Acoustic Cavitation as an Enhancing Mechanism of Low-Frequency Sonophoresis for Transdermal Drug Delivery

Hideo Ueda,*[,a] Mizue Mutoh,[a] Toshinobu Seki,[a,b] Daisuke Kobayashi,[a] and Yasunori Morimoto[a,b]

[a] *Faculty of Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan: and* [b] *Research Institute of TTS Technology; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan.*

Received December 10, 2008; accepted February 24, 2009; published online February 27, 2009

We investigated the role of acoustic cavitation on sonophoretic skin permeation of calcein, a model permeant, across excised hairless rat skin. Three different frequencies (41, 158, 445 kHz) and various intensities (60 to 300 mW/cm^2) of ultrasound were applied. Cavitation generation in degassed and undegassed (normal) water was monitored using a commercially available cavitation meter, then compared with skin permeability from calcein solution consistent of them. In addition, the penetration of a fluorescent dye, rhodamine B, into gelatin gel as a skin alternative was observed to estimate the role of cavitation collapse in the solution at or near the skin surface. Cavitation generation in the undegassed water was dependent on the ultrasound frequency, and the rank order of the cavitation was 41 kHz>158 kHz>445 kHz. At 41 kHz, cavitation generation in degassed water was clearly lower than that in undegassed water. Calcein permeability during ultrasound application correlated well with the cavitation generation in the medium, suggesting the important role of the indirect actions of cavitation collapse which occurred in the applied solution rather than the direct action in the skin. When ultrasound (41 or 158 kHz) was applied to the gelatin gels covered with rhodamine B solution, alteration in the surface configuration, like spots, and the coincident penetration of the dye were observed only at 41 kHz, while no alteration in the surface configuration was evident at 158 kHz. These results suggest that cavitation collapses in the vicinity of the skin surface might be more important for solute penetration in addition to skin permeabilization.

Key words transdermal delivery; sonophoresis; acoustic cavitation; microjet; convective flow

Sonophoresis is a physical technique to enhance transdermal delivery of various drugs using ultrasound energy.[1,2] A wide range of frequencies (20 kHz—16 MHz) have been used for sonophoresis.[3—5] In the recent studies, low-frequency ultrasound (20—100 kHz) has been shown to be more potent in enhancing transdermal delivery compared with high-frequency ultrasound (more than 2 MHz). We previously reported that low-frequency and low-intensity ultrasound (41 kHz, 120 mW/cm^2) was able to increase the permeation of calcein (hydrophilic model solute, MW 623) and water vehicle across excised hairless rat skin.[6] Our results suggested that potential inductions of alterations of the skin barrier properties by ultrasound application and convection specifically occurring during ultrasound application were responsible for the sonophoretic enhancement. However, it is not clear how ultrasound causes such skin alteration and convection across the skin.

Several physical events accompanied by ultrasound application, including thermal effects, radiation pressure, and acoustic cavitation, can contribute to sonophoretic enhancement.[7] These physical events may affect the permeation properties of the skin directly and indirectly. Bommannan *et al.* have demonstrated that the enhancing effect of sonophoresis induced by high-frequency ultrasound (10 MHz, 16 MHz) may be attributed to the direct actions of ultrasound presumably due to cavitational effects in the stratum corneum.[3,8] In contrast, skin conductivity enhancement induced by low-frequency sonophoresis (20 to 100 kHz) can be related to indirect actions, such as mechanical impact, due to the collapse of cavitation bubbles in solution, resulting in disruption of the structure of lipid bilayers in the stratum corneum.[9,10] In addition, the indirect cavitational effect (collapse of cavitation bubbles in the solution) can generate forced convection in the presence of ultrasound.[11]

Acoustic cavitation can be broadly classified as either stable cavitation which corresponds to steady oscillations of bubbles or transient (inertial) cavitation which corresponds to a rapid growth followed by a rapid collapse.[7] Findings obtained by Tezel *et al.* emphasize the importance of transient cavitation at low-frequencies (20—100 kHz), because the amplitude of broadband noise induced by the transient cavitation correlated well with the enhanced skin conductivity.[12] Cavitation collapses at two different locations in the solution give different patterns. Symmetric (isotropic) collapses of transient cavitation in the bulk solution away from the skin surface result in several physical events, such as localized high pressures, a thermal effect, and emitted shock waves.[7] In contrast, asymmetric (anisotropic) collapses in the solution close to the skin surface induce movement of the liquid microjet to the surface with a velocity of up to 100 m/s.[13,14] Kodama and Tomita verified liquid microjet formation in 10—30% hard gelatin gels using high-speed photography.[15] Therefore, indirect actions, such as asymmetric cavitation collapses in solution, are likely to be important for the permeation enhancement during ultrasound application observed in our previous study.

