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In vitro phonophoresis: effect of ultrasound intensity and mode at high frequency on NSAIDs transport across cellulose and rabbit skin membranes

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The objective of this study was to evaluate the effect of intensity, mode, and duration of ultrasound application on the transport of three nonsteroidal anti-inflammatory drugs (NSAIDs) across cellulose membrane and rabbit-skin. Ibuprofen, piroxicam and diclofenac sodium were used as the model drugs. Studies were performed *in vitro* using a modified Franz diffusion assembly adapted to a therapeutic ultrasound transducer. Ultrasound had a significant and positive effect on the transport of the model NSAIDs across cellulose and rabbit skin membranes. Increasing ultrasound intensity from 0.5 to 3.0 W/cm^2 led to a proportional increase in drug transport. Continuous ultrasound mode was more effective in enhancing drug transport than the pulsed mode. Diclofenac sodium had the least flux and permeability coefficient. This was attributed to its comparatively lower pK_a value that renders the drug more ionizable in the buffer solution, consequently reducing its selective penetration through the membranes. This study demonstrated the therapeutic potential of ultrasound in transdermal delivery of NSAIDs and the synergistic effect of temperature and ultrasound operational parameters on drug transport.

1. Introduction

Percutaneous drug absorption is limited due to the resistance imposed by the stratum corneum; the outermost layer of the skin, on drug permeation. Several approaches have been reported to overcome this barrier and to enhance transdermal drug delivery. These include the use of chemical enhancers (Obata et al. 1993; Yoneto et al. 1995), electroporation (Prausnitz 1997), iontophoresis (Wearley and Chien 1990) and phonophoresis or sonophoresis (Tyle and Agrawala 1989; Bommannan et al. 1992; McElnay et al. 1993; Mitragotri et al. 1995). Phonophoresis or sonophoresis is the application of ultrasound waves in transdermal drug delivery.

Phonophoresis, as a stand alone technique in the absence of concurrently applied medications, has been widely used in physical therapy such as the relief of muscle pain (Yueh-Ling 2006).

When the application of phonophoresis is desired a transducer is placed on the target area of the body. The transducer converts electrical energy into ultrasound which is applied in either continuous or pulsed mode. In continuous mode an alternating voltage of appropriate frequency is applied to the transducer resulting in the continuous emission of ultrasound waves of the same frequency. In the pulsed mode short bursts of alternating voltage are repeatedly applied to the transducer resulting in pulsed ultrasound waves. For proper application of ultrasound, however, a coupling agent is placed between the transducer and the tissues being treated. A coupling agent is, in most instances, a gel material that allows the transmission of ultrasound waves. Without couplant the ultrasound is 100% reflected at both the transducer-air interface and air-tissue interface and is rapidly attenuated in air.

Incorporation of drugs into the coupling agent to enhance the therapeutic benefits of ultrasound at low frequency has been reported in the literature (Mitragotri 2000; Mitragotri et al. 2000; Tang et al. 2000). Combining ultrasound with the transdermal delivery of drugs, such as the NSAIDs, is expected to have a dual analgesic effect and to enhance the therapeutic response of the patients (Mitragotri et al. 2000; Tang et al. 2000). This should overcome many of the side effects that have been reported with the oral, rectal and parenteral formulations of NSAIDs including gastrointestinal disturbances, oedema, dizziness, and headache (Babar et al. 1990). In a recent *in vivo* trial, the efficacy of ibuprofen phonophoresis in knee osteoarthritis was examined (Kozanoglu et al. 2003). This study, however, found that ibuprofen phonophoresis was not superior to conventional ultrasound, presumably because ibuprofen was formulated as a cream, which is expected to reduce the efficacy of ultrasound in comparison to an aqueous gel

preparation. Therefore there is a need to improve the efficacy of ultrasound coupled with NSAIDs by optimizing the operational parameters of ultrasound, with emphasis on high frequency applications, and by re-formulating the composition of the couplants. The specific objectives of the present study were to evaluate the effect of intensity, mode, and duration of ultrasound application at high frequency along with the propagated heat effect on the transport of three NSAIDs; ibuprofen, piroxicam, and diclofenac sodium, across cellulose membrane and excised rabbit skin.

