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Effect of squalane on mebendazole loaded Compritol® nanoparticles

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Abstract

The objective of this study is to develop nanostructured lipid formulations (NLF) of Compritol for the delivery of mebendazole. The formulations were prepared with Compritol 888 ATO, squalane, and Pluronic F68. Nine batches with different amounts of modifier, squalane, and drug were prepared. The formulations were characterized by evaluating particle size, morphology, and zeta potential. The thermal properties of the formulations were analyzed by differential scanning calorimetry (DSC). The encapsulation efficiency of each formulation and the drug release rates from each formulation were quantified by UPLC. The particles were spherical and had median particle sizes between 300 and 600 nm (50th percentile). A linear relationship was observed between Compritol/squalane composition and the melting point of the mixture. The DSC scans of the formulations revealed some recrystallization of the drug from the formulations, and the amount of recrystallization correlated with the amount of squalane in the formulation. Approximately 70% efficiency of encapsulation was observed in the formulations with 30% (w/w) squalane, and these formulations also had faster dissolution rates compared to the other formulations. Overall, the formulations with 30% squalane are the preferred formulation for future testing.

Keywords

mebendazole; Compritol; squalane; nanostructured lipid; NLF; NLC

1. Introduction

Mebendazole is a broad-spectrum anthelmintic, commonly used orally for the treatment of gastrointestinal parasitic infections, such as roundworms, hookworms, or tapeworms. It has also shown promise for the treatment of various cancers. Mebendazole has been shown to induce apoptosis in non-small cell lung cancer cells both *in vitro* (0.5 μ M mebendazole in both A549 and H460 cells) and *in vivo* (1 mg/mouse/48h for 3 weeks).[1] Similar observations were also reported with chemo-resistant melanoma cells [2] and adrenocortical carcinoma cells [3]. Bai *et al.* [4] showed that mebendazole improves survival rates in mice

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with glioblastoma multiforme, proving to be more effective than other benzimidazole drugs. Although mebendazole has been used via the oral route as an anthelmintic, the poor aqueous solubility and low bioavailability limit its use as an oral anticancer drug.[5] Mebendazole has three different crystalline forms, polymorphs A, B, and C.[6, 7] Polymorph C is the preferred form for pharmaceutical anthelmintic applications, whereas polymorph A is the least soluble, but most stable, form. While all forms are poorly-water soluble, the solubility of the three different polymorphs differs in physiological media as follows $B > C > A$.[8, 9]

In an attempt to improve the oral bioavailability of poorly soluble drugs, many investigators have utilized nanoparticle formulations. The use of polymeric carrier particles to improve drug passage across the intestinal mucosa is well-documented.[10, 11] Jani *et al.* [12] reported at least 30% absorption of 50 nm polystyrene particles following oral administration to male rats. Histological studies showed that 6–7% of these particles accumulated in the liver, spleen, blood, bone marrow, and kidneys. Particles of size 100–1000 nm were found in the serosal layer of the Peyer's patches. Jenkins *et al.* [13] have reported the size-dependency of uptake over the range of 150 nm to 1000 nm. Besides the size, nanoparticle absorption through the gastrointestinal tract also depends on the composition of the particles. Varshosaz *et al.* [14] have reported enhanced oral bioavailability of buspirone using nanoparticles composed of solid lipids.

Besides enhanced bioavailability, solid lipid nanoparticles (SLN) can also provide a mechanism for the controlled delivery of the substance; however the drug loading capacity of the matrix is limited for many pharmaceutical preparations.[15] This limitation results from the high crystallinity of the lipid material. A method to reduce this crystallinity, and thereby increase the drug loading capacity of the lipid, is to blend these lipids with compatible oils or other modifiers, creating nanostructured lipid carriers (NLC).[16] The resulting composite system has a much higher drug loading capacity than the pure lipid. [15,17] Such a system when mixed with poorly-water soluble drugs may be useful to increase the dissolution rate of these drugs.[18] Additionally, NLC, like SLN, are versatile, stable, and can be manufactured on an industrial scale.[19] In recent years, there has been a significant increase in the use of high melting point lipids, such as glyceryl behenate, to formulate such pharmaceutical preparations.[20] They are desirable because of their biocompatibility, low toxicity, and versatility.[21–23] Compritol 888 ATO is a glycerol behenate and is a high melting point lipid commonly utilized in pharmaceutical applications as a capsule lubricant, an oral excipient, or a sustained release matrix. Because of its relatively high melting point for a lipid product (70°C), Compritol 888 ATO can be formulated into a powder nanostructured lipid formulation (NLF). Other excipients such as lactose or trehalose are included in the formulation as a cryoprotectant.[24] These formulations (NLF) are commonly utilized to enhance oral bioavailability of poorly absorbed drugs.[14, 25–29] Processing of these formulations may be accomplished utilizing several methodologies, including hot emulsification, extrusion, or spray chilling with the final product being in dry powder form.[30,31] Some solid and liquid formulations of mebendazole have already been studied, such as a solid dispersions with low-substituted hydroxypropylcellulose (L-HPC),[32] a tablet formulated with guar gum,[33] complexation with polyethylene glycol (PEG-6000),[34] complexation with Povidone K12 PF,[35] and

