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Formulation and *in vitro* evaluation of niacin-loaded nanoparticles to reduce prostaglandin mediated vasodilatory flushing

D.L. COOPER, J.A. CARMICAL, P.C. PANUS, S. HARIRFOROOSH

Department of Pharmaceutical Sciences, Gatton College of Pharmacy, East Tennessee State University, Johnson City, TN, USA

Abstract. – OBJECTIVE: Niacin, activating G-protein coupled receptor (GPR) 109A, stimulates release of vasodilatory prostaglandins (PGs) such as PGE_2 which can elicit niacin-associated flushing side effects. Poly-lactic-co-glycolic acid (PLGA) and poly-lactic acid (PLA) are used in nanoparticle (NP) drug delivery to reduce adverse effects and modulate drug release. Our study evaluated the *in vitro* effects of niacin-loaded PLGA or PLA-NPs on PGE_2 expression in whole human blood as a model for niacin-induced flushing.

MATERIALS AND METHODS: NPs were formulated using a solvent evaporation process and characterized by size, polydispersity, zeta potential, drug entrapment, morphology, and drug release. NP *in vitro* effects on PGE_2 release were measured via ELISA analysis.

RESULTS: PLGA-NPs demonstrated the lowest NP size (66.7 ± 0.21 nm) with the highest zeta potential and percent drug entrapment (42.00 ± 1.62 mV and 69.09 ± 0.29%, respectively) when compared to PLA-NPs (130.4 ± 0.66 nm, 27.96 ± 0.18 mV, 69.63 ± 0.03 %, respectively). *In vitro* release studies showed that PLGA-NPs underwent significant reductions in cumulative drug release when compared to PLA-NPs ($p < 0.05$). Furthermore, when compared to plain niacin, PLGA-NPs significantly reduced *in vitro* PGE_2 release ($p < 0.05$).

CONCLUSIONS: These results support the use of PLGA-NPs as a novel method of delivery for reducing niacin-associated flushing.

Key Words:
Niacin, Nanoparticles, Prostaglandins, PLGA, PLA, Formulation, Flushing.

Introduction

Niacin, also known as nicotinic acid or vitamin B_3 (Figure 1), has been shown to positively regulate lipoprotein levels[1]. It is one of the few compounds shown to significantly raise HDL levels and regress atherosclerosis[2,3]. The effects of niacin are thought to occur primarily through interactions with the G-protein coupled receptor (GPR)109A[4-6]. Physiologically, niacin functions to alter lipoprotein levels through several mechanisms. In adipocytes, niacin can induce changes in hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) expression levels, in turn inhibiting lipolysis, reducing systemic free fatty acid (FFA) release and subsequent lipoprotein repackaging via hepatic triglyceride (TG) synthesis[7]. Niacin has also demonstrated an ability to inhibit the enzyme responsible for hepatic *de novo* lipogenesis, diacylglycerol acyltransferase 2 (DGAT2)[4]. Inhibition of DGAT2 reduces hepatic TG production, in turn altering production of TG dependent lipoproteins such as very low density lipoproteins (VLDL) and low density lipoproteins (LDL) making it a highly effective drug for treatment of dyslipidemia.

Despite niacin's beneficial effects on lipid regulation, certain adverse side effects are common. The most prevalent adverse effect of niacin use is cutaneous vasodilation, often presented as flushing of the face and extremities. Niacin interactions at the GPR109A receptor can trigger intercellular signaling pathways that increase the expression of vasodilatory prostaglandins (PGs) such as PGD_2 and PGE_2, resulting in what is commonly referred to as the "niacin flush" for patients adhering to niacin therapy, the niacin flush can be intolerable, leading to the discontinuation of treatment[8]. Alternate drug delivery methods, including extended release (ER) niacin, have been reported to reduce flushing effects[9]. Despite the improved effects of ER formulation on niacin-induced flushing, many patients still present with increased flushing symptoms. An 8 week, double-blind, placebo controlled study conducted by Paolini et al[10] showed that

Corresponding Author: Sam Harirforoosh, Pharm.D, Ph.D; e-mail: harirfor@etsu.edu

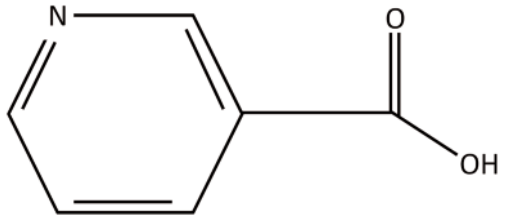

Figure 1. Chemical structure of niacin.

compared to control, patients undergoing ER-niacin treatment experienced greater flushing severity throughout the duration of the study with 40% of patients receiving ER-niacin presenting with moderate or greater flushing severity at least once a week. Furthermore, a retrospective, cohort study[11] analyzing patient compliance followed the prescription pattern of 14,386 patients receiving ER-niacin. The study showed that only 47% of the patient population reached recommended daily dosages and that after one year, only < 15% of patients were still receiving ER-niacin therapy. In another study[12] looking at niacin tolerability, it was shown that 50.8% of patients receiving ER-niacin therapy presented with severe or extreme flushing symptoms. These studies indicate the need for development of a more tolerable means of niacin administration.

Biodegradable polymers such as polylactide-co-glycolide (PLGA) and, polylactic acid (PLA) are among the more popular candidates for fabrication of novel drug delivery systems[13]. PLGA and PLA are commonly used in the development of nanoparticle (NP) delivery systems that present with altered biocompatibility and biodegradation characteristics[14]. Polymer based NPs have been shown to control and prolong the rate of drug release via modulation of NP characteristics such as hydrophilicity and biodegradation[14,15]. Alterations in drug kinetics and release profiles brought forth by polymer encapsulation have been positively correlated with reductions in a number of adverse drug side effects[16-18]. Despite the documented effectiveness of polymeric NPs and their known capabilities of altering systemic drug exposure, no formulation studies have been devised in regard to polymer encapsulated niacin-loaded NPs. The purpose of this study was to develop a novel, NP formulation of entrapped niacin, utilizing the biodegradable polymers PLGA and PLA with the stabilizer, didodecyldimethylammonium bromide (DMAB). In this study, NPs were created through a common emulsion-diffusion-evaporation process[19,20]. The resultant particles were characterized for size, zeta potential, and percentage of niacin entrapment. *In vitro* NP drug release and effects on PGE_2 levels following niacin exposure were also evaluated.