2. Investigations, results and discussion

2.1. Diffusion through cellulose membrane

In vitro transport experiments were performed using the modified diffusion cell assembly illustrated in Fig. 1 and ultrasound parameters listed in Table 1. The effect of different ultrasound intensities at continuous mode on the transport of ibuprofen, piroxicam and diclofenac sodium through cellulose membrane is illustrated in Figs. 2, 3 and 4. The profile of the curves outlined in the Figures is identical indicating that the transport mechanism of the drugs under the influence of continuous ultrasound mode was similar. Transport data were statistically analyzed using JMP IN software (SAS Institute Inc. NC, USA). Statistical analysis revealed that the application of ultrasound had a significant effect on the amount of drug transported across the cellulose membrane (p value <0.05). An increase in ultrasound intensity from 0.5 to 3.0 W/cm^2 resulted in a linear increase in drug transport. This was verified by the linear contrast and ANOVA analyses of variance.

During the first 10 min of sonication there was a rapid increase in the flux of the drugs through the polymeric membrane. This was followed by a slower diffusion over the next 50 min during which ultrasound was turned off. The observed reduction in flux when ultrasound was discontinued indicates that the effect of phonophoresis on cellulose membrane is not permanent. The sudden increase in drug flux through the membrane after 60 min corresponds with the second phase of ultrasound application when ultrasound was turned on for an additional 10 min. To further illustrate the sonic and thermal effects on drug

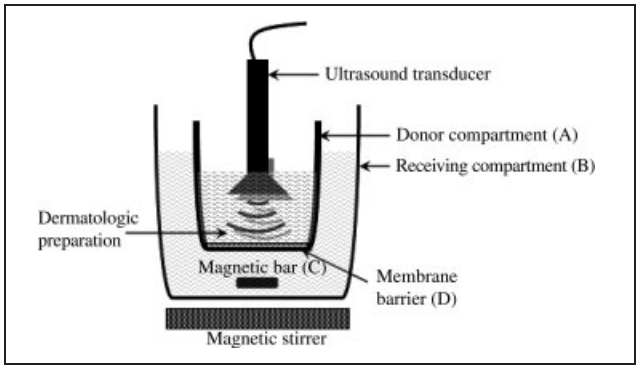

Fig. 1: Schematic diagram of the diffusion apparatus

Table 1: Ultrasound application parameters

Intensities	0.5, 1.5 and 3.0 W/cm^2
Mode	Continuous and pulsed
Frequency	800 kHz
Application	Stationary

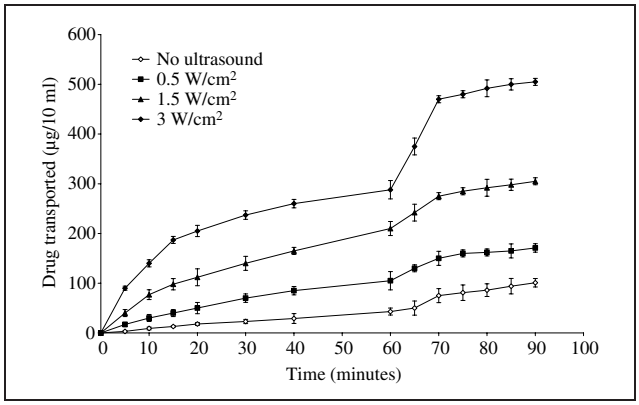

Fig. 2: Effect of continuous ultrasound intensities on ibuprofen transport across cellulose membrane

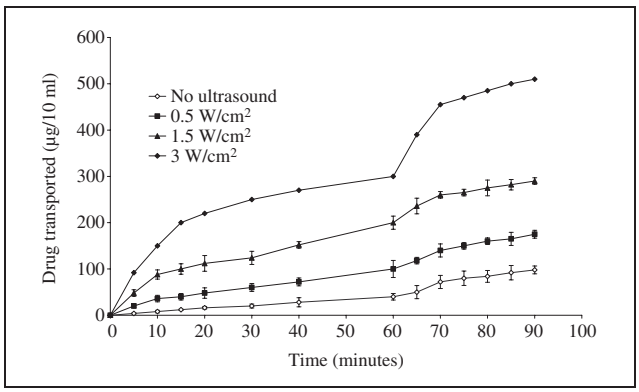

Fig. 3: Effect of continuous ultrasound intensities on piroxicam transport across cellulose membrane