various oil preparations[36]. However, to the best of our knowledge, this is the first study involving the incorporation of mebendazole in a nanostructured lipid formulation (NLF).

The long-term goal of this study is to develop an NLF as a potential dosage form for the delivery of mebendazole in the treatment of cancer. The present study includes the development of different mebendazole formulations and the subsequent analysis of these formulations for physical characteristics, drug loading, and drug release profiles from the formulations. This study has broad reaching applications for the improvement of the bioavailability of mebendazole.

2. Materials and Methods

2.1 Materials

Compritol 888 ATO, obtained as a gift from Gattefosse SAS (Lyon, France), was utilized as the nanoparticle matrix. Squalane, mebendazole, formic acid, lactose, potassium chloride, sodium phosphate monobasic monohydrate, sodium hydroxide, and acetonitrile were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Pluronic F68 was obtained as a gift from BASF (Ludwigshafen, Germany), and hydrogenated soybean phosphatidylcholine (HSPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA).

2.2 Evaluation of thermal properties of modified Compritol

To determine the compatibility and melting point of the lipid matrix Compritol and the matrix modifier squalane, a series of melt mixtures of the two components was generated by blending Compritol and squalane in 500 mg batches containing 0 to 40% w/w squalane, heating until the Compritol melted, stirring for 5 minutes, and cooling to room temperature. After cooling to room temperature, the composites were evaluated visually for uniformity and physical separation of the squalane oil. To further evaluate the compatibility of the two components, thermal analysis of the samples was performed by differential scanning calorimetry (DSC) using a Q2000 DSC (TA Instruments, New Castle, DE, USA). Samples were placed in sealed aluminum pans. The samples were equilibrated at -50°C and heated at a rate of $10^{\circ}\text{C}/\text{min}$ to 350°C .

2.3 Preparation of mebendazole nanostructured lipid formulations (NLF)

A series of nine formulations (NLF) were prepared using Compritol as the matrix lipid, squalane as the modifier, and the active ingredient mebendazole. The NLF were prepared by a hot melt emulsification process.[37,38] This process involved melting of the lipid, followed by emulsification of the lipid in a heated surfactant solution. Solid particles were obtained upon cooling of the material after emulsification. Prior to the preparation of the drug-loaded nanoparticles by this method, a blank batch was prepared using both room temperature cooling of the emulsion and quench cooling using an ice bath. A slightly smaller particle size was observed with the quench cooling process; therefore, this method was adopted for the drug formulations (Figure 1).

Due to the low solubility of mebendazole in lipid, oil, and water, it was first dissolved in formic acid followed by addition of hydrogenated soybean phosphatidylcholine (HSPC) as a

co-solvent, prior to its incorporation into the lipid/oil melt. This was achieved by dissolving 100, 200, or 300 mg mebendazole in a minimum volume of formic acid. After the drug had dissolved, 50 mg of HSPC was added, and the mixture was heated to 85°C to melt the HSPC and evaporate the formic acid. Once the formic acid had evaporated, the HSPC/drug melt was added to the lipid melt which was formulated by mixing a known amount of Compritol with a specified amount of squalane (Table 1). After heating to 85°C, 5 ml of a 1% Pluronic F68 solution was added slowly to the lipid melt, and the mixture was sonicated for 3 minutes using an ultrasonic generator with a 5 mm probe (Vibra Cell VC 100, Sonics and Materials, Inc., Newtown, CT, USA). The output intensity was 40 W. Following this initial emulsification, an additional 15 ml of heated Pluronic solution was added, and the mixture was further sonicated for 2 minutes. Next, the emulsion was removed from the 85°C bath and chilled in an ice bath to harden the particles. A powdered product was obtained by subjecting the particles to four centrifugation wash cycles at 30,000 RPM and by freeze-drying following the addition of 2 ml of a 5% lactose solution as a cryoprotectant. After 48 hours of freeze-drying at -20°C, a powder product (NLF) was obtained.

