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Dependence of low-frequency sonophoresis on ultrasound parameters; distance of the horn and intensity

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Abstract

Sonophoresis at a frequency of 20 kHz has been shown to enhance transdermal drug delivery, a phenomenon referred to as low-frequency sonophoresis. This study provides an investigation of the dependence of low-frequency sonophoresis on various ultrasound parameters, including the distance of the horn from the skin, intensity, and frequency. We performed in vitro experiments with full thickness pig skin to measure enhancements of skin conductivity and drug permeability. Ultrasound was applied to pretreat the skin using a sonicator operating at a frequency of either 20 or 40 kHz. We also measured pitting of aluminum foil to measure cavitation, which is the principal mechanism of low-frequency sonophoresis. The skin conductivity enhancement was found to be inversely proportional to the distance of the horn from the skin. As the intensity increased, skin conductivity enhancement also increased up to a certain threshold, and then dropped off. The intensities (I_{max}) at which maximum enhancement occur are about 14 W/cm^2 for 20 kHz and 17 W/cm^2 for 40 kHz. These findings may be useful in optimizing low-frequency sonophoresis. Overall, the dependence of transport on ultrasound parameters is similar to that of aluminum foil pitting. These results support the role of cavitation in low-frequency sonophoresis.

Keywords: Sonophoresis; Cavitation; Transdermal; Drug delivery

1. Introduction

Transdermal drug delivery offers a beneficial alternative to injections (Bronaugh and Maibach, 1989). However, transdermal drug delivery is limited to a short list of drugs due to the low permeability of drugs through the stratum corneum, the primary barrier of the skin. Various approaches including chemical enhancers (Walters, 1989) sonophoresis (Mitragotri et al., 1995a), iontophoresis (Green et al., 1993; Singh and Bhatia, 1996), and electroporation (Prausnitz et

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al., 1993; Prausnitz, 1996; Edwards et al., 1995) have been investigated to enhance drug transport through the skin. Ultrasound at various frequencies in the range of 20 kHz–16 MHz has been used for sonophoresis. Investigations of sonophoresis can be classified into three categories based on the ultrasound frequency used: high frequency (above 3 MHz) (Bommannan et al., 1992a,b), therapeutic (0.75–3 MHz) (Levy et al., 1989; Mitragotri et al., 1995b), and low frequency ultrasound (below 750 kHz) (Mitragotri et al., 1995c, 1996; Tachibana, 1992).

Therapeutic frequency is the most common frequency range used in over 90% of previous studies (Mitragotri et al., 1995a). However, we found that ultrasound at a frequency of 20 kHz significantly enhances transdermal transport of drugs, a phenomenon referred to as low-frequency sonophoresis (Mitragotri et al., 1995c, 1996). Low-frequency ultrasound has a clear advantage over therapeutic sonophoresis in that cavitational effects including the number density and size of bubbles, which play an important role in low-frequency sonophoresis, are inversely proportional to the ultrasound frequency (Gaertner, 1954). Cavitation is the formation of gaseous cavities in an ultrasound-coupling medium upon ultrasound exposure and involves rapid growth and collapse of a bubble (transient cavitation) or slow oscillatory motion of a bubble (stable cavitation) in the ultrasound field. Oscillations and collapse of cavitation bubbles disorder the lipid bilayers of the stratum corneum, thereby enhancing transport. However, cavitation is a complex phenomenon affected by numerous parameters including ultrasound frequency, intensity, and characteristics of ultrasound coupling medium such as temperature, surface tension, and the number of bubble nuclei (Frederick, 1980). Among these, ultrasonic frequency and intensity are assumed to be the most important parameters for cavitation (Frederick, 1980). There exists a threshold ultrasonic intensity below which cavitation cannot occur. On the other hand, it is difficult to obtain significantly more vigorous cavitation by increasing the intensity due to the appearance of other effects such as acoustic decoupling (Frederick, 1980). Accumulation of the knowledge of the dependence of sonophoresis on various ultrasound parameters is necessary to optimize low-frequency sonophoresis. In this paper, we first show the dependence of low-frequency sonophoresis on the distance of the horn from the skin. We then present our findings on the dependence of low-frequency sonophoresis on ultrasonic intensity and frequency, and the optimum value in terms of ultrasonic intensity. We also present data on low-frequency sonophoresis at 40 kHz.