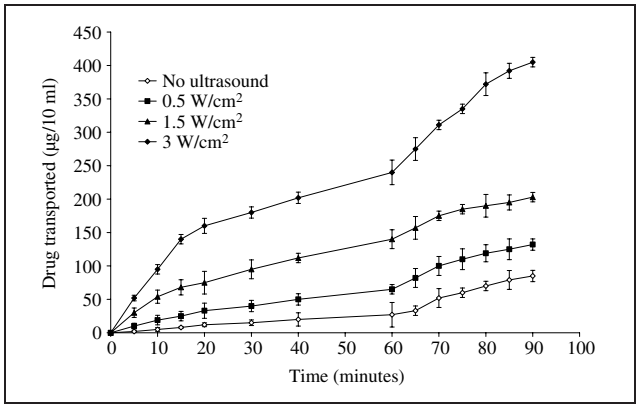

Fig. 4: Effect of continuous ultrasound intensities on diclofinac sodium transport across cellulose membrane

transport, flux (f) and permeability constant (K_p) were estimated for each of the three segments [(0–10 min), (10–60 min), and (60–70 min)] of the concentration time profile given in Figs. 2, 3, and 4. Diffusion rates or flux (f) were estimated from the slope of the drug concentration time curves according to the following equation (Singh et al. 1998).

$$f = {}^{S}/_{A} \qquad (1)$$

where S is the slope of the drug concentration time curve and A is the surface area of the membrane (cm^2). Permeability constants (K_p) were determined by the following equation (Singh et al. 1998).

$$k_P = {}^{f}/_{C_o} \qquad (2)$$

where C_o is the initial concentration of the drug in the donor compartment (l mg/mL). Flux values and the corresponding permeability constants for each drug are given in Tables 2 and 3. Flux and permeability constant increased with increasing ultrasound intensity during the first 10 min of sonication. The increase in drug diffusion with an increase in ultrasound intensities (Figs. 2, 3, and 4) is a result of both thermal and non-thermal events, such as cavitation, radiation pressure and acoustic micro streaming (Median et al. 1995). These events along with the thermal effect; which is an increase in temperature associated with ultrasound application, impacts the diffusion coefficient of drug molecules across the membrane. The extent of these effects, however, depends on the duration and intensity of sonication (Machet et al. 1996). The effect of temperature on drug transport could be deduced from the flux values obtained from the second segment (10–60 min) of the concentration time profiles when no ultrasound was applied (Figs. 2, 3, and 4). For comparison, control experiments were performed in room temperature with no sonic effect. Flux data are given in Tables 2 and 3. As seen from the tables, even though no ultrasound was applied between 10 and 60 min, flux values increased with an increase in the intensity of ultrasound that was applied in the preceding 10 min. While it was concluded that the effect of ultrasound on cellulose membrane is not permanent, the observed increase in flux could only be attributed to the increase in the temperature of the donor compartment generated by ultrasound. The effect of ultrasound intensity on the temperature of the donor compartment is illustrated in Fig. 5. During the first 10 min of continuous sonication, the temperature of the donor compartment gradually increased from 28.5 °C to a maximum temperature of 37 °C, 45.5 °C or 62 °C at ultrasonic intensities of 0.5, 1.5 and 3 W/cm^2, respectively. After ultrasound was turned off the temperature gradually decreased to 28.5 °C. Therefore the observed increase in flux and K_p values with an increase in temperature in comparison to the control study indicates that ultrasound and temperature play a synergistic role on drug transport.

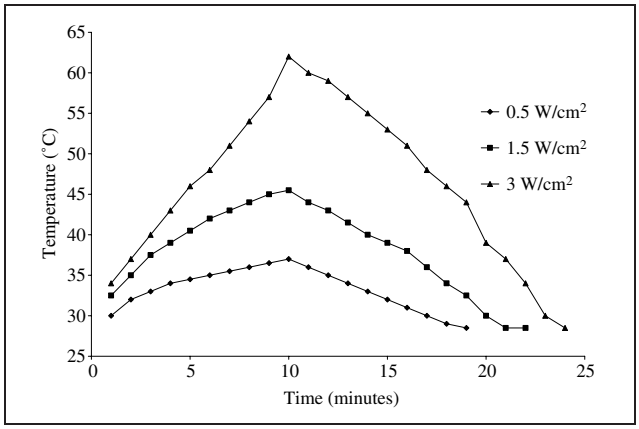

Fig. 5: Effect of ultrasound intensities in continuous mode on the temperature of the donor compartment