2.4 Physical characterization

The resulting NLF were analyzed for surface morphology using a scanning electron microscope (S4800, Hitachi High-Technologies Corporation, Tokyo, Japan). Samples were dispersed in deionized water prior to placement on carbon tape-coated stubs. The samples were gold-coated at 20 mA for 30 seconds. The samples were analyzed at 1 kV, an emission current of 9800 nA, and a working distance of 6.4 mm. The zeta potential of the nanoparticles was measured using a BIC ZetaPlus zeta potential analyzer (Brookhaven Instruments Inc., Holtsville, NY, USA). The analysis was performed by dispersing a small amount of the lipid powder in a 1 mM KCl solution. Particle size measurements were performed using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK).

2.5 Drug content and UPLC analysis

The drug utilized in this study was as obtained from Sigma-Aldrich (Milwaukee, WI, USA). The drug content was determined by first accurately weighing out approximately 10 mg of each sample. After heating the samples to melt the matrix, 0.5 ml of formic acid was added to the sample. The sample was mixed and then diluted with 9.5 ml of deionized water. The samples were analyzed by ultra-high pressure liquid chromatography (UPLC) with UV detection using an Acquity UPLC system (Waters Corporation, Milford MA, USA). The chromatographic analysis was performed using a BEH300 C18 1.7µm 2.1 × 100mm column. The mobile phase was an isocratic system containing 70% of a 50 mM sodium phosphate buffer (pH 6.4) and 30% acetonitrile. The flow rate was 0.1 ml/min, the injection volume was 10 µl, and the run time was 5 minutes. Samples were quantified by UV detection using the peak area at 294 nm.

2.6 In vitro release rate

Evaluation of the rate of release of the formulated mebendazole in phosphate buffer (pH 7.4) was determined for each formulation. The dissolution technique relies on the use of a dialysis membrane to separate the test sample from the bulk test medium. This allows the analysis of drug release independent of the release or deaggregation of the nanoparticles

themselves. Three accurately weighed aliquots, approximately 40 mg for each of the nine formulations, were placed in 10 mm diameter Spectra/Por dialysis bags (40,000 MWCO), and each bag was clamped at one end with a dialysis clamp. These bags were then filled with a 50mM phosphate buffer (pH 7.4) and clamped at the other end. The bags were immediately placed in 50 ml polyethylene centrifuge tubes, and the tubes were filled with 40 ml of the phosphate buffer. The tubes were then placed in a preheated shaker bath at 37°C and 20 RPM. At various sampling times (0.5, 1.5, 3, 7, 24, 48, 120, and 168 h) the 40 ml of the external buffer was removed, the tubes were filled with fresh buffer, and the tubes were returned to the shaker bath. The samples were analyzed by UPLC-UV for mebendazole content.

2.7 Statistical analysis

Statistical comparisons were conducted between samples using ANOVA and the Holm-Sidak method for multiple pair-wise comparisons. Differences between two related parameters were considered statistically significant at $p < 0.05$. SigmaPlot (Systat Software, Inc., San Jose, CA, USA) software was used for all statistical analysis.

3. Results and Discussion

The resulting NLF particles were spheroidal in shape and had fairly uniform surface morphology (Figure 2). Imaging with a Hitachi S4800 scanning electron microscope was moderately difficult due to the delicate nature of the particles and charging of the sample. Gold coating was essential to achieve clear, clean images of the formulations. The batches with 30% drug (A3, B3, and C3) did have a relatively larger median particle size (ranged from 500–600 nm) when compared to the corresponding batches within the same concentration of squalane (Table 2). Analysis of the particle size distribution data further showed that as the amount of drug increased, the particle size distribution shifts towards larger particles, which is especially pronounced at 20% squalane concentration. However, at 30% squalane, the effect of drug concentrations on the particle size was not statistically significant ($p > 0.05$). The diameter of these particles (50th percentile) ranged from 323 to 363 nm.

