mitting easy determination of gene transfection and tissue damage.

To establish the optimal condition of US MB-mediated gene transfer to cornea, 98 different patterns of various US conditions were examined in vitro in preliminary studies because it is difficult in practice to study so many different patterns using rabbit eyes in vivo. First, the duty cycle of US was evaluated. Results clearly showed that a duty cycle of 100% is most effective to transfer genes. However, cell damage was too strong with a duty cycle of 100%. Therefore, a duty cycle of 50% was chosen for our purpose. Second, the amount of MBs perflutren protein (Optison; Amersham Health) was evaluated. Cytotoxicity was highest in 100% MBs, and gene transfer efficiency was highest in 20% MBs. Thus, 20% MBs was chosen. Third, the exposure time of US was evaluated. An exposure time longer than 120 seconds significantly damaged the cells, and an exposure time shorter than 60 seconds could not transfer the gene efficiently. Sixty- and 120-second exposure provided almost identical gene transfer efficiency and cell toxicity. Finally, US power was studied. It is understandable that high US power can transfer the gene to cells but also induce strong cell damage. Considering gene transfer efficiency and cellular damage, a US power of 1 or 2 $W/cm^2$ should be appropriate.

Contrary to in vitro experiments, US alone revealed no significant enhancement of gene transfer compared with controls. Because the cornea is composed of multiple cell layers and abundant extracellular matrix, it is postulated that higher US intensities are needed to produce sufficient microjets to damage cells or inject genes into cells. Adding MB with the plasmids, on the other hand, increased gene transfer efficiency by twofold to threefold. Optic examination showed that GFP was present mainly in keratocytes at the US-targeted regions of the corneal stroma. GFP was not detected in the untreated area of the cornea or other intraocular tissues. It is noteworthy that US induced no immediate corneal damage, such as opacity or defect of corneal/ciliary epithelial cells. Surrounding trabecular meshwork, cells lining Schlemm canal, lens epithelial cells, and retina seemed to be intact.

Previously, Wang et al.[22] reported perflutren protein (Optison; Amersham Health) with DNA could achieve effective gene transfer in muscle. However, effective gene transfer could not be performed in cornea in the present study. Because the cornea has more abundant extracellular materials than muscle, perflutren protein (Optison; Amersham Health) alone might not be effective for transferring DNA to cells.

Viral vector- or liposome-mediated gene transfer methods are effective for transferring genes to almost every cell that comes in contact with vectors[33,34] (e.g., the adenoviral vector). Because the long-term effects of gene transfer on recipient cells remain unclear, the cells to which genes are transferred should be strictly controlled. Especially when transferring genes to the cornea, the pupillary area should be avoided so as not to threaten vision. The present method greatly reduces this concern because of the precise targeting it makes possible.

In previous studies we reported that electroporation-mediated gene transfer can be achieved in rat cornea.[35-37] However, in larger animals, including rabbit, electroporation may be hazardous because the cardiovascular system is sometimes impaired by treatment (data not shown). Therefore, at present it might not be easy to apply electroporation for human gene therapy.

When the vehicle with the gene of interest is injected into tissue through a local gene delivery method such as liposome or viral vector injection, the vehicle spreads in every direction three dimensionally. Accordingly, gene transfer is achieved three dimensionally in a similar way. However, in the treatment of a surface organ such as skin or cornea, two-dimensional gene transfer is sometimes preferable. Using the characteristics of ultrasound, the present method achieved two-dimensional gene transfer.

Thirty percent to 40% of the total corneal area was covered by a 12-$\mu$L plasmid injection. Gene transfer was achieved in the area exposed to US. To expand the area of gene transfer, it was necessary to improve the injection method and the US probe. The present method can be applied to a variety of purposes, such as making it feasible to use highly fragile proteins.[38,39] With the present method, we were able to superficially deliver genes to a targeted tissue surface area two dimensionally. Thus, this sonoporation method could become a valuable modality for therapy and research that require surface-localized drug delivery or gene induction.[40]

Many questions remain before the present method can be applied clinically. The optimal US condition required for efficient gene transfer is highly dependent on the tissue, and the biologic structures responsible for the US effect vary greatly among species. These issues must be carefully explored. Of necessity, there is another limitation to the present study. We examined a rabbit corneal epithelial cell line in an the vitro study, but the gene-transferred cells were mainly keratocytes in the in vivo study. In our preliminary study, rabbit keratocytes were cultured; however, the morphology of these cells soon changed and differentiated into unidentifiable cells. Therefore, we used the rabbit corneal cell line RC-1.

To summarize, our studies show that using US in conjunction with commercially available MBs can enhance gene delivery to cells without damaging tissues. Although this modality was highly dependent on acoustic conditions and bubble concentration, its simplicity and noninvasiveness may provide a new avenue for microinjecting various substances into a wide range of living tissues.

## References


1. Marshall E. Gene therapy's growing pains. *Science.* 1995;269:1050, 1052-1055.
2. Felgner PL, Barenholz Y, Behr JP, et al. Nomenclature for synthetic gene delivery systems. *Hum Gene Ther.* 1997;8:511-512.
3. Marshall E. Gene therapy death prompts review of adenovirus vector. *Science.* 1999;286:2244-2245.
4. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med.* 2002;346:1185-1193.
5. Grisham J. Inquiry into gene therapy widens. *Nat Biotechnol.* 2000;18:254-255.
6. Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle in vivo. *Science.* 1990;247:1465-1468.
7. Wolff JA, Ludtke JJ, Acsadi G, Williams P, Jani A. Long-term persistence of plasmid DNA and foreign gene expression in mouse muscle. *Hum Mol Genet.* 1992;1:363-369.
8. Felgner PL, Gadek TR, Holm M, et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA.* 1987;84:7413-7417.
9. Stechschulte SU, Joussen AM, von Recum HA, et al. Rapid ocular angiogenic control via naked DNA delivery to cornea. *Invest Ophthalmol Vis Sci.* 2001;42:1975-1979.
10. Nishi T, Yoshizato K, Yamashiro S, et al. High-efficiency in vivo gene transfer using intraarterial plasmid DNA injection following in vivo electroporation. *Cancer Res.* 1996;56:1050-1055.
11. Drabick JJ, Glasspool-Malone J, King A, Malone RW. Cutaneous transfection and immune responses to intradermal nucleic acid vaccination are significantly enhanced by in vivo electropermeabilization. *Mol Ther.* 2001;3:249-255.
12. Newman CM, Lawrie A, Brisken AF, Cumberland DC. Ultrasound gene therapy: on the road from concept to reality. *Echocardiography.* 2001;18:339-347.