Materials and Methods

Materials

Nicotinic acid, DMAB, PLA (MW 85,000-160,000 Da), and PLGA (50:50 copolymer compositions; MW 30,000-60,000 Da), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl acetate, acetone, and high performance liquid chromatography (HPLC) grade water were purchased from Fischer Scientific Laboratory (Fair Lawn, NJ, USA). Human blood and PGE_2 ELISA kit were obtained from Innovative Research Inc. (Novi, MI, USA) and Thermo Scientific (Rockford, IL, USA), respectively.

Preparation of PLGA Formulations

Formulation of PLGA based NPs was carried out using an emulsion-diffusion-evaporation process[19]. Organic phase consisting of 50 mg PLGA and 45 mg niacin dissolved in 3 mL ethyl acetate was added to 6 mL HPLC grade water containing 0.1%, 0.25%, 0.5%, or 1% w/v DMAB stabilizer. Suspensions were probe sonicated for 5 minutes at 20 kHz to create the primary emulsion and then diluted

Table I. Method for PLGA niacin formulation.

	Ingredients	Comments	Amount
Organic Phase	PLGA	Polymer	50 mg
	Ethyl Acetate	Primary organic solvent	3 mL
	Niacin	Hydrophilic drug	45 mg
Aqueous Phase	DMAB	Stabilizer	Variable[1]
	HPLC grade water	Primary aqueous solvent	6 mL

[1]DMAB concentrations varied 0.1, 0.25, 0.5, and 1% w/v with respect to solvent

Table II. Method for PLA niacin formulation.

	Ingredients	Comments	Amount
Organic Phase	PLA	Polymer	50 mg
	Dichloromethane	Primary organic solvent	3 mL
	Niacin	Hydrophilic drug	45 mg
Aqueous Phase	DMAB	Stabilizer	Variable[1]
	HPLC grade water	Primary aqueous solvent	6 mL

[1]DMAB concentrations varied 0.1, 0.25, 0.5, and 1% w/v with respect to solvent

with 25 mL HPLC grade water (Table I). Following dilution, suspensions were stirred for 2 hours at 750 rpm to ensure organic phase evaporation, then centrifuged at 10,000 g for 5 minutes. After centrifugation, supernatant was collected and characterized. Formulation of PLA based NPs was carried out using a similar method with dichloromethane as organic solvent (Table II).

NP Characterization

Particle Size, Polydispersity, and Zeta Potential of Nanoparticles

Formula supernatant samples were used for characteristic measurements of NP size, polydispersity index (PDI), and zeta potential. All measurements were performed in triplicate. NP size and PDI were determined by dynamic light scattering using a NICOMP ZLS particle sizer (Particle Sizing Systems, Port Richey, FL, USA). PDI values range from 0 to 1 with higher values representing less homogeneous NP size distribution[21]. Zeta potential was determined using electrophoretic mobility.

Drug Entrapment

Percent drug entrapment was measured by ultraviolet-visible spectroscopy (Eppendorf Biophotometer, Hauppauge, NY, USA). Niacin-loaded NP suspensions (100 µL) were added to acetonitrile (300 µL) and vortex mixed for 15 seconds. After controlling for blank, suspensions were analyzed at 260 nm. Prior to NP analysis, niacin stock solution dissolved in HPLC grade water was used to construct a standard calibration curve (50,000-2,000,000 ng/mL). Percent drug entrapment was calculated using an equation presented in a previous publication[19].

Morphology

To analyze shape and surface morphology, formulations were vortex mixed and 2 µL of NP suspension was placed on a 200 mesh copper grid coated with Formvar (Electron Microscopy Sciences, Hatfield, Pennsylvania). Samples were allowed to dry for 1 hour then examined at 80 kV by transmission electron microscopy (TEM) (Tecnai Philips Transmission Electron Microscope; FEI, Hillsboro, OR, USA).

In vitro Release

Measurement of niacin *in vitro* release was carried out as previously described with slight modification[19,22-24]. Suspensions containing niacin formulated NPs (2 mL) were placed into 15 mL Corning centrifuge tubes containing 8 mL phosphate buffer (pH 7) then positioned on a rotating shaker set at 100 rpm. At varying time points, 2 mL of release medium was removed and replaced with equal volume of fresh medium. Sample aliquots were then centrifuged at 1,500 g for 5 minutes and filtered through a 0.2 micron syringe filter (EMD Millipore, Billerica, MA, USA). Empty nanoparticle suspensions were used to control for blank and analysis was carried out using a UV spectrophotometer set at 260 nm.

In vitro Examination of PGE_2 Expression

Vascularization effects of niacin are mitigated by vasodilatory PGs[4] such as PGE_2. In our study, *in vitro* evaluation of PGE_2 release following niacin exposure was carried out to serve as a model for niacin-induced flushing[25]. For evaluation of PGE_2 release, 1 mL human blood-citrate mixture was placed inside a clean glass tube. Plain niacin or niacin-loaded NP suspensions were added to human blood (5 mM niacin concentration) and incubated for 1 hour. One portion of fresh blood-citrate mixture was also incubated without niacin or NP containing niacin to serve as control. After 1 hour, samples were centrifuged at 3,000 g for 15 minutes. Supernatant was collected and PGE_2 concentrations were evaluated using a PGE_2 ELISA detection kit.

