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Therapeutic ultrasound alters the physicochemical properties of nanostructured lipid carriers

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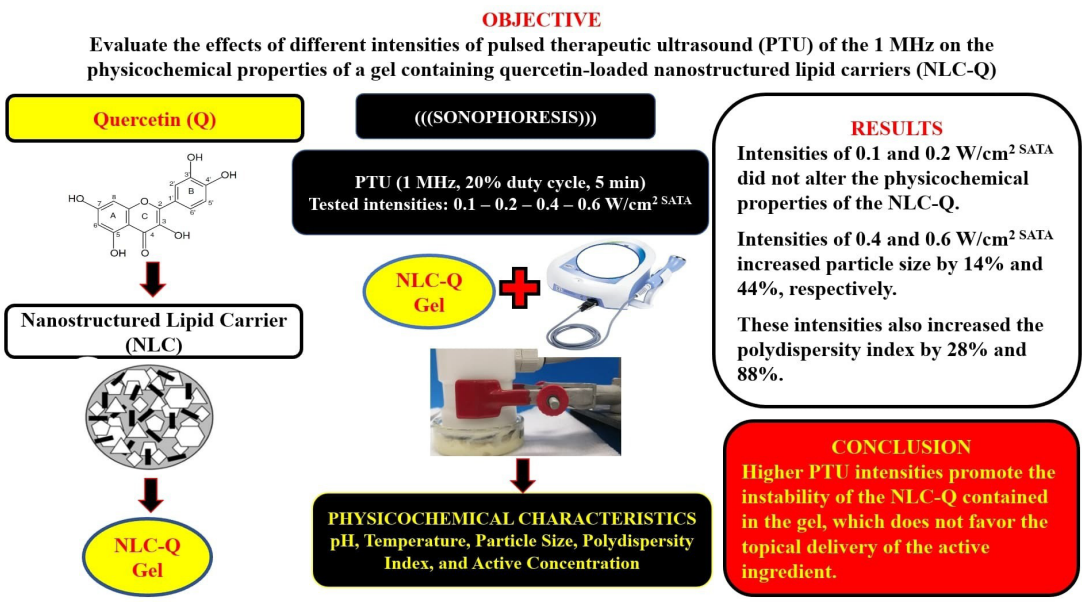
Graphical Abstract



Highlights

- *Ultrasound intensity induces physicochemical changes in lipid-based carriers.*
- *High intensities increase particle size and polydispersity index.*
- *These changes affect active ingredient delivery during sonophoresis.*

Abstract

Quercetin has therapeutic potential in the recovery of musculoskeletal injuries; however, when administered orally, this polyphenol exhibits low absorption. Its skin penetration can be enhanced by nanostructured lipid carriers loaded with quercetin (NLC-Q) applied in combination with pulsed therapeutic ultrasound (PTU). However, different intensities of 1 MHz ultrasound may compromise the physicochemical properties of NLC-Q, and this interaction has not yet been evaluated. The aim of this study was to assess the effects of different 1 MHz PTU intensities on the physicochemical properties of a gel containing nanostructured lipid carriers loaded with quercetin (NLC-Q). NLC-Q was developed using the high shear rate method. PTU (1 MHz, for 5 min, 20% duty cycle) was applied to the gel at intensities of 0.1, 0.2, 0.4, and 0.6 W/cm² SATA (spatial average temporal average intensity). Physicochemical properties (pH, temperature, mean particle size, polydispersity index, and concentrations of quercetin within the nanostructured lipid carriers) were evaluated before and after the application of different PTU intensities. Intensities of 0.1 and 0.2 W/cm² SATA did not alter the physicochemical properties of NLC-Q, while intensities of 0.4 and 0.6 W/cm² SATA increased particle size by 14% and 44%, respectively ($P < 0.001$). These intensities also increased the polydispersity index by 28% and 88% ($P < 0.001$). PTU intensities above 0.4 W/cm² SATA lead to instability of NLC-Q within the gel, which does not favor the topical delivery of the active ingredient.

Keywords: Ultrasound Therapy. Nanoparticle Drug Delivery Systems. Quercetin.

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INTRODUCTION

The administration of drugs through nanostructured lipid carriers (NLCs) improves several issues associated with pharmaceuticals, such as low aqueous solubility, poor drug absorption, rapid metabolism, and elimination from the body, while also reducing drug toxicity[1]. These NLCs are composed of a mixture of solid and liquid lipids, in which part of the solid lipid is replaced, forming an imperfect crystalline structure that allows for higher drug loading, greater retention efficiency, and physical stability during storage[2]. These nanoparticles can increase apparent solubility, control the release rate, and improve the bioavailability of encapsulated lipophilic compounds—features that ensure their wide use as delivery systems for cosmetics, oral and dermal pharmaceutical products, and functional foods[2]. Nanoparticles loaded with quercetin have been developed and experimentally tested for their antioxidant and anti-inflammatory capabilities[3,4,5]. Recent studies have evaluated the antioxidant capacity of quercetin in the treatment of musculoskeletal injuries with and without low-intensity therapeutic ultrasound (sonophoresis)[3,6].