13. Kim HJ, Greenleaf JF, Kinnick RR, Bronk JT, Bolander ME. Ultrasound-mediated transfection of mammalian cells. *Hum Gene Ther.* 1996;7:1339-1346.
14. Bao S, Thrall BD, Miller DL. Transfection of a reporter plasmid into cultured cells by sonoporation in vitro. *Ultrasound Med Biol.* 1997;23:953-959.
15. Wyber JA, Andrews J, D'Emanuele A. The use of sonication for the efficient delivery of plasmid DNA into cells. *Pharm Res.* 1997;14: 750-756.
16. Miller DL, Williams AR, Morris JE, Chrisler WB. Sonoporation of erythrocytes by lithotripter shockwaves in vitro. *Ultrasonics.* 1998;36:947-952.
17. Lawrie A, Brisken AF, Francis SE, et al. Ultrasound enhances reporter gene expression after transfection of vascular cells in vitro. *Circulation.* 1999;99:2617-2620.
18. Manome Y, Nakamura M, Ohno T, Furuhata H. Ultrasound facilitates transduction of naked plasmid DNA into colon carcinoma cells in vitro and in vivo. *Hum Gene Ther.* 2000;11:1521-1528.
19. McDonnell PJ. Excimer laser corneal surgery: new strategies and old enemies. *Invest Ophthalmol Vis Sci.* 1995;36:4-8.
20. Seitz B, Baktanian E, Gordon EM, Anderson WF, LaBree L, McDonnell PJ. Retroviral vector-mediated gene transfer into keratocytes: in vitro effects of polybrene and protamine sulfate. *Graefes Arch Clin Exp Ophthalmol.* 1998;236:602-612.
21. Bennett J, Maguire AM. Gene therapy for ocular disease. *Mol Ther.* 2000;1:501-505.
22. Wang X, Liang HD, Dong B, Lu QL, Blomley MJ. Gene transfer with microbubble ultrasound and plasmid DNA into skeletal muscle of mice: comparison between commercially available microbubble contrast agents. *Radiology.* 2005;237:224-229.
23. Greenleaf WJ, Bolander ME, Sarkar G, Goldring MB, Greenleaf JF. Artificial cavitation nuclei significantly enhance acoustically induced cell transfection. *Ultrasound Med Biol.* 1998;24:587-595.
24. Lu QL, Bou-Gharios G, Partridge TA. Non-viral gene delivery in skeletal muscle: a protein factory. *Gene Ther.* 2003;10:131-142.
25. Blomley M. Which US microbubble contrast agent is best for gene therapy? *Radiology.* 2003;229:297-298.
26. Lawrie A, Brisken AF, Francis SE, Cumberland DC, Crossman DC, Newman CM. Microbubble-enhanced ultrasound for vascular gene delivery. *Gene Ther.* 2000;7:2023-2027.
27. Shohet RV, Chen S, Zhou YT, et al. Echocardiographic destruction of albumin microbubbles directs gene delivery to the myocardium. *Circulation.* 2000;101:2554-2556.
28. Unger EC, Hersh E, Vannan M, McCreery T. Gene delivery using ultrasound contrast agents. *Echocardiography.* 2001;18:355-361.
29. Taniyama Y, Tachibana K, Hiraoka K, et al. Local delivery of plasmid DNA into rat carotid artery using ultrasound. *Circulation.* 2002;105:1233-1239.
30. Taniyama Y, Tachibana K, Hiraoka K, et al. Development of safe and efficient novel nonviral gene transfer using ultrasound: enhancement of transfection efficiency of naked plasmid DNA in skeletal muscle. *Gene Ther.* 2002;9:372-380.
31. Lu QL, Liang HD, Partridge T, Blomley MJ. Microbubble ultrasound improves the efficiency of gene transduction in skeletal muscle in vivo with reduced tissue damage. *Gene Ther.* 2003;10:396-405.
32. Nakashima M, Tachibana K, Iohara K, Ito M, Ishikawa M, Akamine A. Induction of reparative dentin formation by ultrasound-mediated gene delivery of growth/differentiation factor 11. *Hum Gene Ther.* 2003;14:591-597.
33. Vincent MC, Trapnell BC, Baughman RP, Wert SE, Whitsett JA, Iwamoto HS. Adenovirus-mediated gene transfer to the respiratory tract of fetal sheep in utero. *Hum Gene Ther.* 1995;6:1019-1028.
34. Porada CD, Tran N, Eglitis M, et al. In utero gene therapy: transfer and long-term expression of the bacterial neo(r) gene in sheep after direct injection of retroviral vectors into preimmune fetuses. *Hum Gene Ther.* 1998;9:1571-1585.
35. Oshima Y, Sakamoto T, Hisatomi T, et al. Targeted gene transfer to corneal stroma in vivo by electric pulses. *Exp Eye Res.* 2002;74: 191-198.
36. Oshima Y, Sakamoto T, Hisatomi T, Tsutsumi C, Ueno H, Ishibashi T. Gene transfer of soluble TGF-beta type II receptor inhibits experimental proliferative vitreoretinopathy. *Gene Ther.* 2002;9: 1214-1220.
37. Sakamoto T, Oshima Y, Nakagawa K, Ishibashi T, Inomata H, Sueishi K. Target gene transfer of tissue plasminogen activator to cornea by electric pulse inhibits intracameral fibrin formation and corneal cloudiness. *Hum Gene Ther.* 1999;10:2551-2557.
38. Tachibana K, Tachibana S. Transdermal delivery of insulin by ultrasonic vibration. *J Pharm Pharmacol.* 1991;43:270-271.
39. Tachibana K, Uchida T, Ogawa K, Yamashita N, Tamura K. Induction of cell-membrane porosity by ultrasound (Letter). *Lancet.* 1999;353:1409.
40. Tachibana K, Tachibana S. The use of ultrasound for drug delivery. *Echocardiography.* 2001;18:323-328.