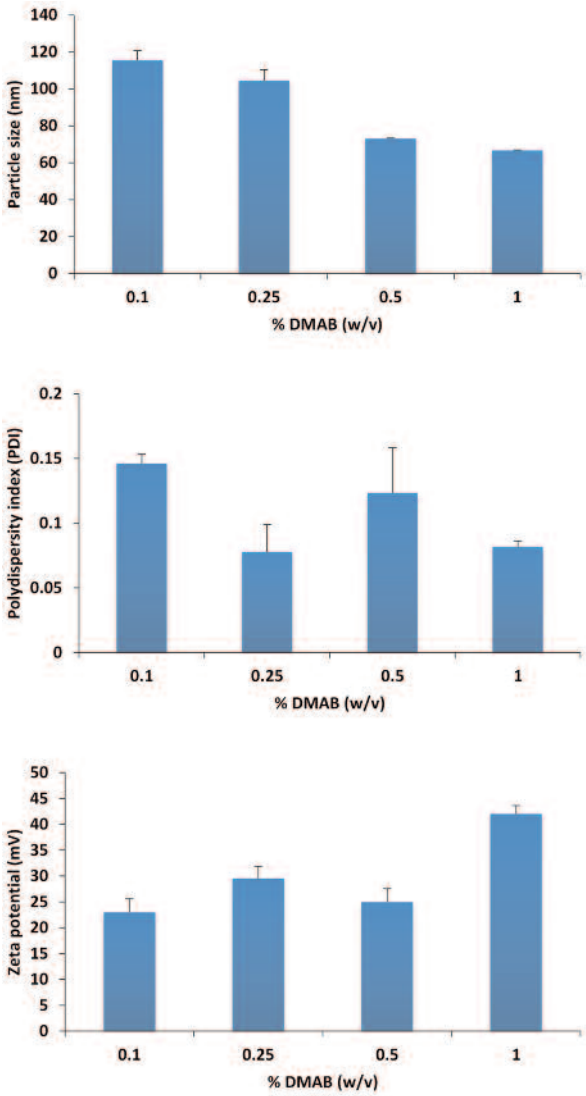

Figure 2. The effect of various DMAB concentrations on niacin-loaded PLGA-NP A) size, B) polydispersity, and C) zeta potential. Values are expressed as mean ± SD, n = 3.

Samples were analyzed per kit instructions and a cloud based data analysis software (MyAssays Ltd, Sussex, England) capable of performing a four parameter logistic fit was used to generate the standard curve.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). The unpaired Student's *t*-test was used to analyze cumulative release data for PLGA- and PLA-NPs using identical stabilizer concentrations. For NP effects on PGE_2 release, one-way ANOVA with post-hoc LSD was used to compare formulation effects. Statistical significance was set at $p < 0.05$.

Results

Effects of DMAB Stabilizer on Niacin-loaded PLGA-NP Size, Polydispersity, and Stability

Characterization studies showed a reduction in particle size with elevated zeta potential using high concentrations of DMAB stabilizer (Figure 2). Higher NP size was seen in PLGA formulations using 0.1% w/v DMAB (115.53 ± 5.24 nm) and 0.25% w/v DMAB (104.43 ± 6.04 nm) formulations. NP size was found to be lowest at 0.5% w/v and 1% w/v DMAB (73.03 ± 0.32 nm and 66.70 ± 0.21 nm, respectively) (Figure 2A). Polydispersity index (PDI) as a measure of size distribution of NP systems, showed narrow particle size distribution across all NP formulations. Formulations of 0.1% DMAB concentrations showed the lowest homogeneity for size distribution, displaying a PDI value of 0.15 ± 0.014 (Figure 2B). The highest level of size homogeneity was seen in formulations using 0.25% DMAB with a minimum PDI of 0.07 ± 0.02 (Figure 2B). Zeta potential increased above 20 mV for all formulations, with the highest measurements seen in formulations using 0.25% w/v DMAB (29.52 ± 2.36 mV) and 1% w/v DMAB (42.01 ± 1.62 mV) concentrations (Figure 2C).

Effects of DMAB Stabilizer on Niacin-loaded PLA-NP Size, Polydispersity, and Stability

The use of PLA polymer in formulations resulted in the formation of larger niacin-loaded NPs than PLGA formulations. The largest particle size was observed using 0.5% DMAB concentrations (212.21 ± 0.56 nm). The lowest average NP size of PLA formulations was achieved using 0.25% DMAB (130.41 ± 0.68 nm) (Figure 3A). Narrow size distribution was achieved across all PLA formulated NP systems. Formulations using either 0.5% or 1% DMAB concentrations demonstrated increased system homogeneity as evident by reduced PDI values (0.11 ± 0.01 and 0.11 ± 0.02, respectively). Formulations with 0.1% DMAB showed the lowest level of homogeneity, displaying a PDI value of 0.15 ± 0.01 (Figure 3B). Zeta potential of PLA based NPs followed a linear increase in relation to stabilizer concentrations, with a peak zeta potential achieved using 1% DMAB concentrations (27.96 ± 0.18 mV) (Figure 3C).

Effects of DMAB stabilizer on NP Entrapment

All PLGA formulations achieved greater than 60% drug entrapment (Table III). The lowest amount and percent of drug entrapped was seen in formulations using 0.5% DMAB (28.42 ± 0.01 mg and 63.15 ± 0.01%, respectively). Peak amount (31.09 ± 0.13 mg) and percent drug entrapment (69.09 ± 0.29%) was achieved using 0.25% w/v DMAB (Table III). No association between amount and percent of drug entrapment was determined in regards to increasing or decreasing stabilizer concentrations.

All PLA formulations achieved greater than 50% drug entrapment (Table IV). Peak NP drug amount and percent entrapment were achieved using 0.25% w/v DMAB concentrations (31.33 ± 0.02 mg and 69.63 ± 0.03%, respectively); while the lowest drug amount and percent entrapment were found in formulations using 0.1% DMAB concentrations (26.27 ± 0.07 mg and 58.38 ± 0.15%, respectively). Similar to PLGA-NPs, there was no noted relationship between stabilizer concentration and drug entrapment in PLA formulated NPs.

