with the lipid matrix.

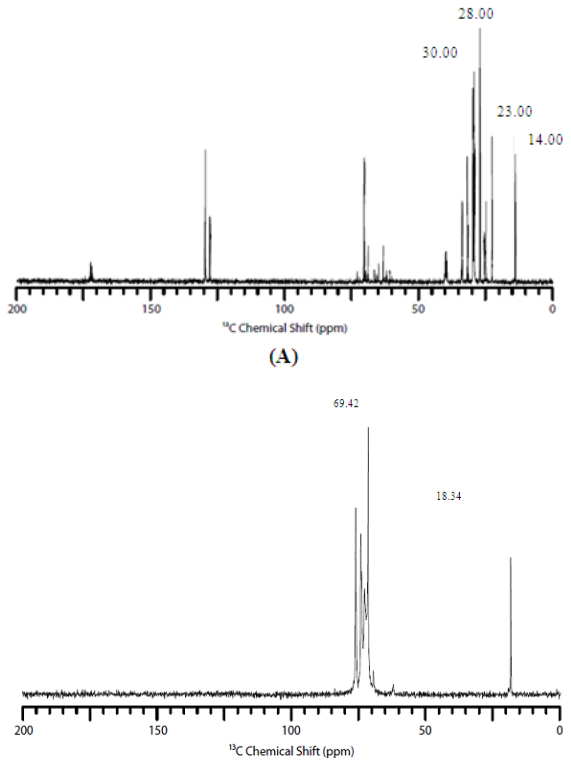


Fig. 9: BNR For Labrafil (A) and Pluoronic (B).

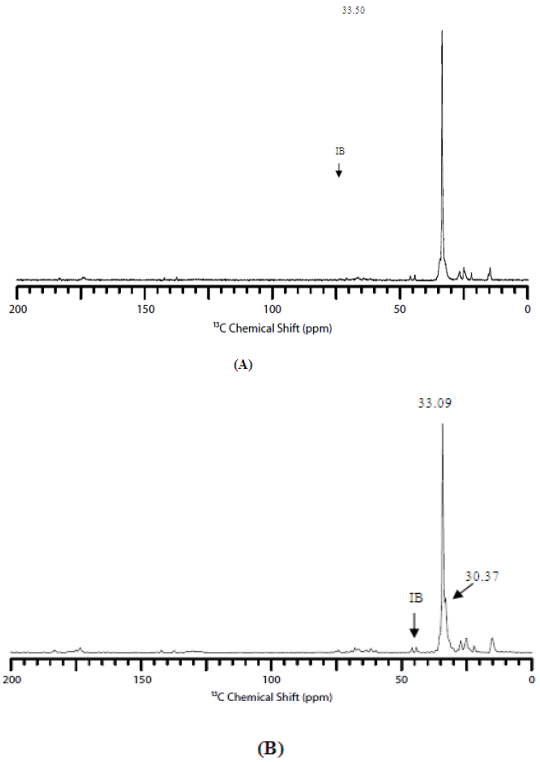


Fig. 10: NMR of SLN containing 15%lipid -1.35%surfactant (A) and NLC containing 15% lipid-1.35% surfactant (B).

CONCLUSIONS

In this research, ibuprofen-loaded lipid particles of very small size (mean < 5µm) were prepared by a simple hot homogenization method. Solid and liquid lipids of biodegradable and biocompatible nature were used as matrices. The effect of lipid phase composition (only solid lipid-SLN versus solid: liquid lipid blend-NLC), lipid phase concentration and surfactant concentration on the entrapment, morphology, particle size and drug release profiles of these particles was evaluated.

The preparation method was effective in obtaining lipid particles of satisfactory drug entrapment efficiency and release controlling properties. Controlled release of the drug was possible with both types of lipid matrices. The particles were spherical in shape.

The addition of the oil Labrafil increased the entrapment capacity of the formulations containing 5% lipid, however, as the lipid concentration increased no significant difference was apparent between SLN and NLC formulations. Higher lipid concentrations (10% and 15%) in both SLN and NLS systems are considered optimal due to their high entrapment capacity and overall low initial burst.