2. Materials and methods

2.1. Materials

Tritium-labeled mannitol (^{3}H-mannitol) was purchased from ARC Research Products. For permeation experiments, ^{3}H-mannitol solution was diluted to 1 μCi/ml with phosphate-buffered saline (PBS, pH 7.4, phosphate concentration = 0.01 M, NaCl concentration = 0.137 M, Sigma Chemicals Co.). In vitro permeation experiments were performed with full thickness pig skin (Yorkshire). After sacrificing the pig, the hair on the back and the abdomen were clipped with clippers, and the skin was harvested immediately. The superfluous tissues such as fat and muscle were removed carefully. Skin with the exception of flawed pieces was cut into small pieces (2×10 cm), and was stored in a -80 °C freezer for up to 12 weeks until the experiments were performed.

2.2. In vitro transdermal experiments

Just prior to experiments, skin was thawed at room temperature and then immediately mounted onto a vertical Franz diffusion cell (Permegear Inc.), which consists of two compartments, a donor and a receiver compartment. The receiver compartment volume was 12.0 ml and the skin area was 1.77 cm^2. The skin was mounted with the stratum corneum side facing the donor compartment. Skin samples having an initial resistivity of < 30 kΩ cm^2 were discarded to ensure the intactness of the barrier function (measurement of skin resistivity described later). The receiver compartment was filled with PBS solution and stirred

with a magnetic stirrer. The diffusion cell was placed in a custom-made lucite mounting block to keep it in place. All experiments were performed at room temperature (25 °C). After ultrasound application (described later), the donor PBS solution was replaced by ^{3}H-mannitol in PBS solution (1 μCi/ml). The concentration of ^{3}H-mannitol in the receiver compartment was measured after 24 h using a scintillation counter (model 2000 CA, Packard). The skin permeability following ultrasonic pretreatment, P_{us}, as well as that without ultrasonic pretreatment (passive permeability), P_p, was calculated based on the following equation:

$$P = V \cdot \frac{\Delta C_r}{A \cdot \Delta t \cdot C_d} \quad (1)$$

where V is the volume of the receiver compartment (12 ml), A is the skin area (1.77 cm^2), ΔC_r is the change in ^{3}H-mannitol concentration of the solution in the receiver compartment in a time Δt (24 h), and C_d is the ^{3}H-mannitol concentration of the solution in the donor compartment. The mannitol permeability enhancement (MPE) is defined as the ratio of P_{us} and P_p, that is:

$$\mathrm{MPE} = \frac{P_{us}}{P_p} \quad (2)$$

2.3. Ultrasound application

Ultrasound was applied for skin pretreatment using a sonicator operating at a frequency of either 20 kHz (VCX400, Horn area of 1.33 cm^2, Sonics & Materials) or 40 kHz (VC140, Horn area of 0.28 cm^2, Sonics & Materials). Before each experiment, the sonicators were tuned according to the respective instruction manuals to ensure that the applied signal optimally matched the resonance frequency of the piezoelectric crystal. The intensities were measured as explained later. The sonicator horn was positioned in the donor chamber between 0.25 and 1 cm above the skin depending on the experimental protocol. The sonicators were operated in a pulse mode (5 s on, 5 s off). The temperature of the donor solution was measured periodically with a thermocouple (Digithermo, VWR Scientific). If necessary, the donor solution was replaced by fresh PBS to maintain the temperature at the skin surface below 40 °C.