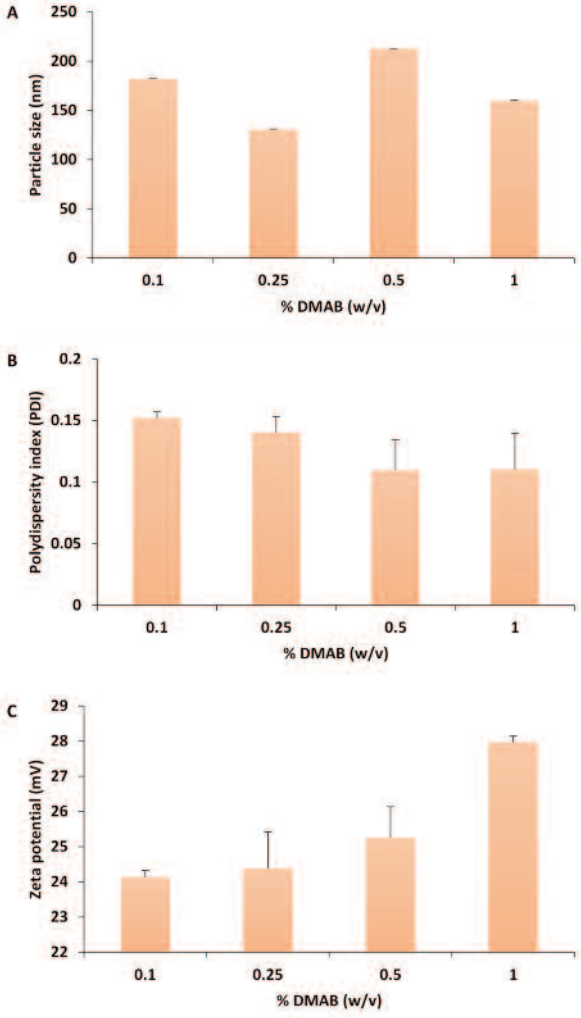

Figure 3. The effect of various DMAB concentrations on niacin-loaded PLA-NP A) size, B) polydispersity, and C) zeta potential. Values are expressed as mean ± SD, n = 3.

Table III. Effects of DMAB concentrations on PLGA-NP niacin entrapment.

DMAB (% w/v)	Amount entrapped (mg)	% EE
0.1	29.72 ± 0.35	66.03 ± 0.77
0.25	31.09 ± 0.13	69.09 ± 0.29
0.5	28.42 ± 0.01	63.15 ± 0.01
1	30.17 ± 0.13	67.05 ± 0.29

All values reported as mean ± SD (n = 3). Amount entrapped per 45 mg niacin. EE is the entrapment efficiency.

Shape and Surface Morphology of Niacin-loaded PLGA-NPs

Morphological features of PLGA-NPs showed well defined, round, separated, spherical particles with smooth surfaces (Figure 4). PLA formulated NPs also presented with well separated, smooth particles typified by a round spherical shape (Figure 5). For both PLGA and PLA formulations, TEM size confirmation was in agreement with similar size parameters obtain during characterization studies performed with the zeta sizer.

In vitro Niacin Release

In vitro release studies were performed on both PLGA and PLA niacin-loaded NPs. Only DMAB stabilizer concentrations of 0.25% and 1% w/v were chosen based on their degree of

Table IV. Effects of DMAB concentrations on PLA-NP niacin entrapment.

DMAB (% w/v)	Amount entrapped (mg)	% EE
0.1	26.27 ± 0.07	58.38 ± 0.15
0.25	31.33 ± 0.02	69.63 ± 0.03
0.5	28.85 ± 0.07	64.35 ± 0.16
1	26.83 ± 0.02	59.62 ± 0.03

All values reported as mean ± SD (n = 3). Amount entrapped per 45 mg niacin. EE is the entrapment efficiency.

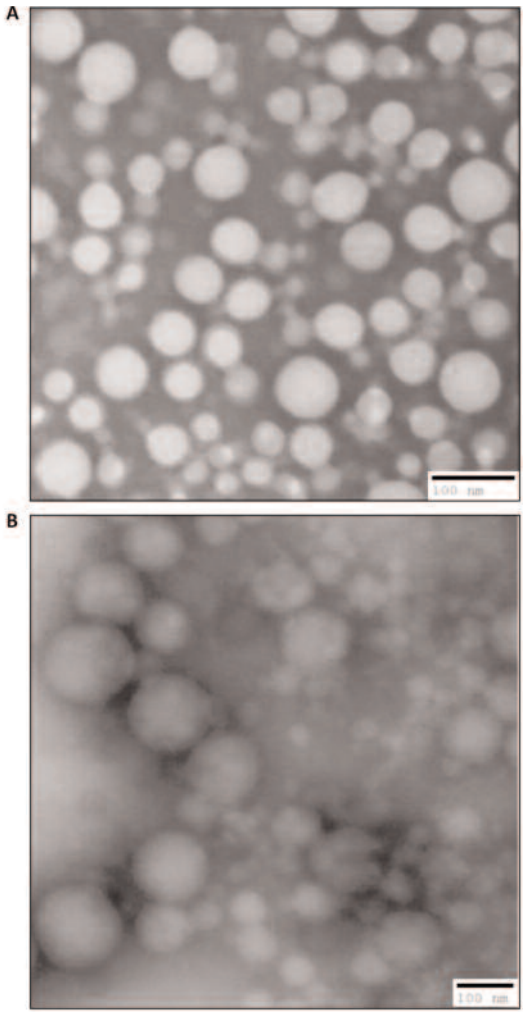

Figure 4. Morphological analysis of niacin-loaded PLGA-NPs. Transmission electron microscopy image of niacin-loaded PLGA-NPs formulated with 0.25% DMAB stabilizer ***(A)***. Transmission electron microscopy image of niacin-loaded PLGA-NPs formulated with 1% DMAB stabilizer ***(B)***.