The purpose of the present study was to investigate and identify the causes of convective flow and skin alterations induced by low-frequency ultrasound. Generation of transient cavitation in solution at various frequencies (41—445 kHz) was monitored using a commercially available cavitation meter to estimate the relationship between cavitation generation and skin transport enhancement of a model hydrophilic molecule under the influence of ultrasound irradiation. Calcein was chosen as a hydrophilic molecule for identification of mechanistic aspect of sonophoresis in the present study because this compound was used as a standard molecule in our previous study.[6,16] Furthermore, the potential generation

* To whom correspondence should be addressed. e-mail: hideo@josai.ac.jp

of a liquid microjet induced by asymmetric collapses of transient cavitation in the solution was evaluated using a hard gelatin gel as a skin alternative.

MATERIALS AND METHODS

Materials Calcein was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), and gelatin powder (mixture of MW 15000—250000) was obtained from Kanto Chemical Co., Inc. (Tokyo). Rhodamine B was obtained from Acros Organics (Geel, Belgium). All other chemicals and solvents were of analytical grade and used without further purification.

Animals Male WBN/ILS-Ht strain hairless rats were obtained from the Life Science Research Center (Josai University, Saitama, Japan). The hairless rats were sacrificed according to the guidelines for animal use approved by the Life Science Research Center, Josai University.

Ultrasound Equipment Three custom-built ultrasound transducers (Dai-ichi High Frequency Co., Ltd., Tokyo, Japan) were used in the sonophoresis study. A function synthesizer (WF1943, NF Corporation, Kanagawa, Japan) along with an amplifier (HSA4012, NF Corporation) was used to drive the transducers. The optimal operating frequencies for the each transducer were 41, 158, and 445 kHz, respectively.[6)]

Measurement of Ultrasound Intensity Ultrasound intensity was calibrated by commonly used calorimetric methods.[9)] A glass beaker thermally insulated with expanded polystyrene was filled with 40 ml of undegassed water. The ultrasound transducer was immersed in the beaker and activated for 15 min. The rate of increase in water temperature was measured to calculate the ultrasound intensity from the following equation:

$$I = \frac{M_{\text{water}} C_{\text{p.water}}}{A} \frac{\Delta T}{\Delta t} \quad (1)$$

where I is the ultrasound intensity (W/cm^2), M_{water} is the mass of water exposed (g), $C_{\text{p.water}}$ is the specific heat of water (4.18 J/g·deg), A is the effective area of the transducer, ΔT is the temperature change of water (deg.), and Δt is the ultrasound application period.

Measurement of Cavitation Generation Generation of transient cavitation in water was measured using a commercially available cavitation meter (KS-8201, Arock Industrial Co., Ltd., Tokyo, Japan) which detects specific cavitation noise. The cavitation generation was represented as the relative cavitation value based on calibration of the cavitation meter. The cavitation generation was measured in degassed and undegassed (normal) water. The degassed water was prepared by vacuum degassing using a vacuum aspirator (FTP-34A, Iwaki Glass Co., Ltd., Tokyo) and immersing a water-containing glass bottle in a sonication bath (Branson 5510, Branson Ultrasonics Co., Danbury, CT, U.S.A.) for 60 min. A plastic tube (30 mm diameter) preventing water leakage with expanded polystyrene was filled with either degassed or undegassed water, and the probes of the cavitation meter and the ultrasound transducer were placed in the water facing each other. The relative cavitation values were measured during ultrasound irradiation for 5 min at intensities ranging from 60—300 mW/cm^2. The measurements at the end of 30 s of the 5 min sonication period were used as cavitation data. The measurements were repeated 3 times.