The therapeutic implications of ultrasound result from thermal, non-thermal (mechanical), and physical effects derived from the absorption of ultrasonic energy[7], and its biological effects depend on application parameters[8]. The main application parameters include the waveform (continuous or pulsed), wave frequency (1 or 3 MHz), intensity (0.1 to 3 W/cm²), duty cycle (%), and application time[8,9,10]. Among the biological effects are increased cell proliferation during tissue repair[11], inflammation reduction[3,11,12,13], attenuation of lipid peroxidation following muscle injury[11], and improvement of endothelial function[8,9,10,13].

Therapeutic ultrasound is also used for drug delivery[7,14], in which the transport and distribution of drugs or therapeutic compounds through the skin and soft tissues occur during or after the influence of ultrasonic disturbance, a process known as sonophoresis[14,15]. The incidence of ultrasonic waves on the skin causes a variation in pressure (expansion, contraction, and distortion of gas bubbles pre-existing in a liquid medium), which leads to acoustic cavitation[16]. These physicochemical effects enhance the transdermal delivery of drugs[14,15]. To enable this process, active ingredients are incorporated into a gel, which is used as a coupling medium to transmit the ultrasonic wave to the skin[14].

Acoustic cavitation combined with the physicochemical properties of drugs contained in gels can enhance the transdermal delivery of active compounds[14]. This occurs in a non-inertial manner (the bubble undergoes repetitive oscillations under an acoustic field but does not collapse) and inertial manner (the bubble oscillates under the acoustic field at higher amplitudes and collapses)[7]. Inertial acoustic cavitation may interfere with the properties of nanoparticles[7] and consequently affect drug delivery[15,17]. The likelihood of this phenomenon depends on the ultrasound frequency, application method, wave type, temporal average intensity, transducer size, and the size and shape of the bubble[17].

To date, no studies have assessed the interaction of different intensities of 1 MHz pulsed therapeutic ultrasound (PTU) on NLCs. This study aimed to evaluate whether different intensities of 1 MHz PTU alter the physicochemical characteristics (pH, temperature, particle size, polydispersity index, and active ingredient concentration) of nanostructured lipid carriers loaded with quercetin (NLC-Q) immersed in a gel.

METHODS

This controlled laboratory study was conducted at the Nanomaterials Production Laboratory of the Universidade Franciscana (UFN), in the state of Rio Grande do Sul, Brazil. Data collection was divided into two stages: the first involved the development of NLC-B (blank) and NLC-Q gels, and the second evaluated the effect of PTU on these nanoparticles.

The nanostructured lipid carriers loaded with quercetin (NLC-Q) were developed according to the established methodology[3,18]. The high shear rate method employed an Ultraturrax® (T-18, IKA®, Brazil) to produce the NLC-Q. A total of 100 mL of NLC-Q was produced following the composition described for the organic phase (Inwitor 900K; 0.8 g [IOI Óleo GmbH, Germany]; Crodamol®: 4.2 g [IPP Pharma, Brazil]; Span 60: 1.0 g [ZTCC,

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China]; Quercetin: 0.1 g [YTBIO, China]) and the aqueous phase (Tween 80: 2.0 g [INLAB, Brazil]; MilliQ Water: 92 mL). Quercetin was added to the other components of the organic phase and stirred for 5 minutes. Then, the remaining aqueous phase components were added to the organic phase and stirred for 10 minutes. The NLC-Q mixture was processed in the Ultraturrax® (T-18, IKA®, Brazil) at 18,000 rpm for 30 minutes, cooled to room temperature, and stored in 100 mL amber bottles.

Next, a volume of 100 mL of Gel-NLC-Q was prepared using the dispersion method. Initially, 0.4 g of Carbopol ETD 2020® (Lubrizol, Brazil) (0.4%), 0.3 g of Germall (115 Ashland, Brazil) (0.3%), and 0.25 g of Triethanolamine (Adcos Professional, Brazil) (0.25%) were weighed. These substances were manually mixed one by one using a pestle in a porcelain crucible, in the following order: Carbopol ETD 2020® (Lubrizol, Brazil) 0.4 g, Germall™ 115 (Ashland, Brazil) 0.3 g, and the emollient solution of Triethanolamine (Adcos Professional, Brazil) 0.25 g. Then, 99.05 mL of the NLC-Q was added to the mixture and stirred manually with the pestle until fully homogenized.

After preparation, three samples of the NLC-Q gel (8 g each) were subjected to centrifugation testing in test tubes (centrifuge model TDL 80-2B, China) for 30 minutes at 3000 rpm. This test increases gravitational force, enhancing particle mobility, which can lead to phase separation, sediment or supernatant formation, and coalescence. Any sign of instability indicates the need to reformulate the product[3,18].