2.4. Measurement of ultrasound intensity

2.4.1. Calorimetric method

The ultrasound intensities were measured using a calorimetric method (Liu et al., 1998). The temperature of 150 g water placed in a beaker was measured with thermocouple (Digithermo, VWR Scientific) during ultrasound exposure. The depth of horn in water was altered between 0.5 and 8.5 cm, depending on the experiment. The measured rate of temperature increase of water was used to determine the intensity of the sonicator as described below:

$$I = \left(\frac{m_{water} C_{P,water}}{A}\right)\frac{dT}{dt} \quad (3)$$

where I is the ultrasound intensity, m_{water} is the mass of water exposed (150 g), $C_{P,water}$ is the specific heat of water (4.18 J/g °C), A is the horn area, and dT/dt is the rate of temperature change of water. The measured intensity in this manuscript corresponds to spatially averaged pulsed average values, that is, the intensity corresponds to that during the ON time of ultrasound.

2.4.2. Hydrophone measurements

Ultrasound pressure measurements were performed using a small needle hydrophone (pinducer, Valpey Fisher (VP-1093)). This pinducer was directly connected to a Dynamic Signal Analyzer (Hewlett Packard model 3562A). Fast Fourier Transform (FFT) was performed on the signal from the pinducer to obtain the magnitude of the signal at 20 kHz. The pinducer was mounted on a platform such that its flat face was parallel to the face of the horn. A glass chamber (1.5 cm in diameter and 2 cm in height) was placed on the platform such that the pinducer is located at the center of the base circle of the cylinder. The chamber was filled with PBS and the ultrasound horn was lowered into the chamber. The distance of the pinducer from the horn was varied between 1 mm and 1 cm. The distance was measured using callipers. At each distance, the horn was activated at various levels and the amplitude of the signal at 20 kHz was measured. The ultrasound intensities were obtained relative to that when the distance of the horn from the pinducer was 1 cm.

2.5. Measurement of skin electrical resistance

Skin conductivity was measured at the start of experiment to verify the initial skin condition and during the experiment to test the effect of ultrasound on skin. In order to measure the electrical resistance of skin, two Ag/AgCl disk electrodes (4 mm in diameter, In vivo metric) were introduced in the diffusion cell, one in the receiver compartment and the other in the donor compartment. A 100 mV AC electric field (10 Hz) was applied across the skin for a short period of time (typically 5 s) using a signal generator (model HP 4116 A, Hewlett Packard), while the electrical current through the skin was measured with an ammeter. The electrical resistance was then calculated from Ohm's law. In order to obtain the actual skin resistance, the PBS resistance was measured separately using the same assembly but without mounting the skin and was subtracted from the measured skin resistance. The skin conductivity following ultrasonic pretreatment, C_{us}, as well as that without ultrasound pretreatment (control), $C_{control}$, were calculated based on Eq. (4):

$$C = \frac{1}{(R_m - R_{PBS})A} \quad (4)$$

where R_m is the measured resistance of skin, R_{PBS} is the PBS resistance (1.18 kΩ), A is the skin area (1.77 cm^2). The skin conductivity enhancement (SCE) is defined as the ratio of C_{us} and $C_{control}$; see Eq. (5):

$$SCE = \frac{C_{us}}{C_{control}} \quad (5)$$

2.6. Pitting of aluminum foil measurement

Pitting of aluminum foil measurement allows quantification of the sonicator's potential to induce cavitation (Okada et al., 1995). Aluminum foils (Reynolds) were mounted onto the diffusion cells in an identical manner to skin. The receiver and the donor compartments were filled with PBS. Ultrasound was applied for 0.5 s at 20 kHz and 5 s at 40 kHz in each experiment. After a continuous application of ultrasound, the foils were removed from the chamber. Indentations on the foil were counted by visual inspection. Exposure times of 0.5 and 5 s were found to be most suitable for reproducible counting of the indentations.