stability, particle size, and percent of drug entrapped. Comparison of percent drug release showed statistical significance between both PLGA- and PLA based NPs using 0.25% w/v or 1% w/v DMAB. At each specific time point, PLGA based NPs formulated at both 0.25% and 1% DMAB demonstrated a reduced rate of release when compared to PLA based NPs (Figure 6 and Figure 7). The initial release (0.5 hours) for PLGA-NPs formulated with 0.25% w/v DMAB reached 17.81 ± 0.02%, while 0.25% w/v DMAB formulated PLA-NPs showed an initial release of 20.53 ± 0.04% ($p < 0.01$). After 48 hours, 0.25% w/v DMAB formulated PLGA- and PLA-NP reached a cumulative release of 67.84 ± 0.08 % and 73.54 ± 0.68%, respectively (Figure 6) ($p < 0.01$). Initial release of niacin was significantly lower in the 1% w/v DMAB PLGA-NP formulated group (18.88 ± 0.03%) in comparison to the 1% w/v DMAB PLA formulated group (21.11 ± 0.03 %) ($p < 0.01$). After 48 hours, 1% w/v DMAB formulated PLGA-NPs had a total cumulative release of 63.47 ± 0.14%, which was significantly lower than the total release of 81.11 ± 0.11% observed in the 1% w/v DMAB PLA formulated group (Figure 7) ($p < 0.01$).

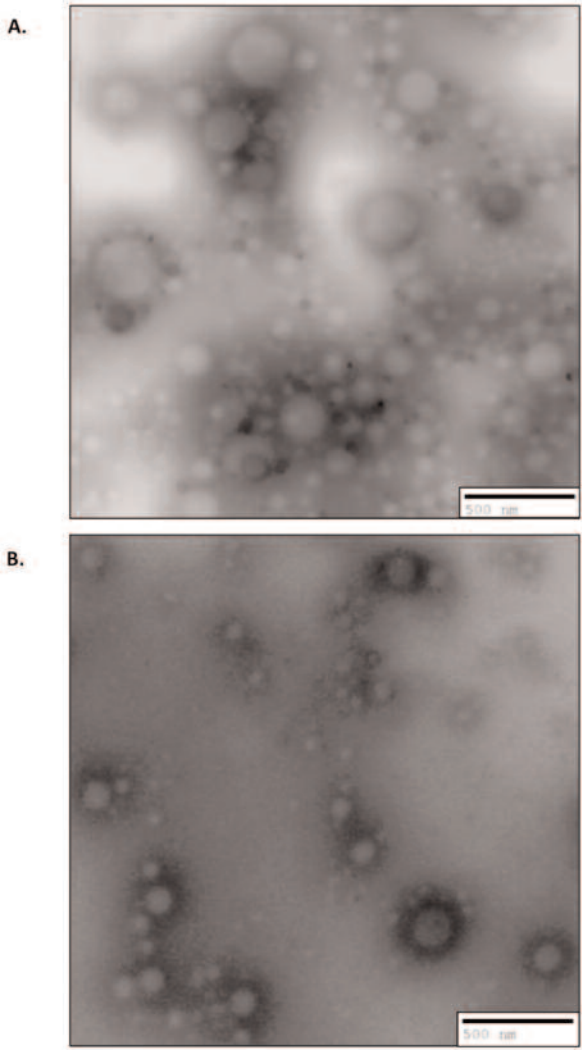

Figure 5. Morphological analysis of niacin-loaded PLA-NPs. Transmission electron microscopy image of niacin-loaded PLA-NPs formulated with 0.25% DMAB stabilizer ***(A)***. Transmission electron microscopy image of niacin-loaded PLA-NPs formulated with 1% DMAB stabilizer ***(B)***.