The therapeutic ultrasound equipment (Prosevem 977, Quark Medical Products, Brazil), 1 MHz, pulsed waveform (2:8 ms) with an ERA (effective radiating area) of 3.8 cm^2 was used. Four round containers measuring 5 cm in diameter by 1 cm in height were each filled with 10 g of gel. The PTU transducer was placed in contact with the gel at the top of the container and remained stationary during the experiment. The distance from the transducer to the bottom of the well was 3 mm, and the application time was 5 minutes. A pulsed waveform was used with a duty cycle of 20%, corresponding to spatial average temporal average (SATA) intensities of 0.1, 0.2, 0.4, and 0.6 $W/cm^{2\ SATA}$ (respectively, the spatial peak temporal average (SPTA) intensities used were 0.5, 1.0, 2.0, and 3.0 $W/cm^{2\ SPTA}$). These intensities were based on a previous study with modifications[8,9,10]. Data for each power level were collected in triplicate. Immediately after PTU irradiation, the gel samples were prepared for characterization tests. A 2 g portion of gel was taken from the surface layer of each well and sent for analysis using the Zetasizer – Nano – ZS (Malvern, USA) and HPLC (Shimadzu, Japan).

The pH of the NLC-Q gel was measured directly in the gel using a pH meter (Ultra Basic, UB-10, USA) previously calibrated with MilliQ water at pH 6.80. These measurements were taken before and after PTU application. To determine particle size and polydispersity index (PdI), a blank sample (NLC-B) and three NLC-Q samples (G1, G2, G3) were analyzed in triplicate. Readings were taken by dynamic light scattering (Zetasizer – Nano – ZS, Malvern, USA) at a fixed scattering angle of 90° at 25 °C. Samples were diluted 500 times (v/v) in MilliQ water and filtered using a 0.45 µm filter to achieve proper scattering, and results were expressed in nanometers[18].

For concentration determination, samples were weighed (0.2044 g), received 4 mL of HPLC-grade acetonitrile, and were placed for 10 minutes in an ultrasonic shaker. Then, 0.48 mL of MilliQ water (pH 2.8) and 0.72 mL of HPLC-grade acetonitrile were added, and the final volume was adjusted to 10 mL in a volumetric flask with acetonitrile. This was vortexed for 10 minutes (Phoenix AP 56 – Brazil) at speed 5. After 10 minutes in the ultrasonic bath (USC-5000, Unique, Brazil) and 8 minutes at 3000 rpm in the centrifuge (Centrifuge TDL 80-2B, China), the solution was filtered using a 45 µm regenerated cellulose filter and placed into labeled vials. These were analyzed in quadruplicate using HPLC (Shimadzu, Japan), equipped with a degasser (DGU-20 HT), quaternary pump (LC-20 AT), automatic injector (SIL-20 HT), column oven (CTO-20A), and photodiode array detector (SPD-M20A)[18].

Temperature was measured in °C (digital thermometer TP 101, China) before and immediately after PTU application, as Initial Temperature °C (before PTU) and Final Temperature °C (after PTU). Temperatures were expressed as deltas (Δ), indicating the difference between pre- and post-PTU application (Δ = before PTU °C – after PTU °C).

Data are presented as means and standard deviations (± SD). Variable distribution was tested using the Shapiro-Wilk normality test. Data with symmetric distribution were compared using one-way ANOVA followed by Tukey's *post hoc* test. Group differences are presented as mean difference (MD), 95% confidence interval (95% CI), and their respective percentages. The significance level was set at 5% ($P < 0.05$).

RESULTS

Table 1 shows the pH results during the study. The pH did not change during the study (P = 0.993).

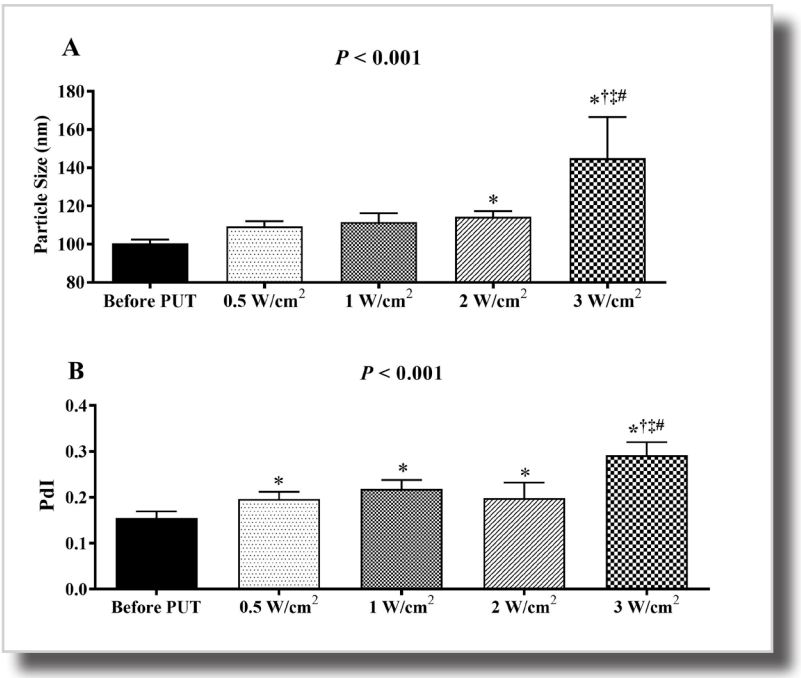
Table 1 - PH data of the different gel formulations before and after the application of different intensities of pulsed therapeutic ultrasound (Santa Maria, RS/Brazil, 2021).