*Cellular and Molecular Biology*™ **51**, Online, OL767-OL784
DOI 10.1170/91

ISSN 1165-158X
2005 Cell. Mol. Biol.™

*Review*

# TRANSDERMAL DRUG DELIVERY USING ULTRASOUND – THEORY, UNDERSTANDING AND CRITICAL ANALYSIS

M. SIVAKUMAR[1✍], K. TACHIBANA[2], A.B. PANDIT[3], K. YASUI[1], T. TUZIUTI[1], A. TOWATA[1] and Y. IIDA[1]

[1✍] Ultrasonic Processing Group, National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Shimoshidami, Moriyama-ku, Nagoya 463-8560, Japan
*Fax: +81 52 736 7400; E-mail: manickam-sivakumar@aist.go.jp; siva_chem@yahoo.com*
[2] Department of Anatomy, School of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan
[3] Chemical Engineering Division, University Institute of Chemical Technology (UICT), Matunga, Mumbai, 400 019, India

*Received January 31, 2005; Accepted May 24, 2005; Published September 2, 2005*

**Abstract** - This review focuses on a unique transdermal drug delivery enhanced by the action of ultrasound, referred as sonophoresis. Sonophoresis is an active form of transdermal delivery which enhances the transport of permeants, such as drugs through cell membranes as a result of ultrasonic energy. Ultrasonic sound waves cause acoustic cavitation, the resultant effects of which microscopically disrupt the lipid bilayers of the stratum corneum and thereby influencing the influx of permeants. Sonophoresis increases the penetration of various low molecular weight drugs as well as high molecular weight proteins. The objective of this review is to account the role of ultrasound parameters and the associated cavitational effects, gained through a number of investigations, in order to facilitate the understanding of this method.

**Key words**: Acoustic cavitation, transdermal, drug delivery, ultrasound, sonophoresis, skin penetration, sonochemical

## INTRODUCTION

Drug delivery techniques were established to deliver or control the amount, rate and sometimes targeting of the drugs to specific body organs in order to optimize its therapeutic effect, patient convenience and dose. Intravenous injection and oral dosing, the two modes of drug delivery in popular use today, have many shortcomings. The recent development of biotechnology drugs based on macromolecules, particularly polypeptide drugs such as interferons and erythropoietin, are a key factor in the growth of the drug-delivery market. Approximately 40 macromolecule drugs are currently being marketed, generating estimated worldwide sales of $20 billion. It is estimated that 400 additional macromolecule drugs are in clinical development. However, at this stage evidence supporting the clinical application of many of these drugs is limited. One reason for this is perhaps because oral administration of these drugs becomes impossible as these drugs break down quickly and are poorly absorbed into the bloodstream. Whereas, intravenous administration of these drugs with chronic application becomes inconvenient, time-consuming and known to result in poor patient-compliance (25). Therefore, the successfulness of new therapeutic biological drugs will depend on a novel technology to enable them to reach their target within the body. An appealing alternative to intravenous and oral drug delivery is "transdermal" or "through the skin" drug delivery, a powerful and a painless tool which avoids the problems inherent with the intravenous and oral delivery methods, with the added benefits of possible sustained controlled release and analyte extraction, for example extracting glucose and other constituents of interstitial fluid across permeabilised skin (21,86,87). These advantages of transdermal systems make them very promising for drug delivery.

**Abbreviations**: **atm**: atmosphere; **Hz**: hertz; **J**: joule; **J/cm$^2$**: joules per square centimeter; **K/sec**: kelvin per second; **kHz**: kilohertz; **MHz**: megahertz; **m/s**: meters per second; **mA/cm$^2$**: milliamperes per square centimeter; **nm**: nanometer; **Pa**: pascal; **SC**: stratum corneum; **W/cm$^2$**: watts per square centimeter

*Why transdermal drug delivery?*

As the body's largest organ, skin is a promising target of drug delivery. However, skin is an extremely effective barrier membrane - perhaps the most effective barrier membrane (40) for the human organism. The skin's

outermost layer, the stratum corneum (SC) represents an impenetrable barrier to molecular transport and thus to most of the drugs from entering the body through the skin.

The stratum corneum is of 10-15 μm in thickness, and is composed of dead keratin-rich cells (corneocytes) and a lipid matrix. The intercellular space is an ordered, impermeable bilayer structure of lipid and aqueous layers, along with fatty acids and cholesterols. Thus, due to the structure and composition, the stratum corneum is almost impermeable.

Three possible pathways, transappendageal, transcellular, and intercellular have been suggested for molecular transport through the SC (110). The transappendageal pathway is primarily through hair follicles. However, the transappendageal skin transport in humans is limited by the small surface area available. The fractional area of hair follicles relative to the skin area is $10^{-2}$-$10^{-5}$ (111). The transcellular pathway requires the substrates to travel through the corneocytes while the intercellular pathway is via the extracellular matrix between the corneocytes. For intercellular skin transport, hydrophilic substrates are rate limited by the lipid environment of the intercellular matrix of the SC (1). On the other hand, lipophilic substrates partition into the intercellular lipids of the SC. However, the rate-limiting step is the partition into the epidermis, which is practically an aqueous environment. Molecular transport through the skin has been described by a solubility-diffusion model (102) and a transfer free energy model (103). Skin penetration also depends on anatomical site, age, sex, skin care, hydration and temperature (75). In addition, the molecular weight (MW) of the substrate affects percutaneous absorption. The diffusion through the SC follows the expression $Da\ (MW)^{-b}$ where the value of $b$ varies between 0.3 and 0.6 (41).