***In Vitro* Sonophoresis Study** Hairless rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and the full thickness skin was excised from the abdomen. The skin was mounted in a glass vertical diffusion chamber (effective diffusion area: 7.065 cm^2) with a water jacket connected to a water bath at 32 °C. The donor compartment (stratum corneum side, 10 ml) was filled with 1 mM calcein dissolved in phosphate buffered saline (PBS, pH 7.4). The receiver compartment (22 ml) was filled with PBS and stirred with a star head magnetic bar driven by a constant speed motor (MC-301, Scinics, Tokyo) at 1200 rpm. The diffusion chamber was left for 12 h to achieve a pseudo-steady-state for solute transport, then the transport experiment was initiated by replacing the liquid in the donor compartment with either degassed or undegassed calcein solution. For ultrasound application, the transducer was positioned 3 mm above the skin surface in the donor compartment. At 3 h after initiating the transport experiment, the chosen frequency of ultrasound was applied to the donor compartment for 30 min. The applied intensities were 60 to 300 mW/cm^2. An appropriate sample volume was withdrawn from the receiver compartment during sonophoresis at predetermined intervals for determination of calcein transport. The volume withdrawn was immediately replaced with an equal volume of fresh PBS.

Calcein Assay A 50 μl sample was diluted 100-fold with potassium dihydrogenphosphate-sodium borate buffer (pH 8.5). The fluorescence intensity of calcein was determined by spectrofluorometer (RF-5000, Shimadzu Corporation, Kyoto, Japan) at an excitation wavelength of 488 nm and an emission wavelength of 515 nm.

Observation of Cavitation Events at Gelatin Surfaces The receiver compartment of the glass vertical diffusion chamber was filled with 30% gelatin gel (20 mm thickness), which had been hardened in the refrigerator for 12 h. The donor compartment (10 ml) was filled with 1 mM rhodamine B dissolved in a 4 : 6 solution of methanol-PBS. Ultrasound was applied for 1 min at an intensity of 120 mW/cm^2. This study was conducted at frequencies of 41 and 158 kHz. Following the ultrasound treatment, the gelatin gel was removed from the diffusion chamber and sliced vertically for examination. The slices obtained were macroscopically monitored by a digital camera (DSC-F55V, Sony Corporation, Tokyo), then examined under a confocal laser microscope. A MRC-600 Lasersharp System (Bio-Rad Laboratories, Richmond, CA, U.S.A.), linked to a Zeiss Axioplan equipped with a Zeiss Neofluar (Carl Zeiss, Oberkochen, Germany), which was set for confocal imaging. A krypton laser was used for fluorescence monitoring.

RESULTS

Generation of Cavitation Bubbles in Bulk Solution under Different Conditions Figure 1a shows the relative cavitation generation in the bulk water at different frequencies (41, 158, 445 kHz). Figure 1b compares the cavitation generation at 41 kHz ultrasound between degassed and undegassed water. At 60 mW/cm^2, the rank order of relative cavitation was 41 kHz>158 kHz>445 kHz. At 41 kHz, when the

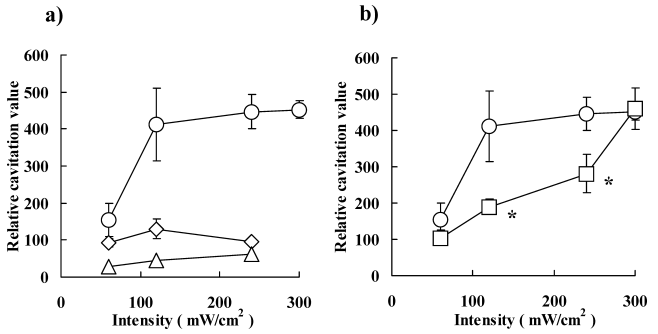

Fig. 1. Relative Cavitation Values Observed in Undegassed Water at Different Frequencies (a) and in Degassed and Undegassed Water at 41 kHz (b)

The applied intensity was 60, 120, 240 and 300 mW/cm^2. Key: ○, 41 kHz (undegassed water); □, 41 kHz (degassed water); ◇, 158 kHz; △, 445 kHz. Each data point represents the mean±S.D. of 8 experiments. * Significantly different from undegassed water ($p<0.05$).