3. Results and discussion

3.1. Ultrasound pretreatment time

Fig. 1A shows the skin conductivity enhancement as a function of ultrasound pretreatment duration (frequency: 20 kHz, distance of the horn from the skin: 1 cm, intensity: 7.44 W/cm^2, and duty cycle: 50%). The error in the data is primarily attributed to the variation in cavitation, which is responsible for enhancing skin permeability. The data in Fig. 1A correspond to ultrasound application when the transducer is at 1 cm from the skin. As the distance between the skin and transducer decreases, the enhancement increases (discussed later). The skin conductivity enhancement is proportional to the pretreatment time. Fig. 1B shows the mannitol permeability enhancement (measured over 24 h after ultrasound pretreatment) under the same ultrasound condition of Fig. 1A. Ultrasound application was performed at various intensities and transducer distances from the skin. At a distance of 0.25 cm, enhancements of up to 1000-fold can be achieved. Data in Fig. 1C corresponding to high values of enhancements represent a skin-transducer distance of 0.25 cm. Under all conditions, the conductivity enhancement was generally related to mannitol permeability (Fig. 1C). This correlation is understandable since transdermal current is determined by the availability of pathways similar to those in the case of transdermal solute flux. However, the exact correlation between skin conductivity and permeability is likely to be different for different solutes.

We have previously shown that cavitation plays an important role in ultrasonic enhancement of transdermal transport (Mitragotri et al., 1995a, 1996). The magnitude of cavitation in a system can be detected by various techniques including (but not limited to) the presence of subharmonic response (indicative of transient as well as stable

cavitation) (Liu et al., 1998), pitting of metal surfaces (indicative of transient cavitation) (Frederick, 1980), and presence of free radicals. Among these, the pitting of metal surface is used here as a preferred method of measuring transient cavitation due to its simplicity. Accordingly, we further measured the number of pits on aluminum foil under the same conditions in order to evaluate the dependence of transient cavitation on ultrasonic parameters.

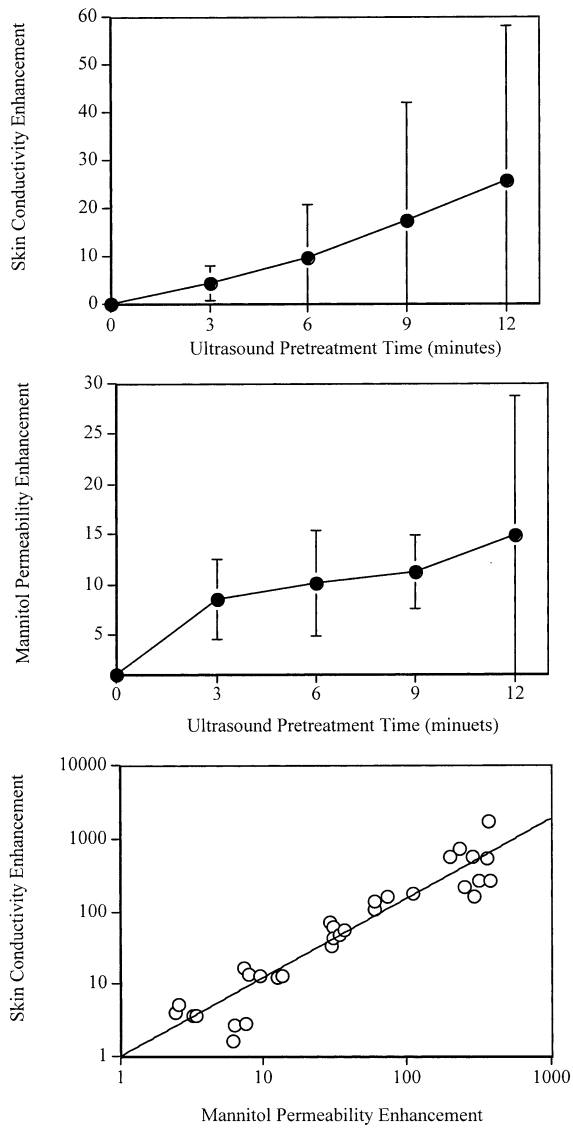

Fig. 1. (A) Variation of the skin conductivity enhancement with time during ultrasound exposure, (B) Variation of the ^{3}H-mannitol permeability enhancement for 24 h with ultrasound pretreatment time (20 kHz, 7.44 W/cm^2, and 5 s pulses applied every 10 s). Vertical bars indicate the value of S.D. ($n = 3$). (C) Relationship between skin conductivity enhancement and mannitol permeability enhancement.