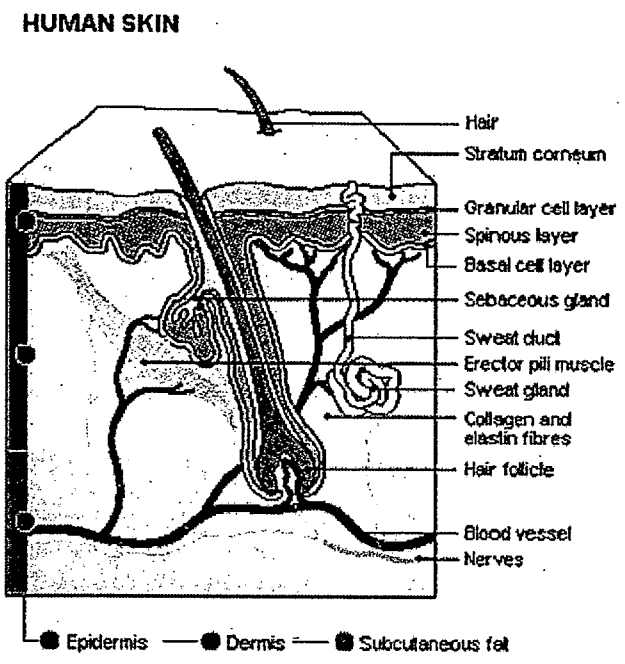


**Fig. 1** Structure of human skin and its components

As a result, the stratum corneum is the rate-determining barrier to percutaneous absorption of most compounds. If a molecule can cross the stratum corneum and the epidermis, it can then reach the dermis and potentially be absorbed by the skin's blood vessels. Fig. 1 shows the structure of human skin and its components and Fig. 2 provides the structure of stratum corneum (27).

Transdermal drug delivery thus involves passive diffusion of a drug substance through the skin and subsequent absorption by the capillary system for systemic distribution. Presently available transdermal patches can be classified into four categories on the basis of their

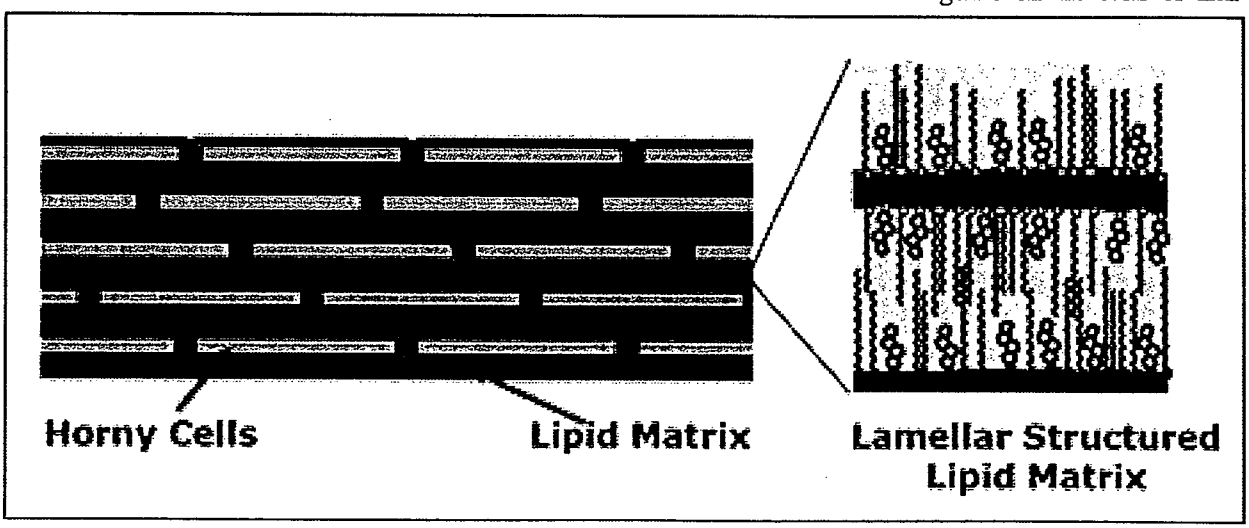


**Fig. 2** Structure of stratum corneum

design: single-layer-drug-in adhesive, multi-layer-drug-in adhesive, reservoir and matrix patches (147). The basic components of these patches are impermeable backing, rate-controlling membrane, liner, adhesive and the drug. These patches can be placed on the arm, on the back, or anywhere that is discreet and effective. Transdermal patches are engineered with safety, security, effectiveness and comfort in mind. Except very few cases, where there are mild skin irritations that occur at the site of the patch application, transdermal patches are a great way to avoid side effects, since a patch's ingredients do not enter the digestive system, therefore, the chance of getting an upset stomach is small (149).

The commercialization of transdermal patches for controlled drug delivery began two decades ago and has resulted in diverse products. There are a number of medications that are available in patch form today and the technology has a proven record of FDA approval. Since the first transdermal patch was approved in 1981 to prevent the nausea and vomiting associated with motion sickness, the FDA has approved, throughout the past 24 years, more than 35 transdermal patch products, spanning 13 molecules (146,148) which are Nitroglycerine for angina, Scopolamine for motion sickness treatment, Fentanyl for pain control, Nicotine for smoking cessation, Estrogen for hormone replacement therapy, Testosterone for male hypogonadism, Clonidine for hypertension treatment, Lidocaine and Prilocaine for topical anaesthesia, Ethinylestradiol and Norelgestromin for contraception, Norethindrone to help reducing the overgrowth of the lining of the uterus and Oxybutynin for overactive bladder.