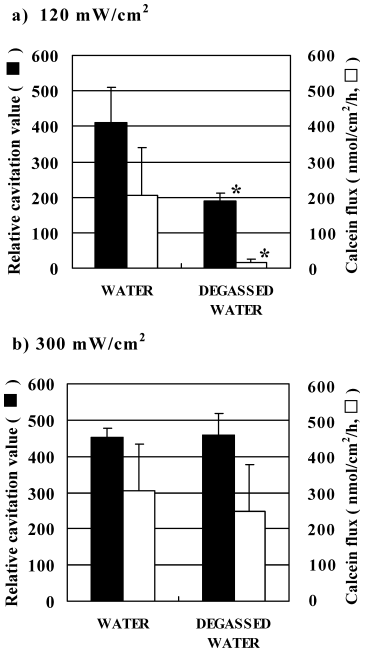

Fig. 2. Comparison of Relative Cavitation Values and Calcein Flux from Degassed and Undegassed Solutions at 41 kHz Ultrasound

(a) 120 mW/cm^2 and (b) 300 mW/cm^2. Each data point represents the mean±S.D. of 3 to 8 experiments. * Significantly different from undegassed water ($p<0.05$).

applied intensity was changed from 60 to 120 mW/cm^2, the cavitation generation increased significantly from a relative cavitation value of 154±45 to 411±98, but a further increase in intensity up to 300 mW/cm^2 did not increase the cavitation generation. At frequencies of 158 and 445 kHz, the relative cavitation values were clearly lower than those obtained from 41 kHz at all intensities, and did not change when the applied intensity was increased up to 240 mW/cm^2 (Fig. 1a). When the relative cavitation values at 41 kHz were compared between normal and degassed water, the values in degassed water were significantly lower at 120 and 240 mW/cm^2, even although they were not significant at 60 and 300 mW/cm^2 (Fig. 1b).

Effect of Cavitation on the Transdermal Transport of Calcein Figures 2a and b compare the relative cavitation values with the calcein flux from degassed and undegassed PBS solution in the presence of 41 kHz ultrasound. In separate experiments, cavitation generation in the PBS solution containing calcein was comparable with that in water (data not shown). At 120 mW/cm^2 (Fig. 2a), the calcein flux from degassed vehicle was significantly reduced to 16±9 nmol/cm^2/h from 206±135 nmol/cm^2/h in normal vehicle which is consistent with the significant reduction in the relative cavitation value (Fig. 2a). However, at 300 mW/cm^2, the calcein flux from degassed vehicle was not altered significantly, and the relative cavitation values in normal and degassed vehicles were comparable (Fig. 2b).

Table 1. Relative Cavitation Generation Values in Bulk Solution and Calcein Permeability across Hairless Rat Skin during Ultrasound Irradiation

	Relative cavitation value	Calcein permeability (cm/s)
41 kHz		
Undegassed		
60 mW/cm^2	154±45	$5.44\times10^{-8}\pm2.08\times10^{-8}$
120 mW/cm^2	411±98	$5.72\times10^{-5}\pm2.86\times10^{-5}$
300 mW/cm^2	453±24	$6.72\pm10^{-5}\pm3.16\times10^{-5}$
Degassed		
120 mW/cm^2	190±21	$4.46\times10^{-6}\pm0.96\times10^{-6}$
300 mW/cm^2	460±59	$6.91\times10^{-5}\pm1.51\times10^{-5}$
158 kHz (undegassed)		
60 mW/cm^2	93±12	$1.54\times10^{-8}\pm0.72\times10^{-8}$ *a)*
120 mW/cm^2	130±26	$3.82\times10^{-8}\pm0.76\times10^{-8}$ *a)*
240 mW/cm^2	96±5	$2.13\times10^{-8}\pm2.88\times10^{-8}$ *a)*
445 kHz (undegassed)		
60 mW/cm^2	28±14	$9.30\times10^{-9}\pm4.68\times10^{-9}$ *a)*
120 mW/cm^2	45±5	$1.12\times10^{-8}\pm0.64\times10^{-8}$ *a)*
240 mW/cm^2	62±6	$6.79\times10^{-9}\pm4.54\times10^{-9}$ *a)*

Mean±S.D. (n=3—8). *a*) The data are taken from ref. 6.