A comparison of the surface charge on the particles revealed no correlation between formulation composition and zeta potential measurements. The average zeta potential measurements were –23.4, –18.8, and –17.7 mV for 10%, 20%, and 30% squalane, respectively. Generally, a zeta potential measurement more negative than ± 30 mV is regarded as a stable dispersal of particles. Those formulations with zeta potential measurements approaching zero have a higher likelihood of particle aggregation. Only formulation A1 achieved the ± 30 mV stability threshold, which corresponds to the formulation with the highest Compritol content. Therefore, squalane and drug content may negatively affect zeta potential and consequently particle stability and/or aggregation.

A comparison between the squalane content and the melting point of the mixture showed a linear relationship (correlation coefficient = 0.9887) between the percentage of squalane in the Compritol/squalane mixture and the melting point of the mixture. This observation indicates good compatibility between the two excipients and good predictability of the

melting point of the solution based on oil content (Figure 3). While thermal analysis alone is not a definitive predictor of compatibility between the two constituents, the miscibility of the components is consistent over the concentration range evaluated, and the presence of two individual melting peaks was never detected over the concentration range. Such inhomogeneity in the solution, such as the separation of the two components, would result in a plateau in the melting point of the solution, which is not present over the concentration range evaluated.

Figure 4a shows the DSC scans for the pure drug, HSPC, and mebendazole in HSPC. Mebendazole appears to be nearly completely dissolved in the HSPC with only slight evidence of the endothermic peak of mebendazole in the MEB+HSPC scan. The scan of pure mebendazole shows an endothermic peak at 260°C and melting endotherm at 325°C. These scans are comparable to those in the literature; Kumar *et al.* [39] report peaks at 250°C and 320°C, and Bustamante *et al.* [40] report peaks at 260.75°C and 315.52°C. These results generally indicate that polymorph A is present.

When formulated with Compritol and squalane, some of the drug is recrystallizing out of the formulation (Figure 4) with an endothermic peaks present at 200–220°C. Also, this is related to the amount of squalane that is present in the formulation; higher the squalane content in the formulation, more is the recrystallized drug present in the sample (Figure 4d). This may indicate that squalane is not the optimal choice at high concentrations, because it may be contributing to the recrystallizing out of the drug from the formulation. A balance must be established to optimize the amount of squalane present to enhance drug loading, while minimizing the amount of free, recrystallized drug present in the formulation. Interestingly, the drug peaks observed in the DSC scans of the formulations appear to indicate that the drug may be polymorph B or C, which characteristically has two endothermic peaks between 200°C and 260°C, as opposed to the one peak observed for polymorph A. The additional endotherm detected at 60–70°C is attributed to enthalpic relaxation of the formulations.

Figure 5 shows the effect of squalane on drug loading. As the percentage of squalane in the formulation increases, the drug loading also increases, even after allowing for the effects of differences in mebendazole content ($p = 0.014$). The percentage of drug in the formulation does not affect encapsulation efficiency ($p > 0.05$). This seems to imply that increasing the amount of drug in the formulation does not impact the overall efficiency of the process.

Overall the *in vitro* drug release studies exhibited good reproducibility amongst the triplicate runs. Analyzing the dissolution profiles in Figure 6 shows that the formulations with 30% squalane had relatively higher drug release compared to the other formulations. In fact, the formulations with the highest squalane content (30%) had a significantly different dissolution ($p < 0.001$) profile compared to the formulations with less squalane, regardless of the content of drug in the formulation. This may be due to the higher drug loading in these formulations. Within the batches containing 30% squalane, the formulations with higher drug content showed increased drug release over time. All the formulations exhibited slow release as a percentage of total drug in the formulations. The drug release may be controlled by drug/polymer compatibility, and drug release may be improved by a

significantly higher drug loading or design of a faster disintegrating polymer formulation. The alternative is delivery of the drug as an intact lipid nanoparticle. However, mebendazole cytotoxicity in this form has not been studied.

The amount of modifier in the formulation had the biggest impact on drug encapsulation and drug dissolution rate in this study. Overall, those formulations with 30% squalane are the preferred formulation for future testing, because formulations C1–C3 demonstrated the highest drug loading. This result is expected because the formulations with higher modifier percentages should, in turn, have a lower lipid crystallinity and hence a greater loading capacity. Although squalane appears compatible with Compritol, its use results in recrystallization of the drug. A possible approach to decrease this recrystallization would be to increase the percentage of HSPC in the formulation, given the observation that HSPC mixed well with the Compritol in our formulations. The HSPC could lower the melting point of the formulation, and/or reduce its crystallinity. This reduced crystallinity may translate into an increased drug loading capacity.