Of the NSAIDs evaluated in this study, diclofenac sodium had the lowest permeability constant. This could be due to its lower pK_a (3.78) value and therefore a larger proportion of the drug exists in the dissociated (ionized) form in the donor compartment, which reduces its capacity to penetrate through the hydrophobic cellulose membrane. The relatively higher pK_a values of ibuprofen (5.2) and piroxicam (5.3 and 6.3) causes the donor solution to retain a higher proportion of the drug in the undissociated form. Therefore, the selective diffusion of ibuprofen and piroxicam through the cellulose barrier and into the recipient solution was greater than that of diclofenac sodium.

To demonstrate the effect of ultrasonic mode on drug transport across the membrane, sonication was applied in pulsed or continuous mode at 0.5 and 1.5 W/cm^2 intensities for up to 40 min. Piroxicam was used as the model drug in this study. The effect of ultrasound mode is shown in Fig. 6. As seen from the figure, piroxicam diffusion at continuous mode was higher than its diffusion at pulsed mode of equal intensity. To further illustrate this effect, diffusion rates or flux (f) were calculated from the slopes of the graph and the permeability constants (K_p) were calculated by applying Eq. (2). Flux and K_p values are listed in Table 4. The higher flux and K_p values observed with the continuous mode demonstrate its effect and efficiency as a practical technique for the enhancement of drug transport.

Table 2: NSAID's Flux values across cellulose membrane as a function of ultrasound intensity at continuous mode

Ultrasound intensities	Flux (F) × 100 µg/10 mL · min · cm^2		
	Ultrasound (initial 10 min)	No ultrasound (10–60 min)	Ultrasound (terminal 10 min)
DICLOFENAC			
Control	1.8	1.52	8.8
0.5 W/cm^2	6.71	3.2	12.36
1.5 W/cm^2	19.1	7.14	9.89
3.0 W/cm^2	33.55	9.17	25.07
IBUPROFEN			
Control	3.14	2.35	11.19
0.5 W/cm^2	10.63	5.36	15.96
1.5 W/cm^2	27.16	9.21	23.03
3.0 W/cm^2	49.48	9.21	64.21
PIROXICAM			
Control	2.82	2.22	11.33
0.5 W/cm^2	12.68	4.54	14.12
1.5 W/cm^2	31.11	7.75	21.19
3.0 W/cm^2	54.04	9.44	54.68

Table 3: NSAID's permeability coefficients across cellulose membrane as a function of ultrasound intensity at continuous mode

Ultrasound intensities	K_p (cm^2 · min^{-1} × 10^{-6})		
	Ultrasound (initial 10 min)	No ultrasound (10–60 min)	Ultrasound (terminal 10 min)
DICLOFENAC			
Control	1.8	1.52	8.8
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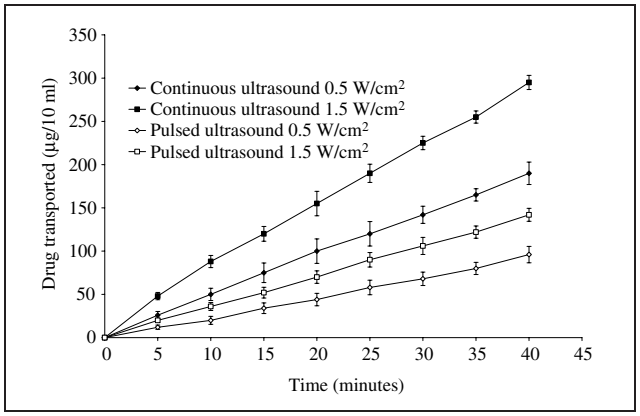

Fig. 6: Effect of continuous and pulsed ultrasound mode on piroxicam transport across cellulose membrane