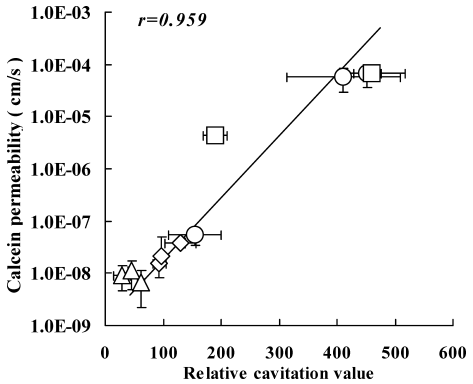

Fig. 3. Relationship between the Relative Cavitation Value and the Calcein Permeability across Hairless Rat Skin

Key: ○, 41 kHz (undegassed water); □, 41 kHz (degassed water); ◇, 158 kHz; △, 445 kHz. Each data point represents the mean±S.D. of 3—8 experiments.

Table 1 summarizes the relative cavitation values and permeability data under different experimental conditions. Figure 3 shows a relationship between the relative cavitation value and calcein permeability during ultrasound irradiation based on the data in Table 1. Calcein permeability in the presence of ultrasound correlated well with the relative cavitaion value (r=0.959, $p<0.05$).

Corroboration of Cavitation Generation in Gelatin Gel as a Skin Alternative Figures 4a and b show cross-sectional images of gelatin gel obtained by digital camera after ultrasound application for 1 min at 41 kHz and 158 kHz with an intensity of 120 mW/cm^2. When 41 kHz ultrasound was

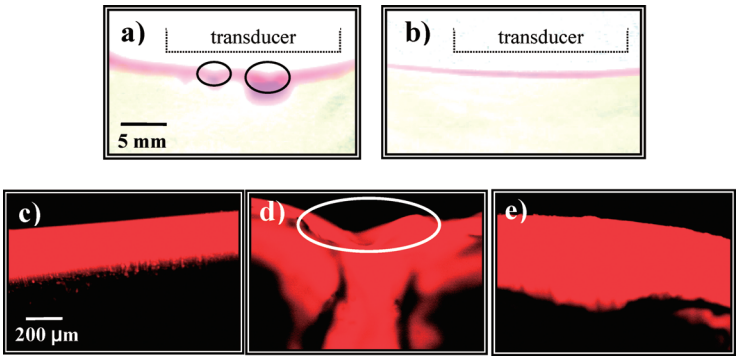

Fig. 4. Cross-Sectional Images of Gelatin Gel Obtained by Digital Camera (a, b) and Confocal Laser Scanning Microscopy (c—e)

The gelatin gel covered with rhodamine B solution was subjected to sonication at 41 kHz (a, d) or 158 kHz ultrasound (b, e) at 120 mW/cm^2 for 1 min. Panel c was a negative control without ultrasound application. Dashed lines in panel a and b indicate the position and size of the transducer (18 mm). Ellipsoidal circles show spots generated by ultrasound application. All confocal images were obtained at a magnification of ×100.

applied, localized penetration of rhodamine B staining was observed under the ultrasound transducer area (18 mm in diameter, Fig. 4a). In contrast, localized penetration of the staining was not observed at 158 kHz. Figures 4c, d, and e are confocal images of cross-sections of rhodamine B-stained gelatin gels. After application of 41 kHz ultrasound, spots were observed on the gelatin gel (ellipsoidal circles in Fig. 4d). The localized penetration of the staining was coincident with the spots generated by the 41 kHz ultrasound. On the other hand, the gelatin surface after application of 158 kHz ultarasound (Fig. 4e) was similar to that without ultrasound (Fig. 4c), although penetration depth seemed to be deeper than that without ultrasound.

DISCUSSION

We previously reported that both structural alteration of the stratum corneum and convective solvent flow were responsible for the sonophoretic enhancement induced by 41 kHz ultrasound in excised hairless rat skin.[6] In the present study, we demonstrated the potential contribution of acoustic cavitation on the sonophoretic enhancement seen in previous observations.