Variables	Intensity (W/cm² SATA)	pH
NLC-B gel before PTU	0.0	5.37 ± 0.02
Gel NLC-Q antes PTU	0.0	5.35 ± 0.02
NLC-Q gel before PTU	0.1	5.40 ± 0.02
	0.2	5.37 ± 0.03
	0.4	5.32 ± 0.02
	0.6	5.33 ± 0.02
NLC-B gel after PTU	0.1	5.31 ± 0.07
	0.2	5.32 ± 0.02
	0.4	5.34 ± 0.06
	0.6	5.34 ± 0.01

Data presented as mean and standard deviation (± SD). pH: Hydrogen potential; SATA: spatial average temporal intensity; PTU: Pulsed therapeutic ultrasound; NLC-B: Gel with blank nanostructured lipid carrier; NLC-Q gel: Gel with nanostructured lipid carrier loaded with quercetin.

The results of particle size before and after the application of different PTU intensities are shown in Figure 1A. The NLC-B gel before PTU application (107.4 ± 2.1 nm) showed an increase (P < 0.001) of 14% in size after applying an intensity of 0.4 W/cm² SATA (MD: 14; 95% CI: 2 to 26 nm) and 44% at the intensity of 0.6 W/cm² SATA (MD: 45; 95% CI: 32 to 57 nm). The intensity of 0.6 W/cm² SATA resulted in an increase in particle size in the NLC-Q gel (P < 0.001) compared to the intensities of 0.1 W/cm² SATA (MD: 36; 95% CI: 23 to 49 nm), 0.2 W/cm² SATA (MD: 34; 95% CI: 20 to 46 nm) and 0.4 W/cm² SATA (MD: 31; 95% CI: 18 to 44 nm), corresponding to increases of 32%, 30%, and 27%, respectively.

Figure 1 - Particle size and polydispersity index (PdI) data of the NLC-Q gel before and after the application of different intensities of pulsed therapeutic ultrasound (Santa Maria, RS/Brazil, 2021).



Data presented as mean and standard deviation (± SD). PdI: Polydispersion Index; SATA: spatial averaged–temporal intensity. * P < 0.05 *vs* Gel with Quercetin before PTU application; † P < 0.05 *vs* Gel with Quercetin after application of PTU at 0.1 W/cm² SATA; ‡ P < 0.05 vs Gel with Quercetin after application of PTU at 0.2 W/cm² SATA; # P < 0.05 vs Gel with Quercetin after application of PTU at 0.4 W/cm² SATA.

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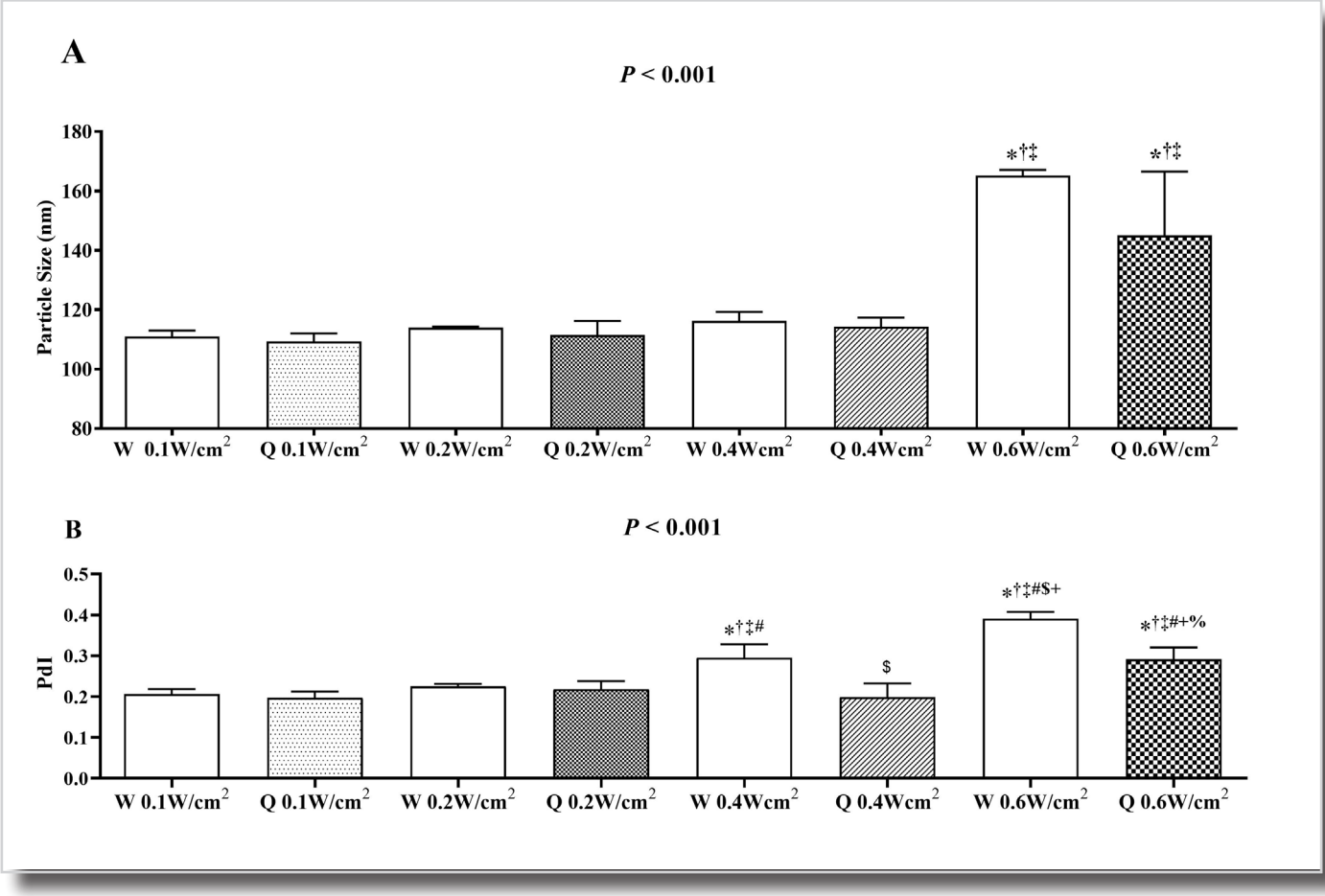
The results of the polydispersity index (PdI), before and after the application of different PTU intensities, are shown in Figure 1B. PTU application increased PdI (P < 0.001) by 27% at the intensity of 0.1 W/cm² SATA (MD: 0.06; 95% CI: 0.01 to 0.07), 41% at 0.2 W/cm² SATA (MD: 0.04; 95% CI: 0.03 to 0.09), 28% at 0.4 W/cm² SATA (MD: 0.04; 95% CI: 0.01 to 0.7), and 88% at 0.6 W/cm² SATA (MD: 1.14; 95% CI: 0.11 to 0.17). The intensity of 0.6 W/cm² SATA increased PdI by 48% (compared to 0.1 W/cm² SATA), 33% (0.2 W/cm² SATA) and 47% (0.4 W/cm² SATA) in relation to the other PTU intensities.