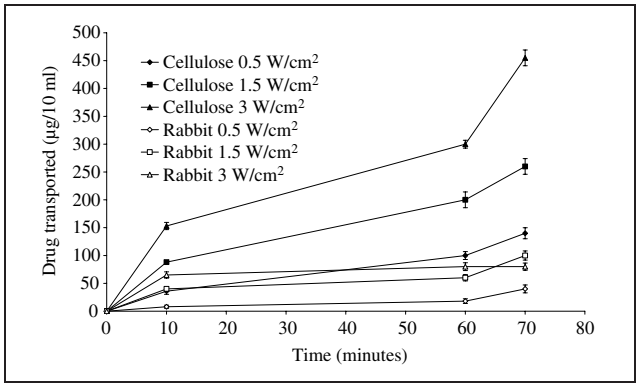

Fig. 7: Piroxicam transport across cellulose and rabbit skin membranes under the influence of continuous ultrasound mode

2.2. Diffusion through rabbit skin

Excised rabbit skin was used to study the effect of continuous ultrasound on piroxicam transport across animal membrane and to establish an *in vitro* procedure for the application of phonophoresis in transdermal drug delivery. The results of the permeability study are given in Fig. 7. Ultrasound increased the diffusion of the drug across the rabbit skin. This effect was dependent on both the mode and intensity of the applied ultrasound. Fig. 7 shows the increase in drug transported across the rabbit skin and cellulose barrier with an increase in ultrasound intensities. The amounts of the drug transported across rabbit skin was, however, significantly lower than the amount of drug transported through the cellulose membrane. It is probable that dermal retention of the drug in the rabbit skin could diminish percutaneous transport mediated by ultrasound.

Unlike cellulose membrane where an absolute increase in drug diffusion was observed with an increase in intensity, only 0.5 and 1.5 W/cm^2 intensities were tolerated by the rabbit skin. When an intensity of 3 W/cm^2 was applied, an increase in drug release was observed in the initial 10 min. After 60 min, however, there was no further increase in drug transport. This could be due to the heat generated in the donor cell compartment caused by the second 10 min of sonication. The effect of temperature on permeability was reported in the literature and was attributed to the softening effect of temperature on membrane lipids and thereby facilitating further membrane disruption by phonophoresis (Gustavo et al. 2003). In a study by Yamashita (1997) who examined skin surface by scanning electron microscopy after ultrasound application it was reported that cells of the stratum corneum were almost completely removed at low frequency and at intensity of 0.5 W/cm^2. These observations support the notion that a synergistic effect exists between the physical cavitation induced by ultrasound and temperature, which could explain the increase in permeability up to 60 min with an increase in ultrasound intensity. Further increase in temperature after the second ultrasound application at high intensity, however, might have caused deformities in skin structure; which includes fat, proteins and other hydrophilic moieties, rendering the skin less permeable or impermeable even under the influence of ultrasound (Kost et al. 1990).

In conclusion, the effect of phonophoresis on the *in vitro* transport of drugs across polymeric or biological membranes could be readily evaluated using the assembly described in this study. One of the advantages of the reported method is the larger scale of the setting that allows the incorporation of commercially available ultrasound transducers. While an increase in ultrasound intensity resulted in an increase in NSAIDs flux across the membranes, the effect of ultrasound was not permanent. Rather this effect was dependent on the duration of application. This signifies that ultrasound application at high frequency could be used for transdermal drug delivery. While this conclusion might hold true for polymeric membranes, extreme intensities might have negative and lasting effect on biological membranes. This was inferred from the effect of ultrasound on rabbit skin at the intensity of 3 W/cm^2, which showed that no change in transport was observed when ultrasound was re-applied after 60 min. It is therefore essential to consider the physiological effects of ultrasound at high frequencies when considering its application in clinical practice. Such effects could be examined by microscopic analysis, which might reveal undesirable ultrasound-induced structural damages to the skin. This study also demonstrated that formulation parameters, such as the pK_a and solubility of the drug, are critical for effective ultrasound application. The effect of temperature generated by ultrasound on the stability and solubility of the drug incorporated into the couplant is another factor that should be considered in future studies.