The capacity to generate cavitation bubbles in bulk water was examined using various frequencies and intensities as well as in degassed and undegassed water. The increase in the relative cavitation values in undegassed water was dependent on the ultrasound frequency in the range examined (41—445 kHz). The rank order of the relative cavitation values (41 kHz>158 kHz>445 kHz) indicates that the cavitation bubbles are more easily generated at the lower frequency used in this study (Fig. 1a). This finding is consistent with the general finding that the cavitation activity varies inversely with ultrasound frequency.[17] In case of 41 kHz, the relative cavitation values in the undegassed water were saturated over 120 mW/cm^2, while the values in the degassed water increased depending on the applied intensity up to 300 mW/cm^2 (Fig. 1b). This saturated phenomenon is due to the potential presence of a bubble cloud in the medium near the ultrasonic horn. It has been reported that the ultrasound acoustic amplitude of 29 kHz in the medium from a horn tip with a diameter of 10 mm and a flat surface is lowered due to the reduction in the acoustic radiation resistance by the presence of a bubble cloud.[18] Because cavitation bubbles are much more easily produced in undegassed water compared with degassed water, abundant generation of cavitation bubbles in the undegassed water may prevent the increase in the ultrasonic amplitude and the accompanying bubble generation when ultrasonic power input is increased.

The cavitation generation measured in the present study seems to be responsible for the enhanced sonophoretic permeation. The calcein flux from the degassed solution in which cavitation generation was produced was reduced significantly compared with that from the undegassed solution when 41 kHz ultrasound was applied at 120 mW/cm^2 (Fig. 2a). In contrast, no significant difference in calcein flux was observed between degassed and undegassed solutions at 300 mW/cm^2 (Fig. 2b). In addition, a good correlation between calcein permeability in the presence of ultrasound and the relative cavitaton values based on the data in Table 1 suggests that the indirect action of cavitation bubbles generated in the solution plays an important role in the enhancing effect induced by low-frequency and -intensity ultrasound (Fig. 3). The particular importance of the indirect action of cavitation on the skin permeability enhancement is consistent with the findings observed in other reports in the literature.[9,10]

There are at least three modes of cavitation bubble–stratum corneum interactions including shock wave emission, impact of the liquid microjet on the stratum corneum, and liquid microjet penetration into the stratum corneum.[19] Regardless of the relative role of these interactions, Tezel and Mitragotori have shown that collapses of cavitation bubbles close to the skin surface are important for sonophoretic permeation enhancement.[19] Tang *et al.* have also demonstrated that asymmetric collapses of transient cavitation bubbles occurring in the vicinity of the skin surface are more important for inducing skin permeabilization compared with symmetric collapses occurring in the solution away from the skin.[10] In the previous study, we observed penetration of 40 kDa of fluorescein isothiocyanate (FITC)-labeled dextran and rhodamine B into the hairless rat skin after application of 41 kHz ultrasound using the confocal microscopy.[16] However, potential contribution of cavitational events to the penetration of those compounds into the skin was not clear in this study. On the other hand, Kodama and Tomita used gelatin gel as an alternative of biological tissues to investigate generation of liquid jet induced by cavitation bubbles at surface.[15] In the present study, the cavitational events were investigated

using a gelatin gel as a skin alternative.

As shown in Figs. 4a and d, several spots (alterations in surface configuration) were observed on the gelatin surface after application of 41 kHz ultrasound at 120 mW/cm^2 for 1 min. In contrast, no change in the surface configuration of the gelatin was evident after application of 158 kHz ultrasound (Fig. 4e). Because cavitation generation at 120 mW/cm^2 at 41 kHz was clearly higher than that at 158 kHz (Fig. 1a), the impact of liquid microjet caused by asymmetric cavitation collapses in the vicinity of the gelatin gel may be involved in the induction of alterations in surface configuration. The observed spots in gelatin gel surface may be responsible for skin damages induced by ultrasound. Kodama and Tomita described that cavitation bubble-shock wave interaction had a potential to cause biological tissue damage based on their observation in gelatin gel study.[15] Although the mechanical properties, such as mechanical strength, elasticity, and hardness, differed between the gelatin gel and the skin, the cavitational events may be related to change in skin barrier properties indicated by the reduction in skin electrical resistance obtained in our previous study.[6] Thus, cavitation generation can be an important factor in the safety point of view, as well as enhancing mechanism.