Although the transdermal approach has many advantages, but, yet this approach has not developed as widely as was once predicted. This is due to that current transdermal patch designs are not capable of transporting large molecular drug through the skin barrier, especially peptides and proteins (of molecular weight more than 500 Da, for example, insulin - 6000 Da), which includes many drugs that are marketed or will emerge from the biotechnology industry. The reason, as mentioned earlier, is due to the difficult-to-cross stratum corneum. The physiochemical nature of this pathway dictates that only lipophilic drugs such as estradiol ($\log K_{o/w} = 2.58$) will readily diffuse through stratum corneum. In addition, the main disadvantage of traditional transdermal delivery is that therapeutic plasma levels are obtained very slowly (6 to 8 hr after application in the case of scopolamine) (107).

*Techniques to enhance transdermal drug delivery*

Transdermal delivery of drugs of much higher molecular weight (>500 Da) thus requires skin permeation enhancement mechanisms. In the quest to facilitate and increase the rate of delivery of higher molecular weight drugs through epidermis, several avenues have been proposed. These include the use of chemical enhancers (7,24,25,118,137,139), iontophoresis (7,18,35,108,142), electroporation (25,34,99,104,105), microneedles (7,148), laser ablation (49,64,95), pressure waves or shock waves (28,53), magnetic field (magnetophoresis) (57,94,109), photomechanical waves (60-62,76,77) and RF ablation (151).

– *Chemical enhancers*: Numerous compounds have been evaluated as chemical enhancers for penetration enhancing activity, including sulphoxides (such as dimethyl-sulphoxide, DMSO), azones (e.g. laurocapram), pyrrolidones (for example 2-pyrrolidone, 2P), alcohols and alkanols (ethanol or decanol), glycols (for example propylene glycol, PG, a common excipient in topically applied dosage forms), surfactants (also common in dosage forms) and terpenes (139). Using a chemical enhancer, for example, nerolidol (a terpene) has been shown to enhance 5-fluorouracil permeability over 20-fold through human skin *in vitro* (24). These chemical enhancers temporarily alter the barrier properties of the stratum corneum that enhances the drug flux (7,118). However, due to the incredibly slow permeability coefficients of the macromolecules, the enhancement effects required to ensure the delivery of pharmacologically effective concentrations are likely to beyond the capability of chemical enhancers tolerated by the skin (25).

– *Iontophoresis*: This method is about a century old technique and refers to the facilitated movement of ions of soluble salts through the application of a small electric current (usually 0.5 $mA/cm^2$) in order to drive the solute molecules into the skin (108). The effect of simple electrorepulsion is known to be one of the main mechanisms by which iontophoresis produces its enhancement effects, though other factors including the increased permeability of the stratum corneum by the generation of small pores (142) in the presence of a flow of an electric current and electroosmosis of uncharged and larger water soluble molecules are also possible (7). There have been numerous research applications of iontophoresis in topical drug delivery for lower molecular weight solutes (<500 Da) (7,108). Iontophoresis using specially designed current can give about 400 % better penetration compared to simple topical application (35).

– *Electroporation*: Electroporation is a mechanical method used to introduce macromolecules into a host cell through the cell membrane. In this procedure, application of a large electric pulse (10 μs-100 ms) temporarily disturbs the phospholipid bilayer and causes the formation of transient pores in the membrane, allowing macromolecules like DNA to pass into the cell. Prausnitz and Weaver and Preat have done intensive work for enhancing the transdermal drug delivery using this technique (104). The electrical resistance of the skin is reported to drop as much as three orders of magnitude within microseconds of administration of an

electrical pulse (99,105). Increases in transdermal penetration of up to 104-fold have been reported *in vitro* for various sizes of molecules. To date there appear to be no clinical studies assessing the ability of the technique to facilitate transdermal drug delivery (25).

– *Microneedles*: A newer and potentially more promising technology for macromolecule delivery is microneedle-enhanced delivery. These systems use an array of tiny needle-like structures to open pores in the stratum corneum and facilitate drug transport. The structures are small enough that they do not penetrate into the dermis and thus do not reach the nerve endings, so there is no sensation of pain. The structures can be either solid (serving as a pre-treatment prior to patch application), solid with drug coated directly on the outside of the needles, or hollow to facilitate fluidic transport through the needles and into the lower epidermis. These systems have been reported to greatly enhance (up to 100,000-fold) the permeation of macromolecules through skin (7,148).

– *Laser ablation*: The use of lasers to remove the stratum corneum barrier by controlled ablation (49) has also been investigated as a means of enhancing topical drug delivery. In 1991, Nelson *et al.* (95) reported that mid-infrared laser (1 J/cm$^2$) ablation of pig stratum corneum enhanced the permeation of both hydrocortisone and interferon. Lee *et al.* (64) found that stratum corneum ablation with low intensity (0.35-0.45 J; 0.91-1.17 J/cm$^2$) erbium:yttrium-aluminum-garnet (YAG) laser (light emission at 2940 nm) increased the permeability of both lipophilic and hydrophilic drugs through nude mouse skin *in vitro*. However, the structural changes caused by this technique still need to be assessed for safety and reversibility, particularly at the higher intensities that may be needed to enhance the penetration of large molecular weight solutes where evidence of deeper level ablation effects exist (64).

– *Pressure waves or shock waves*: Pressure waves, which are generated by intense laser radiation, can permeabilize the stratum corneum (SC) as well as the cell membrane. These pressure waves are compression waves and thus exclude biological effects induced by cavitation. Their amplitude is in the hundreds of atmospheres (bar) while the duration is in the range of nanoseconds to a few microseconds. The pressure waves interact with cells and tissue in ways that are probably different from those of ultrasound. Furthermore, the interactions of the pressure waves with tissue are specific and depend on their characteristics, such as peak pressure, rise time and duration. A single pressure wave is sufficient to permeabilize the SC and allow the transport of macromolecules into the epidermis and dermis. Cell permeabilization using these waves may be a way of introducing macromolecules and small polar molecules into the cytoplasm, and may have applications in gene therapy and anticancer drug delivery. In addition, drugs delivered into the epidermis can enter the vasculature and produce a systemic effect. For example, insulin delivered by pressure waves resulted in reducing the blood glucose level over many hours. The application of pressure waves does not cause any pain or discomfort and the barrier function of the SC always recovers (28).