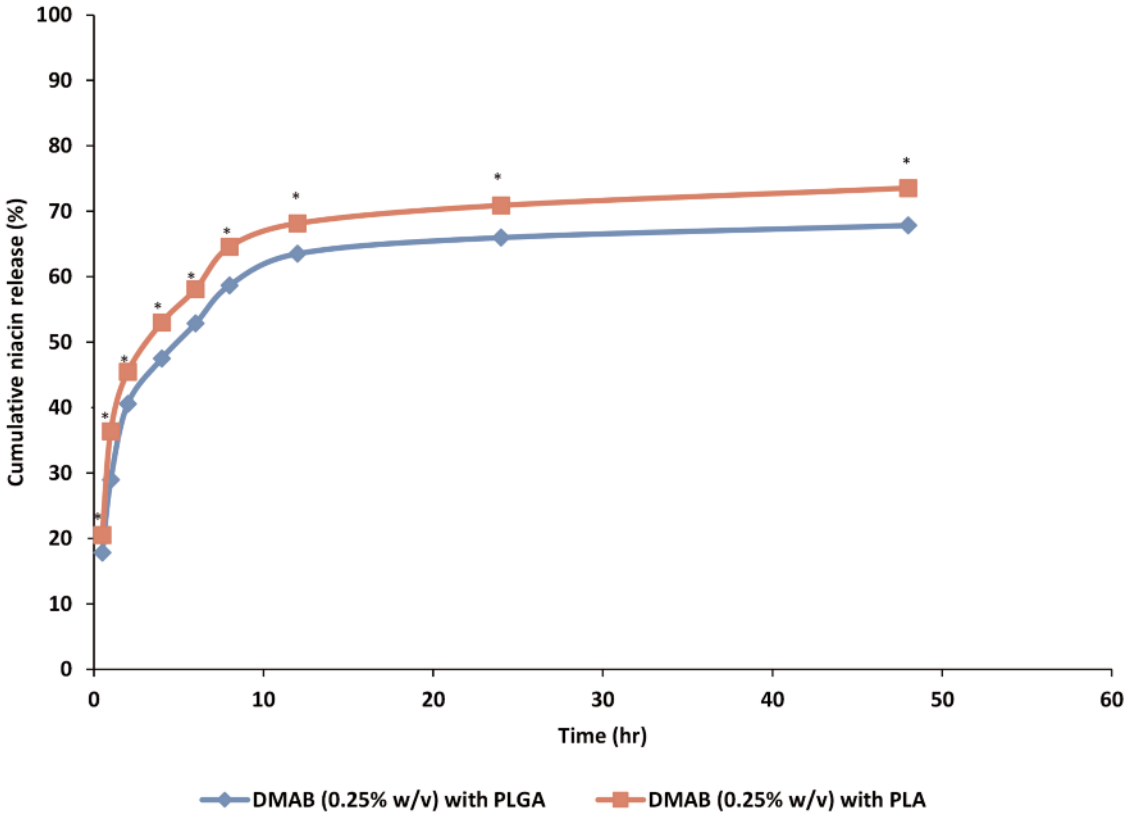

Figure 6. PLGA and PLA niacin-loaded NP *in vitro* drug release study with 0.25% DMAB stabilizer concentration. *In vitro* release profile of niacin in phosphate buffer (pH 7) from 0.25% DMAB formulated NPs (mean ± SD, n = 3, $^{*}p < 0.05$).

NP in vitro Effects on Prostaglandin Release

To measure *in vitro* effects of niacin-loaded NP formulations on prostaglandin levels, experiments evaluating extent of PGE_2 release were carried out using whole human blood samples. One-way ANOVA demonstrated marginal differences among treatment groups ($p = 0.06$). When compared to control (234.84 ± 73.01 pg/mL), PGE_2 expression assessed 1 hour after exposure of plain niacin formula showed a significant increase in PGE_2 blood concentrations (804.78 ± 95.16 pg/mL) ($p = 0.01$). All niacin-loaded PLGA-NP formulations experienced no significant increase in PGE_2 concentrations when compared to control. When compared to plain niacin, the use of 0.25% (389.09 ± 13.93 pg/mL, $p = 0.03$), 0.5% (291.08 ± 325.93 pg/mL, $p = 0.01$), and 1% (415.99 ± 4.04 pg/mL, $p = 0.03$) DMAB formulated niacin-loaded PLGA-NPs showed a significant reduction in PGE_2 blood concentrations (Figure 8). The use of niacin-loaded PLA-NP formulations showed no significant difference in PGE_2 expression when compared to plain niacin formula (data not shown).

Discussion

Niacin has been used for decades as a method to effectively treat dyslipidemia and associated cardiovascular diseases. To date, it remains one of the few available drugs shown to favorably affect multiple lipoprotein parameters[3]. Adverse flushing side effects associated with high dose niacin consumption has restricted the use of this highly valuable, lipid regulating drug. To help mediate vasodilatory effects of niacin, extended and prolonged oral delivery systems have been developed[26]. These unique delivery systems have shown improvements in the control of niacin-induced vasodilation, yet perpetuation of the niacin flush still persists as one of the more commonly reported side effects[10,27]. Over the years, polymeric NPs have been effectively used in the development of controlled drug delivery systems[28]. Highly biodegradable polymers such as PLGA

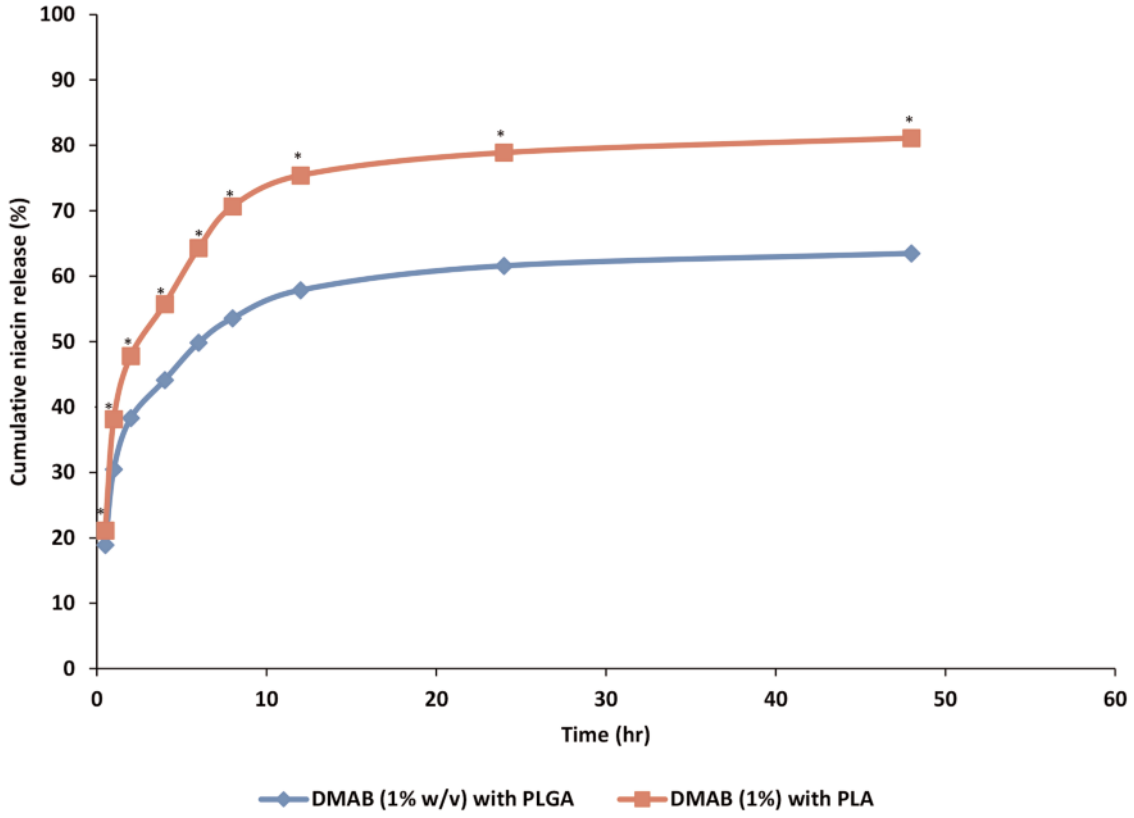

Figure 7. PLGA and PLA niacin-loaded NP *in vitro* drug release study with 1% DMAB stabilizer concentration. *In vitro* release profile of niacin in phosphate buffer (pH 7) from 1% DMAB formulated NPs (mean ± SD, n = 3, $^{*}p < 0.05$).