3. Experimental

3.1. Chemicals

Ibuprofen (pK_a 5.2) was obtained from BASF Corp. (Mount Olive, NJ, USA). Diclofenac sodium (pK_a 3.78), monobasic sodium phosphate and anhydrous dibasic sodium phosphate were purchased from Sigma Chemical Company (St. Louis, MO). Piroxicam (pK_a 5.3 and 6.3) was obtained from

Table 4: Effect of continuous and pulsed modes on piroxicam flux and permeability coefficient across the cellulose membrane

Intensities	Continuous mode		Pulsed mode	
	Flux (F) × 100 µg/10 mL · min · cm^2	K_p cm^2 · min^{-1} · 10^{-6}	Flux (F) × 100 µg/10 mL · min · cm^2	K_p cm^2 · min^{-1} · 10^{-6}
0.5 W/cm^2	17.09	17.09	8.34	8.34
1.5 W/cm^2	25.25	25.25	12.39	12.39

Pfizer, UK. Cellulose membrane (Spectrapor-3) was obtained from Spectrum Medical Instruments (Los Angeles, CA). All chemicals and raw materials were used as received without further processing.

3.2. Diffusion cell

Permeation experiments were performed using a modified Franz diffusion cell. A cross-sectional schematic view of the diffusion assembly is given in Fig. 1. The modified cell allows vertical separation of a solute donor reservoir compartment from the receptor compartment. The cellulose membrane or the skin barrier (D) was mounted on the mouth of the donor cell (A) between the cell flange and the faceplate. The length of the donor cell was 10 cm with an inside diameter of 6 cm. The volume of the donor cell was 246.4 mL. The total barrier surface area available for diffusion was 28.31 cm^2. The donor cell (A) was positioned in the middle of a 500 mL beaker (B), which served as the receiver compartment. Stirring of the receiver compartment was provided by placing a stirring bar (C) 5 cm from the surface of the barrier. The magnetic stirring bar was driven by an external magnetic stirrer (ER 10, GDR) at a constant speed of 900 rpm. Rabbit skin samples were obtained from shaved dorsal skin of about 45 days old female rabbits. The skin was excised just before the experiments. The subcutaneous fat was trimmed off and the skin was cleaned using saline solution to remove all visceral debris. When the skin was not immediately used, it was stored at −20 °C and used within three months.

3.3. Vehicle preparation and assay

The volume of the solution in the donor compartment was 25 ml. This solution was prepared by solubilizing ibuprofen, piroxicam, or diclofenac sodium in pH 7.4 phosphate buffer (pH 7.4) at a concentration of 1 mg/mL. The vehicle in the receiving compartment was 400 mL of pH 7.4 phosphate buffer. During the experiments 2 mL samples were withdrawn at different time intervals from the receiving compartment and replaced with a fresh phosphate buffer. The quantity of the drug diffused through the cellulose membrane and rabbit skin was determined spectrophotometrically (UV spectrophotometer, Perkin Elmer, USA) at 229, 355, and 270 nm for ibuprofen, piroxicam, and diclofenac sodium, respectively. A blank phosphate buffer solution was used as reference material. Diffusion experiments were carried out in triplicate unless otherwise stated.

3.4. Mounting the polymer and skin membranes

Rabbit skin tissues were mounted on the mouth of the donor compartment with its inside surface facing the receiving vehicle in the receiving compartment. The skin was equilibrated for 30 min in the receiving vehicle. The cellulose membrane was first wetted by a running tap water, rinsed three times with distilled water, and then dried with a filter paper. Subsequently the membrane was mounted on the mouth of the donor compartment and allowed to equilibrate for 30 min in the recipient vehicle before the start of the transport experiment.

3.5. Ultrasound application

An ultrasonic therapy apparatus (CSL-I, Shanghai, China) was used in this study. It was equipped with a flat tip probe with an applicator surface area of 7.071 cm^2. The probe was centrally positioned in the donor compartment and submerged into the donor solution about 3 cm from the barrier surface. The ultrasound was applied under the operating conditions listed in Table 1. The ultrasound was applied in two phases. Initially, it was applied for 10 min. After the initial the ultrasound applicator was turned off and the study was allowed to proceed for 50 min without ultrasound. After 60 min from the beginning of the experiment the applicator was turned on and the ultrasound was applied for additional 10 min after which the experiments were terminated.

To measure the effect of sonication on the temperature of the donor compartment, ultrasound was applied for a period of 10 min during which the temperature of the donor solution was recorded. Temperature was continuously measured for additional 14 min after the sonication was stopped. A thermocouple (Shemadzu Instruments, Tokyo, Japan) was used during and after sonication. It was positioned in the donor solution close to the mounted diffusion barriers. The temperature of the donor solution was measured at three ultrasound intensities (0.5, 1.5 and 3 W/cm^2).

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