Kodama and co-workers argued that shock waves permeabilize membrane by inducing relative displacement between the cell and the surrounding fluid (53). They have used three different shock-wave sources for the generation of these waves; argon fluoride excimer laser, ruby laser, and shock tube. The uptake of two fluorophores, calcein (MW: 622 Da) and fluorescein isothiocyanate (FITC) - dextran (MW: 71,600 Da), into HL-60 human promyelocytic leukemia cells was investigated. The intracellular fluorescence was measured by a spectrofluorometer, and the cells were examined by confocal fluorescence microscopy. A single shock wave generated by the shock tube delivered both fluorophores into approximately 50 % of the cells ($p$, 0.01), whereas shock waves from the lasers did not. The cell survival fraction was 0.95. Confocal microscopy showed that, in the case of calcein, there was a uniform fluorescence throughout the cell, whereas, in the case of FITC-dextran, the fluorescence was sometimes in the nucleus and at other times not. They concluded that the impulse of the shock wave (i.e. the pressure integrated over time), rather than the peak pressure, was a dominant factor for causing fluorophore uptake into living cells, and that shock waves might have changed the permeability of the nuclear membrane and transferred molecules directly into the nucleus.

– *Magnetophoresis*: Limited work probed the ability of magnetic fields to move diamagnetic materials through skin (57,94). Santini *et al.* (109) have discussed the interesting idea of employing intelligent systems based on magnetism or microchip technology to deliver drugs in controlled, pulsatile mode.

– *Photomechanical waves*: Photomechanical waves (PW's) are also known as laser generated stress waves. In this technique, a drug solution placed on the skin and covered by a black polystyrene target, is irradiated with a laser pulse. The resultant photomechanical wave stresses the horny layer and enhances drug delivery (60). The mechanism(s) by which PW's increase the permeability of the stratum corneum is not entirely clear. Microscopic studies have indicated that changes in the lacunar system are visible following exposure of human stratum corneum to a PW (77) and that expansion of this system is suggested to result in the formation of transient channels through the stratum corneum (76). The largest molecule that has been reported to be delivered through rat skin to date has been 40 kDa (61). Suggestion has been raised that many clinically important proteins such as insulin (6,000 Da) and hematopoietin (48,000 Da) are within, or close to, the delivery capability range of PW's (62), however, this relatively new technique

does not yet seem to have produced any human clinical data.
– *RF ablation*: This technique uses radio-frequency (RF) electrical current and creates microscopic passages in the stratum corneum and outer epidermis via a process called cell ablation. This technique is typically performed at frequencies above 100 kHz that do not stimulate the muscles and nerves. The pre-clinical and clinical trials using this technique for a variety of drugs, including hGH (a large 22 kDa protein), demonstrating high therapeutic drug delivery levels and excellent bioavailability (151).

In addition to the above methods, a number of other methods have also been studied for the transdermal delivery of various drugs. Thermal poration, an approach that has been used to deliver conventional drugs (115) and vaccines (16) to animals and to extract interstitial fluid glucose from human being (38). High-velocity jet injectors are also receiving increased attention (46). Clinically insulin has been delivered using this technique (17). Jet injectors are presently on the market and a number of companies are developing new devices (106).

## SONOPHORESIS

Apart from a few dermal patches, we have still not realized the enormous advantages of transdermal delivery over the traditional routes for giving drugs. In the continuous search of newer and efficient techniques, "sonophoresis" in recent years has become a promising approach and it has become the subject of many studies. Sonophoresis is similar to iontophoresis. However, the problem with iontophoresis is that it only works if the molecule can be ionised into positive and negative charged components. This is not an impediment for sonophoresis.

Sonophoresis is a transdermal drug delivery technology in which enhancement of the transport of permeants occurs by the application of ultrasound. Simply this process can be referred to as the use of the energy of sound waves in order to drive the movement (flux) of chemicals transcutaneously through the skin by means of changing the barrier properties of the skin (2,8,10,34). Passing intense ultrasound waves results in cavitation which is the main mechanism for sonophoretic enhancement.

*Ultrasonic domain*

It's necessary to understand what sound is before one can understand ultrasound. Sound is the propagation of pressure wave through some physical elastic medium. Usually the medium is air, but a liquid works well too. A vacuum doesn't. The longitudinal pressure waves are generated due to mechanical disturbances. Mechanical energy is being converted to a wave form that radiates energy away from the disturbance, transferring energy to the medium and to objects that the wave contacts. Human hearing is limited (20 Hz to 18 kHz). If the vibrational frequency is too fast, too high a frequency (>18 kHz), we can't hear it. Thus, ultrasound is a sound wave where vibrations are too fast for us to hear. The broad classification of ultrasound as sound above 18 kHz and up to 10 MHz can be subdivided into three distinct regions; low frequency or power (18-100 kHz), medium frequency or therapeutic ultrasound (0.7-3 MHz) and high frequency or diagnostic ultrasound (3-10 MHz) (58, 73).

The application of ultrasound waves in chemistry, sonochemistry, is well documented (122) and from many chemical reaction studies it has been found that it has an influence on reaction rate kinetics and the sonochemical effects results in reduction in reaction time, higher yield and saving in energy (116,117). Sonochemistry normally uses frequencies between 20 and 40 kHz. The origin of sonochemical effects in liquids due to ultrasound principally derives from acoustic cavitation which serves as an effective means of concentrating the diffuse energy of sound (125). Since acoustic cavitation in liquids can also be generated well above 40 kHz, recent researches into sonochemistry use a much broader range.