The results for particle size and PdI of the NLC-B and NLC-Q gels after the application of different PTU intensities are shown in Figure 2. The particle size in the NLC-B and NLC-Q gels did not change after ultrasound application at intensities of 0.1 W/cm² SATA, 0.2 W/cm² SATA and 0.4 W/cm² SATA. However, PTU application at 0.6 W/cm² SATA significantly increased (P < 0.001) the particle size in both NLC-B and NLC-Q gels compared to the other evaluated intensities. This increase was approximately 50% for the NLC-B gel and 35% for the NLC-Q gel, with no significant difference between these two gels (P > 0.05, MD: 20.1; 95% CI: -1.3 to 41.5 nm) at this intensity (Figure 2A data).

Figure 2 - Particle size and polydispersity index (PdI) data of nanostructured lipid carriers with blank gel (NLC-B) and nanostructured lipid carriers with quercetin gel (NLC-Q) after application of different intensities of pulsed therapeutic ultrasound (Santa Maria, RS/Brazil, 2021).

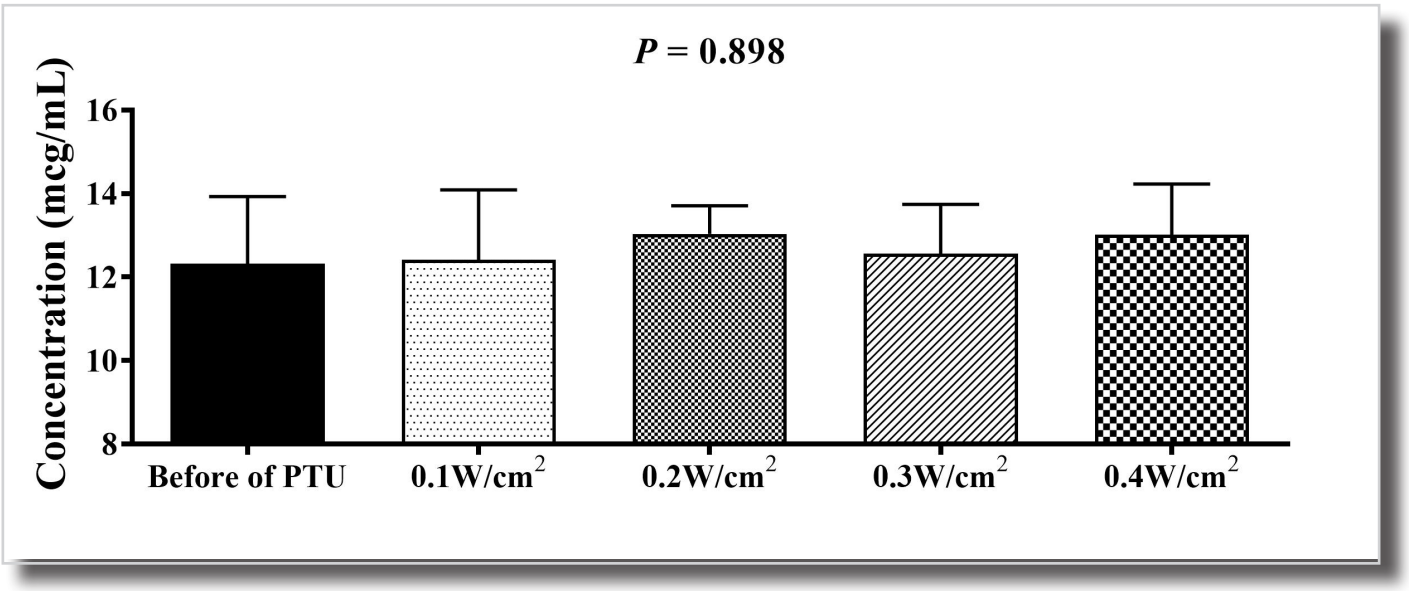


Data presented as mean and standard deviation (± SD). PTU intensity in SATA: spatial average temporal intensity. NLC-B: Nanostructured Lipid Carriers with Blank Gel; NLC-Q: Nanostructured Lipid Carriers with Quercetin Gel. PdI: polydispersity index; * P < 0.05 vs Blank Gel (with NLC-B) after ultrasound application at 0.1 W/cm² SATA; † P < 0.05 vs Quercetin Gel (with NLC-Q) after ultrasound application at 0.1 W/cm² SATA; ‡ P < 0.05 vs Blank Gel (with NLC-B) after ultrasound application at 0.2 W/cm² SATA; # P < 0.05 vs Quercetin Gel (with NLC-Q) after ultrasound application at 0.2 W/cm² SATA; $ P < 0.05 vs Blank Gel (with NLC-B) after ultrasound application at 0.4 W/cm² SATA; + P < 0.05 vs Quercetin Gel (with NLC-Q) after ultrasound application at 0.4 W/cm² SATA; % P < 0.05 vs Blank Gel (with NLC-B) after ultrasound application at 0.6 W/cm² SATA.