4. Conclusion

We have presented a method for the incorporation of mebendazole, a broad spectrum anthelmintic which may also be used as a possible anticancer agent, in nanostructured lipid formulations (NLF). The particles were small, uniform, and loaded with an appreciable amount of drug. This formulation of mebendazole lends flexibility to the dosing of the drug by allowing the selection of the preferred dissolution rate of the drug (*e.g.* by adjusting the Compritol and squalane content to get the desired rate and release profile) and potentially stabilizes the drug as the more active polymorph C. It is possible that further increasing the HSPC content may lead to higher drug loading. The particle size, drug loading, and biocompatibility of these formulations could increase the anticancer efficacy of mebendazole following oral administration.

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Figure 1.
Particle size distribution profiles of two blank Compritol formulations using (A) room temperature cooling and (B) ice bath cooling.

Figure 2. SEM micrograph of representative Compritol/squalane NLF with mebendazole. The samples were gold-coated at 20 mA for 30 seconds. The samples were analyzed at 1 kV with an emission current of 9800 nA with a working distance of 6.4 mm.

Figure 3.

Effect of squalane on the melting point of Compritol/squalane binary mixture. The mixture was placed in sealed aluminum pans, equilibrated at -50°C and heated at a rate of $10^{\circ}\text{C}/\text{min}$ to 350°C .

Figure 4.

DSC thermograms of (a) mebendazole, HSPC, and a mixture of 200mg mebendazole and 50 mg HSPC; (b) mebendazole with HSPC (A), Compritol with 10% squalane (B), and formulations A1, A2, and A3; (c) mebendazole with HSPC (A), Compritol with 20% squalane (B), and formulations B1, B2, and B3; (d) mebendazole with HSPC (A), Compritol with 30% squalane (B), and formulations C1, C2, and C3.

Figure 5.

Effect of the total amount of mebendazole and squalane in a given formulation on the efficiency of encapsulation. Efficiency of encapsulation was calculated based on the ratio of the amount of mebendazole measured and the theoretical amount in a 10 mg sample.

Figure 6. Dissolution profiles of mebendazole from the nine formulations with (a) 10% drug (formulations A1, B1, and C1), (b) 20% drug (formulations A2, B2, and C2), and (c) 30% drug (formulations A3, B3, and C3).

Table 1

Formulations for the nine batches of mebendazole-loaded NLC

Formulation	Compritol (mg)	Modifier % ^a	Squalane (mg)	Drug % ^b	Drug (mg)	HSPC (mg)	Lactose (mg)	Actual Drug Content % (%RSD) ^c
A1	765	10	85	10	100	50	100	1.70 (20.9)
A2	675	10	75	20	200	50	100	6.08 (9.4)
A3	585	10	65	30	300	50	100	5.86 (15.3)
B1	680	20	170	10	100	50	100	3.70 (0.7)
B2	600	20	150	20	200	50	100	12.99 (4.5)
B3	520	20	130	30	300	50	100	18.86 (2.4)
C1	595	30	255	10	100	50	100	7.18 (4.9)
C2	525	30	225	20	200	50	100	12.45 (7.5)
C3	455	30	195	30	300	50	100	21.28 (7.9)

^a Percentage (w/w) of squalane relative to total content of blank formulation (*i.e.* Compritol and squalane)

^b Percentage (w/w) of mebendazole relative to total content of drug-loaded, dispersed NLF (*i.e.* Compritol, squalane, mebendazole, and HSPC)

^c Relative standard deviation of encapsulation efficiency measurements (n = 3)

Table 2

Particle size and zeta potential of mebendazole formulations

Formulation	Zeta Potential (mV)	Median Particle Diameter (nm)
A1	-31.3 ± 1.3	482
A2	-20.1 ± 1.0	391
A3	-18.64 ± 2.5	503
B1	-8.82 ± 0.6	229
B2	-22.64 ± 1.5	371
B3	-24.82 ± 0.5	601
C1	-14.12 ± 1.5	326
C2	-20.91 ± 1.6	331
C3	-18.02 ± 2.6	363