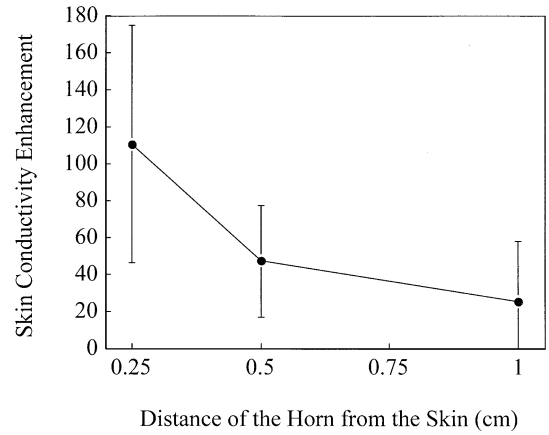

Fig. 2. Dependence of skin conductivity enhancement on the distance of the horn from the skin (20 kHz of frequency, 7.44 W/cm^2 of intensity, 50% of duty cycle, application time of 12 min). Vertical bars indicate the value of S.D. ($n = 3$).

3.2. The distance of the horn from the skin

Fig. 2 shows the dependence of skin conductivity enhancement on the distance of the horn from the skin (Application time of 12 min, Duty cycle of 5 s on/5 s off, frequency of 20 kHz and intensity of 7.44 W/cm^2). Skin conductivity enhancement increases with a decrease in the distance of the horn from the skin. We performed additional measurements to assess the role of the distance of the horn from the skin. In these experiments, we measured the number of pits on aluminum foil (ultrasound intensity of 7.44 W/cm^2) as a function of the distance of the horn (in the range of 0.25–4.00 cm) from the aluminum foil. Fig. 3 shows the correlation between cavitation events measured by pitting of aluminum foil and the distance of the horn from the aluminum foil. The Figure shows that the number of pits is inversely proportional to the distance. These data support the role of cavitation in low-frequency sonophoresis. The results suggest that the distance of the horn from the skin is an important parameter in determining the efficiency of sonophoresis.

As a possible explanation for the distance dependence of cavitation and sonophoresis, we hypothesized that the horn may be considered as a point source of ultrasound. A source with a radius less than the wavelength of the emitted wave can be considered as a point source. The radiation of ultrasound from a point source is isotropic which gives rise to a spherical wave. In that case, the sound pressure is inversely proportional to the distance from the sound source (Kinsler et al., 1982). Acoustic pressure, p, at a distance, r from a point source is expressed as:

$$p = A \cdot \exp\left(j\left(\frac{\omega t - 2\pi r}{\lambda}\right)\right)\frac{1}{r} \quad (6)$$

where A is a constant, ω is the angular frequency, t is the time, and λ is the wavelength. Note that in most cases involving high-frequency sonophoresis, the wavelength is much shorter than the radius of the horn, hence planar waves are more important rather than spherical waves.

Eq. (6) shows that bringing the horn close to the skin may increase the pressure amplitude at the skin and hence improve the effectiveness of low frequency. To assess this hypothesis, we performed ultrasound intensity measurements with a pinducer. Fig. 4 shows the dependence of intensity on the distance. The relative dependence of intensity on the distance with the pinducer is similar to that of skin conductivity enhancement and pitting. Since decreasing the distance of the horn from the skin increased the enhancements, subsequent experiments were at a distance of 0.25 cm instead of 1 cm.

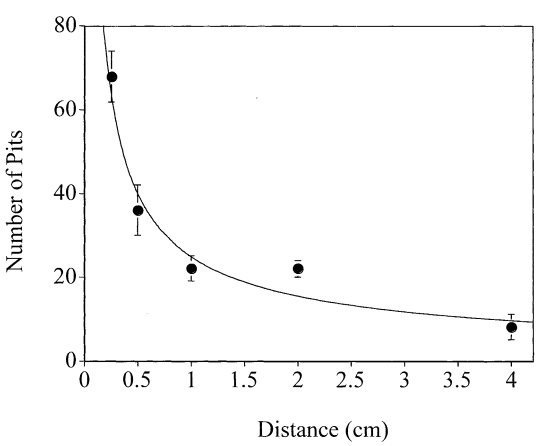

Fig. 3. Dependence of number of pits on the distance of the horn from the aluminum foil (Fitting curve: $y = 24.9x^{-0.7}$, $r = 0.961$). Vertical bars indicate the value of S.D. ($n = 6$).