The PdI did not change after the application of PTU at intensities of 0.1 and 0.2 W/cm$^{2\ SATA}$. However, there was an increase ($P < 0.001$) at intensities of 0.4 W/cm$^{2\ SATA}$ (NLC-B) and 0.6 W/cm$^{2\ SATA}$ (NLC-B and NLC-Q), respectively by approximately 40%, 85%, and 40% compared to the other gel intensities evaluated. The PdI at 0.4 W/cm$^{2\ SATA}$ PTU for NLC-B gel was 40% higher ($P < 0.05$) than that of the NLC-Q gel. The same occurred at the pulsed ultrasound intensity of 0.6 W/cm$^{2\ SATA}$, where the NLC-B was 75% higher than the NLC-Q gel. The intensity of 0.6 W/cm$^{2\ SATA}$ for the NLC-B gel was 75% higher than that of 0.4 W/cm$^{2\ SATA}$ for the same gel. However, there was no difference between 0.4 W/cm$^{2\ SATA}$ for the NLC-B gel and 0.6 W/cm$^{2\ SATA}$ for the NLC-Q gel ($P > 0.05$) (Figure 2B).

The quercetin concentrations in the nanoparticles before and after PTU application are shown in Figure 3. The different PTU intensities did not alter ($P = 0.898$) the concentrations of quercetin (µg/mL) in the NLC-Q.

Figure 3 - Quercetin concentration data in the gels before and after application of different intensities of pulsed therapeutic ultrasound (Santa Maria, RS/Brazil, 2021).



Data presented as mean and standard deviation (± SD). NLC-Q: gel containing nanostructured lipid carriers loaded with quercetin; PTU: pulsed therapeutic ultrasound; SATA: spatial average temporal intensity.

The temperature variation data are presented in Figure 4. The intensities of 0.1, 0.2, and 0.4 W/cm$^{2\ SATA}$ did not alter the temperature of the NLC-Q gel. However, the intensity of 0.6 W/cm$^{2\ SATA}$ increased the temperature variation delta of the NLC-Q gel approximately threefold compared to 0.1 W/cm$^{2\ SATA}$ (MD: 1; 95% CI: 0.1 to 2.1 °C; $P < 0.030$), but did not differ from the other intensities evaluated (0.2 and 0.4 W/cm$^{2\ SATA}$).