and PLA have been approved for human use by the Food and Drug Administration[29]. The application of these polymeric materials has ranged from development of artificial cartilage and skin, to the creation of NP delivery systems used in the delivery of various bioactive compounds[29]. PLGA and PLA polymers have been commonly used for the development of sustained drug delivery systems[13,30]. Furthermore, polymeric NPs have been shown to control drug release by altering the rate of NP biodegradation and matrix erosion, leading to reduced or sustained systemic drug delivery over a prolonged period of time. Due to altered drug release characteristics of polymeric NP systems, we hypothesized that use of polymer encapsulated niacin NP systems could reduce flushing effects associated with niacin consumption. Therefore, the primary objective of our research was the development and optimization of a novel, reformulated, polymeric NP delivery system for niacin that could minimize associated flushing effects and function as an effective replacement for traditional oral niacin delivery.

Particle size is an important characteristic for optimization of effective NP systems, as size can influence cellular uptake, tissue diffusion, bioavailability, and/or rate of degradation[31]. Many factors, including the type of polymer used as well as addition of effective stabilizers, can affect particle size. In our study, we found that PLGA-NPs demonstrated a reduction of particle size in relation to DMAB concentrations. As DMAB concentrations increased, particle size decreased with the smallest average particle size seen at 1% w/v DMAB concentrations (Figure 2A). Consequently, use of PLA in NP formulations showed no association between particle size and DMAB concentrations (Figure 3A). A possible explanation for these observations could be based on polymer molecular weight[31,32]. The PLGA polymers represented in our study have a molecular weight of 30,000-60,000 Da, while the molecular weight of its PLA counterpart were 85,000-160,000 Da. Alterations in polymer type during the formulation process resulted in a 55,000-100,000 Da difference in molecular weight. It is possible that this

shift in molecular weight resulted in an increase in polymer suspension viscosity[32], which would result in a net reduction in particle dispersion following physical agitation during the formulation process. Reduced efficiency of emulsion droplet dispersion utilizing the same agitation parameters would result in an increase in NP size.

Zeta potential represents a measure of system stability based on net charge and systemic attraction or repulsion of developed NPs[33]. In our study, NPs developed using both PLGA and PLA polymers demonstrated high, positively charged, zeta potential values (Figure 2C and Figure 3C, respectively). DMAB is a highly cationic stabilizer used in our drug formulation process[19,34]. Previous studies have shown that inclusion of DMAB into formulation parameters resulted in a net increase in charged NP systems[35,36]. PLA formulated NPs showed a concentration dependent increase in zeta potential in relation to DMAB concentration, indicating that as stabilizer concentration increased, inclusion of stabilizer into NP shell can increase as well. Overall, in both PLGA and PLA formulated NPs, the use of DMAB resulted in a net increase of cationic DMAB inclusion within the NP surface, thus giving highly positive surface charges to our newly formulated NP systems.

NP drug entrapment did not present a consistent pattern. For both PLGA and PLA formulated NPs, drug entrapment was highest utilizing 0.25% w/v DMAB concentration. The lowest level of drug entrapment was seen in PLGA and PLA formulations using 0.5% and 0.1%, respectively (Table III and Table IV). The process of drug entrapment is highly dependent on solvent diffusion and a drug's partition co-efficient[31]. In comparison to PLGA entrapment, it is possible that increased solvent viscosity of the higher molecular weight PLA led to an overall reduction in drug entrapment via reduced solvent diffusion into the aqueous media. The rate of solvent diffusion may act to slow the degree of polymer precipitation, effectively increasing the time allowed for drug partition into the aqueous phase, leading to a net reduction of drug entrapment.

Morphological examination of 1% and 0.25% formulations via TEM showed NPs that were mostly spherical in shape, with distinct and well defined borders that displayed moderate particle separation (Figure 4 and Figure 5). These visual verifications were consistent with our characteristic studies performed with the zeta sizer. TEM