Ultrasound waves which alternately compress and stretch the molecular spacing of the medium through which it passes. Thus, the average distance between the molecules in a liquid will vary as the molecules oscillate about their mean position. If a large negative pressure, i.e. sufficiently below ambient is applied to the liquid so that the distance between the molecules exceeds the critical molecular distance necessary to hold the liquid intact, the liquid will break down and voids will be created, i.e. cavitation bubbles will form. When produced in a sound field at sufficiently high power, the formation of cavitation bubbles will be initiated during the rarefaction cycle. Those bubbles will grow over a few cycles taking in some vapour or gas from the medium to an equilibrium size which matches the frequency of bubble resonance to that of the sound frequency applied. The acoustic field experienced by an individual bubble is not stable because of the interference of other bubbles forming and resonating around it. As a result some bubbles suffer sudden expansion to an unstable size and collapse violently. It is the fate of these bubbles when they collapse results in the generation of intense local heating, high pressures, and very short lifetimes (73). These hotspots have temperatures of roughly 5000°C, pressures of about 1000 atm, and heating and cooling rates greater than $10^9$ K/sec (5,123). Thus, cavitation can produce extraordinary physical and chemical conditions in an otherwise cold liquid (125).

There are two basic types of cavitation: stable and transient (inertial) (29). In transient or inertial cavitation, bubbles grow above their resonant size and then collapse violently. A series of effects can occur as a result of this type of cavitation: shock waves may be propagated; acoustic energy is often converted to heat yielding high microscopic

temperatures (3,36); high fluid velocities can be generated; and free radicals might be formed (31). Stable or non-inertial cavitation, a less violent event, refers to bubbles that pulsate about some equilibrium radius and often persist for many acoustic cycles (65). As a result of these oscillations, streaming of surrounding liquid occurs and mechanical stresses create mixing of the medium.

The use of ultrasound in medicine has also gained popularity due to its potential applications both in therapy as well as in medical diagnosis in recent years. Medical ultrasound generally uses frequencies between 1 and 10 million hertz (1-10 MHz). Potentially, both stable and transient cavitation may induce membrane permeabilization. Liu *et al.* (67) reported that disruption of red blood cell membranes by ultrasound correlates better with the occurrence of stable cavitation. On the other hand, other investigators (33,79) postulated that ultrasound-induced cell damage results from inertial (transient) cavitation.

Tezel *et al.* (135) have reported detailed investigations of the occurrence of cavitation during low-frequency sonophoresis. Cavitation was monitored by recording pressure amplitudes of subharmonic emission and broadband noise at four different ultrasound frequencies in the range of 20-100 kHz and at various intensities in the range of 0-2.6 W/cm$^2$. Enhancement of skin conductivity, in the presence of sodium lauryl sulfate (SLS), was also measured under the same ultrasound conditions. Enhancement of skin conductivity correlated well with the amplitude of broadband noise, which suggests the role of transient cavitation in low-frequency sonophoresis. No correlation was found between the subharmonic pressure amplitude and conductivity enhancement. Results of Sundaram *et al.* (121) further support the role of transient cavitation in ultrasound mediated membrane permeabilization. They have developed a mathematical model to relate the effect of ultrasound with the number of transient cavitation events. The model also allowed assessment of the role of various stages of transient cavitation, including bubble expansion, collapse, and subsequent shock wave formation, in reversible as well as irreversible membrane permeabilization. Bubble expansion and collapse, as well as shock wave, were found to contribute toward membrane permeabilization. However, a systematic dependence of membrane permeabilization on ultrasound or cavitation parameters is not yet known (121).

*Generation of ultrasound*

An ultrasound generator generates electrical oscillations of ultrasonic frequency (e.g. above 20 kHz). Transducer, an electro-mechanical component is a device that converts one form of energy into another. The major component of a transducer is a crystal of piezo-electric material. The transducer sends out sound waves which are partially reflected by the medium in which they are propagating, the other part penetrates and propagates into the medium. During its propagation, a wave is partially scattered and absorbed by the medium, resulting in attenuation of the emitted wave; the lost energy is converted into heat.

*Sonophoresis method*

The basic principle of application of ultrasound for transdermal drug delivery is to use an ultrasound source at the skin with the drug interspersed (in solution) between the transducer and the skin surface. A voltage is then applied to the ultrasound transducer at the resonance frequency of the piezo-electric crystal. This results in various effects as discussed in the following section and thereby facilitates the delivery of the drug through the skin barrier. To ensure the effective transmission of ultrasound, a coupling medium (generally a gel or water) is interposed along with the drug component between the transducer and the skin. The viscosity of the coupling agent and the quantity of the gas dissolved in the medium may significantly affect the transdermal transport (81). A decrease in sonophoretic transport of insulin and vasopressin was reported *in vitro* when molecules were administered in a gel as compared to administering them as saline solution (145). The reason for this reduction has been explained by boundary layers that form within the gel owing to the relatively rapid rate of molecular transport across the (ultrasonically) permeated stratum corneum as well as poor diffusive mass transfer between the skin and gel. The following Fig. 3 further provides a clear evidence where exposing hairless mouse to ultrasound in presence of evans blue gives intense staining of the skin, when the administration is in the form of liquid. Alternatively, administering in the form of gel gives a less intense staining of the skin. Whereas, administration without the application of ultrasound leads to very less staining of the skin (Tachibana, unpublished work) (Fig. 3).

Similar findings were reported with lidocaine *in vivo* in hairless mice (128). Tachibana and Tachibana (126) have used ultrasonic vibration to deliver insulin through the skin of hairless mice fasted overnight and partially immersed in an aqueous solution of insulin (20 units/ml). The skin surface was exposed to ultrasonic vibration in two ultrasonic energy ranges (3000-5000 Pa and 5000-8000 Pa) at 48 kHz for 5 min. Blood glucose concentration was measured before and after exposure to insulin and ultrasonic vibration. In the group subjected to the lower energy vibrations, blood glucose fell to reach 34 ± 11.9% of control values in 120 min, while when the animals were exposed to higher energy vibrations, the fall in blood glucose was 22.4 ± 3.9% of control values at 120 min. The values remained low for the length of the experiment (240 min). Those exposed to insulin alone or ultrasonic vibration alone revealed no significant change in blood glucose concentration. They have postulated that ultrasonic vibration may alter skin permeability resulting in the absorption of insulin. That the

blood glucose decrease was greater at the higher of the two energy ranges, suggests this factor could control insulin delivery. Mitragotri *et al.* (83) have found that low-frequency ultrasound did not induce a long-term loss of the barrier properties of the skin (*in vitro*) or damage to living skin of hairless rats. At a mechanistic level, they have hypothesized that application of low-frequency ultrasound enhances transdermal transport through aqueous channels in the SC generated by cavitation-induced bilayer disordering. Support for this hypothesis has also been provided using experimental and theoretical analyses of low-frequency sonophoresis.