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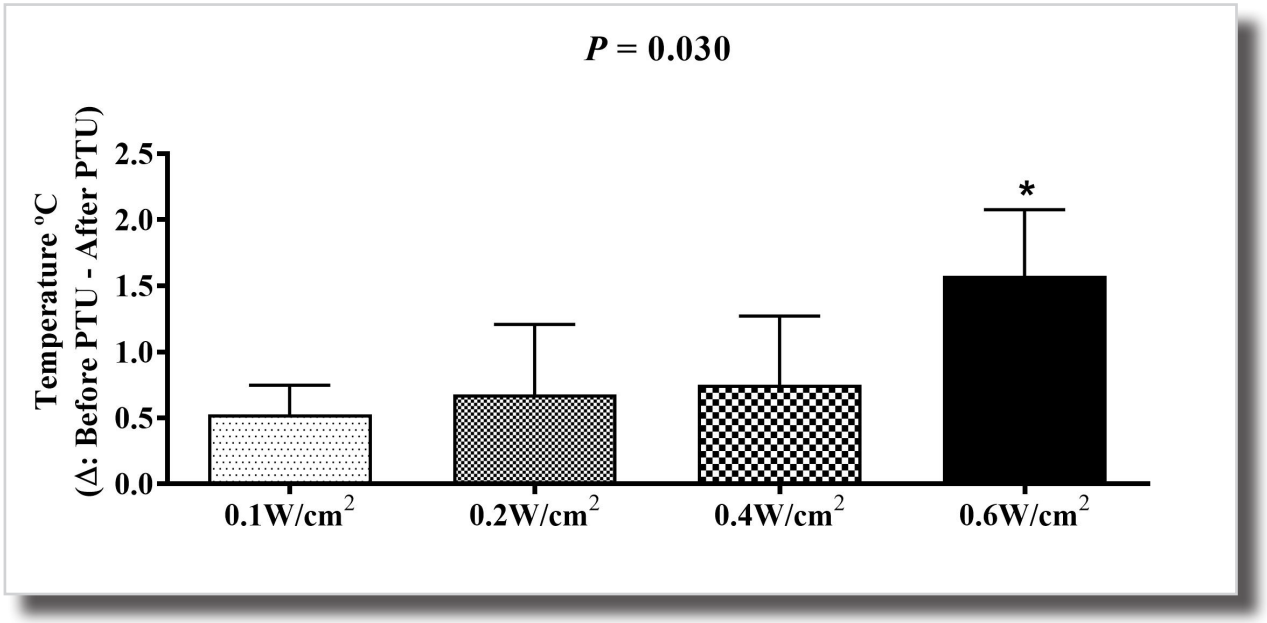
Figure 4 - Temperature variation data (Δ: before PTU application – after PTU application) following the application of different intensities of pulsed ultrasound (Santa Maria, RS/Brazil, 2021).



Data presented as mean and standard deviation (± SD). NLC-Q: nanostructured lipid carriers loaded with quercetin; PTU: pulsed therapeutic ultrasound; SATA: spatial average temporal intensity. * P < 0.05 vs PTU application at 0.1 W/cm$^{2\ SATA}$.

DISCUSSION

The different intensities of 1 MHz PTU applied to a gel containing lipid nanoparticles loaded with quercetin (NLC-Q) did not alter the pH or the quercetin concentrations within the gel. However, intensities of 0.4 W/cm$^{2\ SATA}$ and 0.6 W/cm$^{2\ SATA}$ (equivalent to 2 W/cm$^{2\ SPTA}$ and 3 W/cm$^{2\ SPTA}$, respectively) increased both particle size and PdI. These changes were more pronounced at 0.6 W/cm$^{2\ SATA}$, which also raised the temperature of the NLC-Q gel.

The pH values of the NLC-Q gel in this study ranged from 5.31 (minimum) to 5.37 (maximum). These values are close to those of human skin (between 5.4 and 5.9), which is desirable in topical or transdermal formulations to avoid irritation effects[19]. The pressure generated by ultrasonic waves can mediate chemical phenomena[20] and potentially alter pH[21], which did not occur here, demonstrating the stability of the NLC-Q gel across the PTU intensities investigated (0.1, 0.2, 0.4, and 0.6 W/cm$^{2\ SATA}$).

The concentrations of quercetin in the NLC-Q gel remained unchanged after application of the different PTU intensities. It can be inferred that these intensities induced non-inertial cavitation, as the shear stress did not rupture the lipid layer of the NLCs[7]. Moreover, the quercetin concentration within the NLCs remained stable throughout the study.

Particle size and PdI are variables that influence the stability, solubility, release rate, and biological performance of NLCs[22]. In the present study, PTU intensities of 0.1 W/cm$^{2\ SATA}$ and 0.2 W/cm$^{2\ SATA}$ did not alter the particle size of the NLC-Q gel, indicating its stability at these intensities. However, intensities of 0.4 W/cm$^{2\ SATA}$ and 0.6 W/cm$^{2\ SATA}$ increased the particle size. For cutaneous use, particle sizes should range between 50 and 300 nm, as this facilitates penetration through the skin barrier, enhances cellular absorption, and ensures a quicker onset of action[22]. In this study, although higher PTU intensities altered particle size (pre-PTU size: 100.4 ± 2.1 nm; post-PTU at 0.4 W/cm$^{2\ SATA}$: 114 ± 2.3 nm; post-PTU at 0.6 W/cm$^{2\ SATA}$: 145 ± 21.5 nm), the values remained within the recommended range for cutaneous application[22].