Higher surfactant concentration seemed to reduce the burst effect (a desirable attribute in controlled release delivery) and stabilize the system more than a low surfactant concentration. The addition of Labrafil to the lipid matrix did not significantly modify the drug release rate when compared to the similar solid lipid formulation. No correlation was identified between mean particles size and dissolution results, indicating that formulation composition was the determinant factor on drug release profile. Ibuprofen release from the lipid matrix follows the Higuchi model of diffusion.

X-Ray diffraction studies demonstrated the coexistence of amorphous and crystalline drug within the lipid matrix. The addition of Labrafil modified the crystallinity of both ibuprofen and the solid lipid leading to a more amorphous structure. NMR studies confirmed close interaction of the drug with the lipids. These studies suggest that the drug is well embedded within the lipid matrix

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENTS

The authors wish to thank RCMI, Mefical Sciences Campus at University of Puerto Rico for supporting the travel cost and yje training on the SSNMR. Also, the authors wish to to thank Dr. Eric Munson at the University of Kentucky for giving the training on SSNMR.

REFERENCES

1. Casadei M, Cerreto F, Cesa S, Grianmuzo M, Feeney M, Marianecci C, Paolicelli P. Solid lipid nanoparticles incorporated in dextran hydrogels. Int J Pharm 2006;140-6.
2. Barthelemy P, Laforet J, Joachim F. Compritol 888 ATO:an innovative hot melt agent for prolonged release drug formulations. Eur J Pharm Biopharm 1999;87-90.
3. Umer M, Gornullo U, Yaner G, Altmkurt T. A new approach for preparing controlled release Ketoprofen tablets using beeswax. J Farmaco 2005;27-31.
4. Das S, Kong W, Tan R. Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles ((SLNs):development, characterization and comparative evaluations of clotramazole loaded SLNs and NLCs. Eur J Pharm Sci 2013;139-51.
5. Garcia-Fuentas M, Alonzo M, Torres D. Design and characterization of a new drug nanocarrier made of solid-liquid lipid mixtures. J Colloid Ibterface Sci 2005;590-6.
6. Pardelike J, Weber S, Matsko N, Zimmer A. Formation of a physical stable delivery by simply autoclaving nanostructured lipid carriers (NLC). Int J Pharm 2012;22-7.
7. Cimi M, Bragagni N, Mura P. Development of new delivery system consisting of drug in cyclodextrin nanostructured lipid carriers for Ketoprofen topical delivery. Eur J Pharm Biopharm 2012:46-53.
8. KheradmandiniaS, Vasheg-Farahani E, Nostrati, MAtyabi F. Characterization of Ketoprofen loaded solid lipid nanoparticles made of beeswax and carnuba wax. J Nanomedicine 2010;753-9.
9. Martins S, Tho I, Ferreira D, Souto E, Brandl M. Physicochemical properties of lipid nanoparticles. Effect of lipid and surfactant composition. J Drug Dev Ind Pharm 2011;815-24.
10. Portta S, Minemi S, Nukala R, Peinado C, Lamprou D, Uequhart A, Douroumis D. Preparation and characterization of Ibuprofen

solid lipid nanoparticles with enhanced solubility. J Microcapsulation 2011;74-81.

11. Puglia C, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, Rocci M. Lipid nanoparticles for prolonged topical delivery:An *in vitro in vivo* investigation. Int J Pharm 2008;295-304.
12. Martins S, Tho I, Ferreira D, Souto E, Brandl M. Multivariance design for the evaluation of lipid and surfactant composition effect for optimization of lipid nanoparticles. Eur J Pharm Sc 20i2;613-23.
13. Zhang Q, Jiang X, Jiang W, Su L, Shi Z. Preparation of nimodipine loaded microemulsion for intestinal delivery and evaluation on the targeting efficiency to braim. Int J Pharm 2004;85-96.
14. Basak S, Kumar K, Ramalingram M. Design and release characteristics of Jsustained release tablets containing Metformin hydrochloride. J Brazilian Pharm Sci 2008;477-83.
15. Jana U, Mohanty AK, PAL SL, Manna PK, Mohanta GP. Preparation and *In vitro* characterization of felodipine loaded Eudragit RS 100 nanoparticles. Int J Pharm Pharm Sci 2014;564-7.