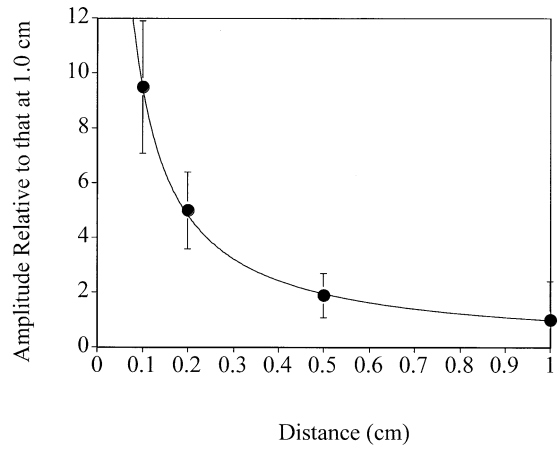

Fig. 4. Dependence of ultrasound intensity on the distance of the horn from the pinducer (Fitting curve: $y = 0.99x^{-0.99}$, $r = 1.000$). Vertical bars indicate the value of S.D. ($n = 5$).

3.3. Dependence of skin conductivity enhancement and cavitation on ultrasound intensity and frequency

Intensity is an important parameter for cavitation. The relation between cavitation and intensity is complex. For example, there exists a threshold level of intensity below which cavitation will not occur and it is difficult to obtain significantly more vigorous cavitation by increasing intensity (Frederick, 1980). We have previously reported that skin conductivity enhancement is proportional to intensities up to 14 W/cm^2 (Ultrasound frequency: 20 kHz, distance the horn from the skin: 1cm.) (Mitragotri et al., 2000). However, the linear relationship between skin conductivity enhancement and intensity may break down at higher intensities due to the presence of a cavitation cloud. This cloud is generated near the ultrasound source due to cavitation and reduces amount of energy delivered to the skin. To understand the intensity dependence of sonophoresis, we performed experiments in the intensity range of 2.39–33.46 W/cm^2 at 20 kHz and 0.40–43.3

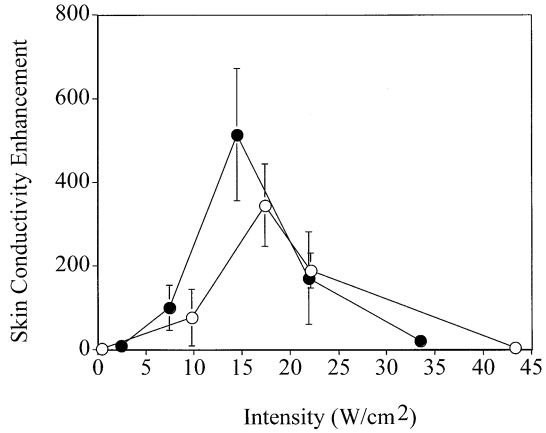

Fig. 5. Dependence of skin conductivity enhancement on ultrasound intensity at two frequencies (●) 20 kHz in the intensity range of 2.39–33.46 W/cm^2, (○) 40 kHz in the intensity range of 0.40–43.30 W/cm^2. Ultrasound was applied for 12 min in either case (5 s pulses applied every 10 s). Vertical bars indicate the value of S.D. ($n = 3$).