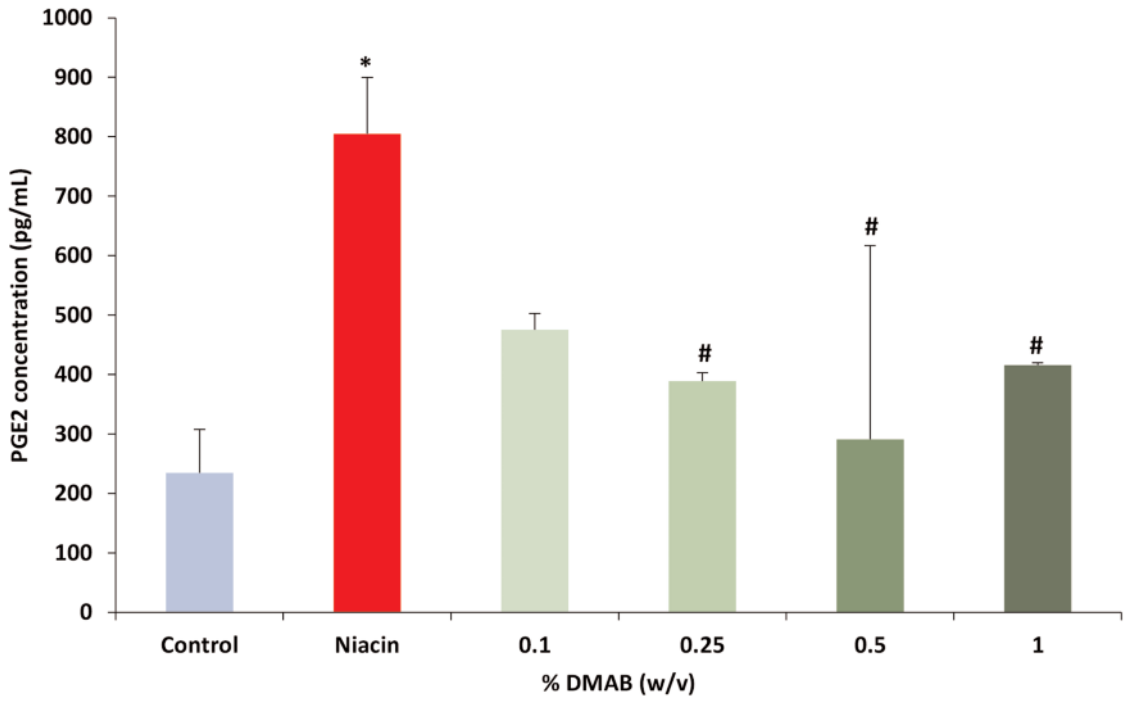

Figure 8. Effects of niacin-loaded PLGA-NP formulations on PGE2 release (mean ± SD, n = 2, $^{*}p < 0.05$, significantly different from control, $^{\#}p < 0.05$, significantly different from plain niacin formulation).

scale measurement of NPs where also in range with our particle size observations. It is important to note that particles with a larger charge undergo an increasing degree of repulsion in comparison to lower charged particles[37]. The degree of repulsion effectively functions to mitigate particle aggregation and increase system separation and stability, suggesting the use of DMAB for stable formulation of both PLGA and PLA based niacin-loaded NPs.

Niacin release from PLGA- and PLA-NPs demonstrated differing results in relation to polymer type. When formulated with 0.25% or 1% DMAB concentrations, initial drug release were significantly reduced in the PLGA group in comparison to PLA formulated NPs. Furthermore, overall cumulative drug release was significantly lower in PLGA formulated NPs (Figure 6 and Figure 7). Past studies have suggested correlation between drug release and drug entrapment[38]. When high concentrations of drug are entrapped within a NP shell, crystallization of the drug can occur. This process can result in increased crystallized drug formation within the inner core of the NP shell. In turn, crystallized drug should dissolve, diffuse, and release more slowly than an equally dispersed drug[39]. When comparing the amount of niacin released from PLGA- and PLA-NPs against the degree of drug entrapment, it can be speculated that niacin release from 0.25% and 1% DMAB formulated PLGA-NPs undergoes significant reduction, possibly as a result of an increased concentration of crystallized drug entrapped within the NP shell. Furthermore, the higher burst release observed for PLA formulations can be attributed to reduced particle size, a short diffusion path, and/or compositional matrices interactions of the formulation[40,41]. All formulations showed adequate reduction in particle size, with the largest particle size obtained in PLA formulation at 1% DMAB (159.53 ± 27.96 nm) (Figure 2 and Figure 3). However, this does not explain why PLGA formulations of a much smaller size yielded a significant reduction in drug release following 0.25% and 1% DMAB formulations. It is possible that formulations with PLA polymers could result in a shortened diffusion path through the polymer matrix that allow higher amounts of niacin to leak out of the NP shell during initial entrapment measurements[40,41]. Furthermore, altered matrices interactions of differing NP formulations may increase drug adhesion to the NP wall leading to delayed or reduced drug release[42].

ELISA assay of *in vitro* experiments using human blood detected significant increases in concentrations of PGE_2 in samples treated with plain niacin, when compared to control (non-treated blood samples) ($p = 0.01$). Increased *in vitro* PG release in response to niacin exposure has been shown to occur below 5 mM concentrations[25]. The use of identical concentrations of niacin (5 mM) loaded into PLGA-NPs demonstrated reductive effects in PGE_2 release when compared to plain niacin (Figure 8). Niacin-loaded PLGA-NPs showed significant reductions in PGE_2 release when formulated at 0.25%, 0.5%, and 1% DMAB concentrations. Enhanced NP characteristics such as size and zeta potential correlated favorably with noted reductions in PGE_2 release. All formulations that demonstrated a significance reduction in PGE_2 presented with more favorable NP characteristics than the non-significant 0.1% w/v DMAB formulation. PLGA formulations that produced a significant net reduction in PGE_2 release presented with particles sizes of 104 nm or less and zeta potential at or above 25 mV; while the non-significant formulation (0.1% w/v DMAB) achieved a particle size and zeta potential of 115 nm and 23 mV, respectively (Figure 2). No formulations using PLA polymers showed any significant reductions in PGE_2 concentrations when compared to plain niacin (data not shown). It is worth noting that characteristic studies performed on niacin-loaded PLA-NPs showed larger NP size parameters and reduced zeta potential when compared to PLGA-NP formulations (Figure 3). Taking into account changes seen in the PGE_2 expression between polymer formulations, a logical assumption can be made that the role of size and zeta potential as a measure of system stability plays a vital role in the control of niacin-induced PGE_2 expression. Furthermore, the elucidation of release patterns seen with both formulations indicates that PLGA formulated niacin-loaded NPs function to reduce the rate and extent of niacin exposure, thus potentially leading to a net reductive effect in PGE_2 release.

Conclusions

This study used a simplified solvent diffusion evaporation technique to formulated PLGA and PLA niacin-loaded NPs. The results of our characteristic work demonstrated effective formulation of small, highly stable, niacin entrapped NPs for both polymer types. *In vitro* release experiments

showed significantly reduced niacin release of PLGA formulated NPs in comparison to PLA formulated NPs. Furthermore, *in vitro* studies using whole human blood demonstrated niacin-loaded PLGA-NP effectiveness at significantly reducing PGE_2 release in comparison to plain niacin treatment. Effects noted in our *in vitro* studies may indicate the use of niacin-loaded PLGA-NP for controlled release of niacin. A conclusion can be made that the altered state of niacin release seen in PLGA formulations effectively reduced PGE_2 release. Therefore, our results may indicate the use of PLGA formulated niacin-loaded NPs for the reduction of PG-induced niacin flushing. Further investigations are warranted to elucidate optimum niacin NP formulation and its effects on niacin induce vasodilation.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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