On the other hand, another important finding from Figs. 4a and d is the coincidence of the spot at the surface and the localized penetration of rhodamine B inside the gelatin gel. If the asymmetric collapses of cavitation bubbles occur in the vicinity of the gelatin surface, potential penetration of the liquid microjet into the gelatin gel can occur as well as the impact of the microjet.[19,20] The penetration of the microjet can be induced only in the presence of ultrasound energy. Thus, such a cavitational interaction may be involved in the induction of convective flow across the skin observed during ultrasound application in our previous study.[6,16] In contrast, overall penetration of rhodamine B after application of 158 kHz ultrasound (Fig. 4e) seemed to be slightly deeper compared to that in control (Fig. 4c). Other ultrasonic phenomena, such as oscillation of the molecules and induction of shearing stress, may be dominant in penetration enhancement in case of 158 kHz.[21]

In conclusion, the indirect action of the cavitational events in solution plays an important role in enhancement of sonophoretic solute permeation. In particular, cavitation collapses in the solution in the vicinity of the skin surface seem to be more important for induction of skin permeabilization and convective solvent flow. Further detailed studies are required to establish suitable sonophoresis conditions balancing the effectiveness and safety for clinical application.

REFERENCES

1) "Transdermal Drug Delivery: Second Edition, Revised and Expanded," ed. by Guy R. H., Hadgraft J., Marcel Dekker, New York, 2003.
2) Mitragotri S., Kost J., *Adv. Drug Deliv. Rev.*, **56**, 589—601 (2004).
3) Bommannan D., Okuyama H., Stauffer P., Guy R. H., *Pharm. Res.*, **9**, 559—564 (1992).
4) Mitragotri S., Blankschtein D., Langer R., *Science*, **269**, 850—853 (1995).
5) Tezel A., Sens A., Tuchscherer J., Mitragotri S., *Pharm. Res.*, **18**, 1694—1700 (2001).
6) Mutoh M., Ueda H., Nakamura Y., Hirayama K., Atobe M., Kobayashi D., Morimoto Y., *J. Controlled Release*, **92**, 137—146 (2003).
7) "Ultrasound: Its Chemical, Physical, and Biological Effects," ed. by Suslick K. S., VCH Publishers, New York, 1988.
8) Bommannan D., Menon G. K., Okuyama H., Elias P. M. Guy R. H., *Pharm. Res.*, **9**, 1043—1047 (1992).
9) Mitragotri S., Farrell J., Tang H., Terahara T., Kost J., Langer R., *J. Controlled Release*, **63**, 41—52 (2000).
10) Tang H., Wang C. C. J., Blankschtein D., Langer R., *Pharm. Res.*, **19**, 1160—1169 (2002).
11) Tang H., Mitragotri S., Blankschtein D., Langer R., *J. Pharm. Sci.*, **90**, 543—566 (2001).
12) Tezel A., Sens A., Mitragotri S., *J. Pharm. Sci.*, **91**, 444—453 (2002).
13) Birkin P. R., Silva-Martinez S., *J. Electroanal. Chem.*, **416**, 127—138 (1996).
14) "Sonochemistry: Theory, Applications and Uses of Ultrasound in Chemistry," ed. by Mason T. J., Lorimer J. P., Ellis Horwood, Chichester, 2004.
15) Kodama T., Tomita Y., *Appl. Phys. B*, **70**, 139—149 (2000).
16) Morimoto Y., Mutoh M., Ueda H., Fang L., Hirayama K., Atobe M., Kobayashi D., *J. Controlled Release*, **103**, 587—597 (2005).
17) Lavon I., Kost J., *Drug Discov. Today*, **9**, 670—676 (2004).
18) Yasui K., Iida Y., Tuziuti T., Kozuka T., Towata A., *Phys. Rev. E*, **77**, 016609 (2008).
19) Tezel A., Mitragotri S., *Biophys. J.*, **85**, 3502—3512 (2003).
20) Lauterborn W., Hentschel W., *Ultrasonics*, **60**, 260—269 (1985).
21) Camel E., "Percutaneous Penetration Enhances," ed. by Smith E. W., Maibach H. I., CRC Press, Boca Raton, 1995, pp. 369—382.