W/cm^2 at 40 kHz. Fig. 5 shows the dependence of skin conductivity enhancement on ultrasound intensity. As the intensity increased, enhancement also increased up to a certain point, then dropped off. The intensity (I_{max}) at which enhancement is maximum occurs at about 14 W/cm^2 for 20 kHz

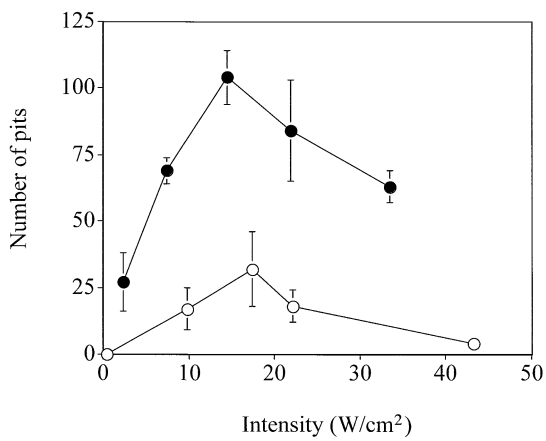

Fig. 6. Dependence of number of pits on ultrasound intensity at two frequencies (●) 20 kHz in the intensity range of 2.39–33.46 W/cm^2, ultrasound was applied for 0.5 s continuously; (○) 40 kHz in the intensity range of 0.40–43.30 W/cm^2, ultrasound was applied for 5 s continuously. Vertical bars indicate the value of S.D. ($n = 6$).

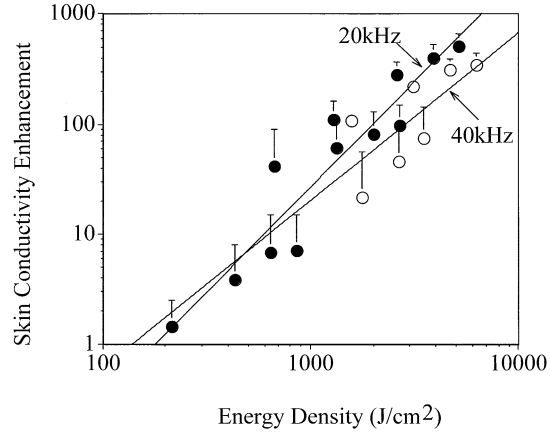

Fig. 7. Dependence of low-frequency sonophoresis on energy density at two frequencies (●) 20 kHz, (○) 40 kHz. Vertical bars indicate the value of S.D. ($n = 3$ or 5).

and about 17 W/cm^2 for 40 kHz. Fig. 6 shows the dependence of the number of pits on ultrasound intensity. The behavior of the number of pits is similar to skin conductivity enhancement, suggesting that the increase in enhancement up to I_{max} is attributed to the higher energy levels delivered to the skin and the decrease in the enhancement beyond the I_{max} is due to acoustic decoupling, a process which decreases the intensity 'seen' by the skin due to the presence of the cavitation cloud.

3.4. Dependence of low frequency sonophoresis on ultrasound energy dose

We have previously reported that the skin conductivity enhancement is directly proportional to the incident ultrasound energy density (E = Intensity × Application Time × Duty Cycle) (Mitragotri et al., 2000). Fig. 7 shows the dependence of sonophoretic skin conductivity enhancement on the total energy density of ultrasound at two frequencies (at a distance of 0.25 cm, at the intensity values $< I_{max}$). The Figure shows that there exists a threshold ultrasound energy density below which the effect of ultrasound on skin conductivity can not be detected and beyond which the conductivity varies linearly with the energy density. The threshold energy density for affecting skin permeability value was approximately 200 J/cm^2 at either frequency. The differ-

ence in the curve at 20 and 40 kHz was not statistically significant ($P > 0.05$).

4. Conclusions

The data presented here offer information for optimizing ultrasound parameters used in sonophoresis. Specifically, the data show that the distance of the horn from the skin affects the efficacy of sonophoresis due to the dependence of ultrasound intensity on the distance. Therefore, the relationship between the enhancement and ultrasound intensity as well as energy density also depends on the distance between the horn and skin. Overall, the dependence of skin conductivity enhancement on ultrasound parameters is similar to that of cavitation (measured by aluminum foil). Further studies should focus on: (i) optimization of ultrasound frequency in low frequency sonophoresis; (ii) in vivo testing of dependence of sonophoresis on ultrasound parameters; and (iii) evaluation of safety of low frequency ultrasound in skin applications.