The intensity of ultrasonic waves is one of the variables responsible for producing thermal and/or mechanical (non-thermal) effects that influence therapeutic responses[7]. These effects interact with gas bubbles, causing them to contract and expand, generating the phenomenon of acoustic cavitation[16]. The oscillation of bubbles may lead to heat

production, microfluidic flow, and shear stress in the medium through which the acoustic field travels[7,16]. In the present study, higher intensities (0.4 and 0.6 W/cm$^{2\ SATA}$) caused acoustic cavitation and altered the particle size of the NLC-Q. The increase in temperature observed at 0.6 W/cm$^{2\ SATA}$ also contributed to modifications in the lipid structure of the NLC. Local temperature depends on the ultrasound beam area relative to its frequency, intensity, exposure time, and the tissue's capacity to dissipate heat[17]. The stationary application of the transducer is another factor that can promote temperature increase[17]. The recommended ultrasound intensity for active ingredient delivery ranges from 0.3 to 3 W/cm[19] in continuous mode; however, pulsed waveforms can attenuate thermal effects[21]. Therefore, pulsed wave application seems more appropriate for sonophoresis, since continuous waves promote greater temperature increases, which can alter NLC-Q particle size in the gel.

The different PTU intensities evaluated in this study altered the PdI of the NLC-Q gel. The PdI is an indicator of nanoparticle stability, with recommended values between 0.1 and 0.25[22]. In this study, PTU increased the PdI at three lower intensities without surpassing the recommended threshold[22]. However, at 0.6 W/cm$^{2\ SATA}$ (PdI: 0.29 ± 0.03), the value exceeded the accepted limit[22]. Based on this criterion, this intensity is not advisable for sonophoresis with NLC-Q gels, as it destabilizes the nanoparticle. Notably, the particle size and PdI values were lower for NLC-Q compared to NLC-B, suggesting that the active ingredient, in this case quercetin, influences these variables. Quercetin possesses antioxidant activity[5,23], which may attenuate pro-oxidant molecular products derived from pyrolysis or thermolysis that occur during high-intensity PTU application[24,25].

The thermal and mechanical effects caused by ultrasonic waves in NLCs influenced both particle size and PdI in this study. These changes are likely due to inertial acoustic cavitation, as higher intensities may have caused bubble collapse[7]. The type of acoustic cavitation depends on the amplitude and frequency of ultrasound waves, as well as the properties of the bubble material and particle size[24]. Kooiman and colleagues[25] investigated ultrasound-cell interactions and suggested that cavitation is the primary driver of therapeutic effects and active ingredient delivery. Ultrasound exposure increases internal bubble pressure, making it highly responsive and resulting in mechanical, thermal, and chemical effects in its surrounding environment[24]. Molecules may undergo chemical reactions during the bubble expansion phase, such as pyrolysis or thermolysis due to elevated temperature, producing radicals like H^+ and OH^- (hydroxyl radicals). These radicals may combine into molecular products such as H_2O_2 (hydrogen peroxide), H_2 or H_2O[21]. On the other hand, the antioxidant actions of quercetin on reactive oxygen and nitrogen species[5], may neutralize these molecular products, particularly OH^- [5,23], demonstrating that this active compound protects NLCs from destabilization at PTU intensities below 0.4 W/cm$^{2\ SATA}$. In this study, 0.6 W/cm$^{2\ SATA}$ increased temperature, which contributed to the enlargement of particle size and PdI in NLC-Q. Specifically for PdI, these values surpassed the recommended upper limit (0.25)[22]. Such temperature increases following ultrasound exposure are dependent on application parameters[8,9] and have been reported in previous studies[7,17].

This study has some limitations, including the use of only one ultrasound frequency (1 MHz), one waveform (pulsed), one active ingredient (quercetin) in the nanoparticles, stationary transducer application, and a fixed exposure time (5 minutes). Future studies examining PTU interactions with different nanoparticle-based gels will help define appropriate parameters for the clinical use of sonophoresis.

CONCLUSION

The intensities of 0.1 W/cm$^{2\ SATA}$ (0.5 W/cm$^{2\ SPTA}$) and 0.2 W/cm$^{2\ SATA}$ (1 W/cm$^{2\ SPTA}$) of pulsed therapeutic ultrasound (PTU) did not alter the physicochemical properties of NLC-Q, as assessed by changes in pH, particle size, PdI, and quercetin concentrations within the NLCs. The intensity of 0.4 W/cm$^{2\ SATA}$ (2 W/cm$^{2\ SPTA}$) modified particle size and PdI but did not affect the cutaneous penetration capacity of NLC-Q. Conversely, the intensity of 0.6 W/cm$^{2\ SATA}$ (3 W/cm$^{2\ SPTA}$) altered both particle size and PdI, causing instability in the NLC-Q gel, which may hinder the penetration of the active ingredient into the skin, making it unsuitable for sonophoresis application. Therefore, PTU intensities above 0.4 W/cm$^{2\ SATA}$ (2 W/cm$^{2\ SPTA}$) are not recommended for sonophoresis with NLC-Q gel. In clinical or experimental research involving sonophoresis, the interaction between PTU intensity and the active ingredient encapsulated in NLCs should be evaluated beforehand.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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