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References

Bommannan, D., Okuyama, H., Stauffer, P., Guy, R.H., 1992a. Sonophoresis. I. The use of high-frequency ultrasound to enhance transdermal drug delivery. Pharm. Res. 9, 559–564.

Bommannan, D., Menon, G.K., Okuyama, H., Elias, P.M., Guy, R.H., 1992b. Sonophoresis. II. Examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. Pharm. Res. 9, 1043–1047.

Bronaugh, R.L., Maibach, H.I. (Eds.), 1989. Percutaneous Absorption: Mechanism–Methodology–Drug Delivery. Marcel Dekker, New York, pp. 1–12.

Edwards, D.A., Prausnitz, M.R., Langer, R., Weaver, J.C., 1995. Analysis of enhanced transdermal transport by skin electroporation. J. Control. Rel. 34, 211–221.

Frederick, J.R., 1980. Ultrasound Engineering. Wiley, New York.

Gaertner, W., 1954. Frequency dependence of acoustic cavitation. J. Acoust. Soc. Am. 26, 977–980.

Green, P., Flanagan, M., Shroot, B., Guy, R.H, 1993. Iontophoretic drug delivery. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York, pp. 311–333.

Kinsler, L., Frey, A., Coppens, A., Sanders, J., 1982. Fundamentals of Acoustics. Wiley, New York.

Levy, D., Kost, J., Meshulam, Y., Langer, R., 1989. Effect of ultrasound on transdermal delivery to rats and guinea pigs. J. Clin. Invest. 83, 2074–2078.

Liu, J., Lewis, T., Prausnitz, M.R., 1998. Non-Invasive assessment and control of ultrasound-induced membrane permeabilization. Pharm. Res. 15, 918–923.

Mitragotri, S., Blankschtein, D., Langer, R., 1995a. Sonophoresis: ultrasound mediated transdermal drug delivery. In: Swarbrick, J., Boylan, J. (Eds.), Encyclopedia of Pharmaceutical Technology, vol. 14. Marcel Dekker, New York, pp. 103–122.

Mitragotri, S., Edwards, D., Blankschtein, D., Langer, R., 1995b. A mechanistic study of ultrasonically-enhanced transdermal drug delivery. J. Pharm. Sci. 84, 697–706.

Mitragotri, S., Blankschtein, D., Langer, R., 1995c. Ultrasound-mediated transdermal protein delivery. Science 269, 850–853.

Mitragotri, S., Blankschtein, D., Langer, R., 1996. Transdermal drug delivery using low-frequency sonophoresis. Pharm. Res. 13, 411–420.

Mitragotri, S., Farrell, J., Tang, H., Terahara, T., Kost, J., Langer, R., 2000. Determination of threshold energy dose for ultrasound induced transdermal drug transport. J. Control. Rel. 63, 41–52.

Okada, T., Iwai, Y., Hattori, S., Tanimura, N., 1995. Relation between impact load and the damage produced by cavitation bubble collapse. Wear 184 (2), 231–239.

Prausnitz, M.R., 1996. Do high-voltage pulses cause changes in skin structure? J. Control. Rel. 40, 321–326.

Prausnitz, M.R., Bose, V.G., Langer, R., Weaver, J.C., 1993. Electroporation of mammalian skin—a mechanism to enhance transdermal drug delivery. Proc. Natl. Acad. Sci. 90, 10504–10508.

Singh, J., Bhatia, K.S., 1996. Topical Iontophoretic drug delivery: pathways, principles, factors, and skin irritation. Med. Res. Rev. 16, 285–296.

Tachibana, K., 1992. Transdermal delivery of insulin to alloxan-diabetic rabbits by ultrasound exposure. Pharm. Res. 9, 952–954.

Walters, K.A., 1989. Developmental issues and research initiatives. In: Hadgraft, J., Guy, R.H. (Eds.), Transdermal Drug Delivery. Marcel Dekker, New York.