Over the last several years, research on low-frequency sonophoresis generally classified into two categories; simultaneous sonophoresis and pretreatment sonophoresis. Simultaneous sonophoresis; this approach corresponds to a simultaneous application of drug and ultrasound to the skin. This was the first mode in which low-frequency sonophoresis was shown to be effective. This method enhances transdermal transport in two ways: a) enhanced diffusion through structural alterations of the skin, and b) convection induced by ultrasound. Transdermal transport enhancement induced by this type of sonophoresis decreases after ultrasound is turned off (89). Although this method can be used to achieve a temporal control over skin permeability, it requires that the patients use a wearable ultrasound device for drug delivery. In the pretreatment method, a short application of ultrasound is used to permeabilize skin prior to drug delivery. The skin remains in a state of high permeability for several hours. Drugs can be delivered through permeabilized skin during this period. In this approach, the patient does not need to wear the ultrasound device (91). Katz *et al.* (51) used this pretreatment method with low-frequency ultrasound to shorten the lag-time for analgesic agent to be effective.

*Selection of ultrasonic parameters*

The phenomenon of bubble growth and collapse and hence the lifespan of these bubbles are dependent on various ultrasonic parameters, for example, amplitude of the acoustic pressure, environmental pressure, frequency, characteristics of the liquid (such as viscosity, vapour pressure, etc.) and existence of dissolved gas in the liquid. Thus, ultrasound assisted transdermal drug delivery is affected by different parameters of ultrasound out of which some of the important parameters that affects the phenomenon to a great extent are discussed below.

– *Frequency*: The frequency F of an emitted ultrasonic wave depends on the thickness of the piezo-electric crystal. Attenuation of an acoustic wave is inversely proportional to its frequency. Thus, with an increase in frequency, the ultrasound penetrates to a greater depth into and under the skin. Since, the outer layer of epidermis, the stratum corneum, is the main barrier to percutaneous penetration of drugs, it initially seemed logical to concentrate the ultrasonic energy on this skin layer using very high frequency (10-20 MHz) in order to achieve a higher transdermal enhancement (10,11). In addition, these high frequencies were tried in early trials in order to avoid potential safety issues (58).

Bommannan *et al.* (11) have made theoretical investigations as well as experimental verifications about ultrasound propagation in tissue and predicted that higher

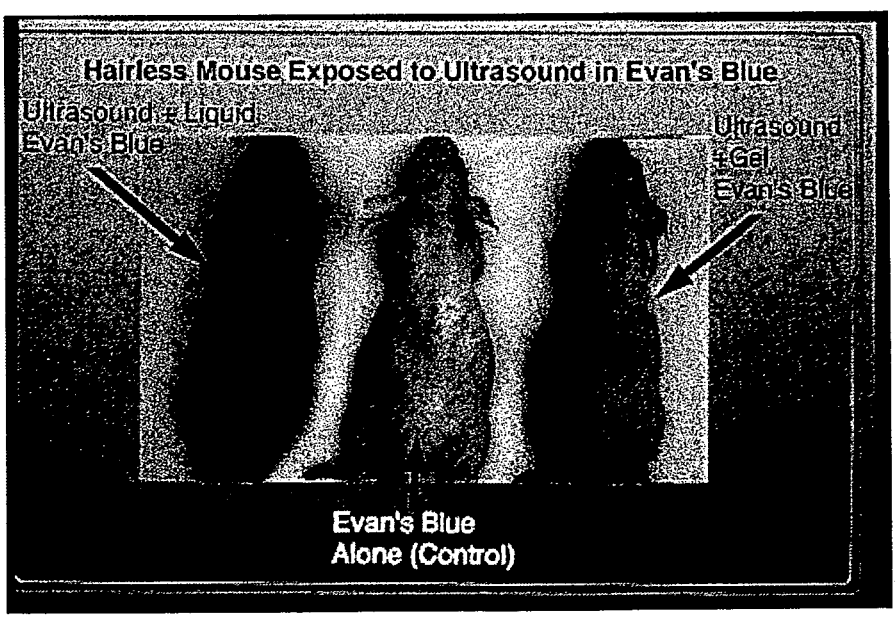


**Fig. 3** Effect of ultrasound and the form of drug in staining the hairless mouse skin

frequency ultrasound (>1 MHz) increases the concentration of energy deposition in the stratum corneum. In their experiments, salicylic acid was subjected to passive transdermal delivery under the influence of ultrasound at 2, 10 and 16 MHz frequencies. Sonophoresis for 20 min at 2 MHz caused no significant increase in salicylic acid delivery over passive diffusion. Treatment with ultrasound at 10 and 16 MHz, on the other hand, significantly elevated salicylic acid transport, by 4-fold and 2.5-fold, respectively. A shorter period of 5 min of sonophoresis again resulted in enhanced transdermal transport at higher frequencies. They found that the enhancing effect of sonophoresis is due to a direct effect of ultrasound on the stratum corneum.

Mitragotri *et al.* (82) reported that the sonophoretic enhancement in the therapeutic frequency range varies inversely with ultrasound frequency. They found that 1-MHz ultrasound enhances transdermal transport of estradiol across human cadaver skin *in vitro* by 13-fold, but that 3-MHz ultrasound at the same intensity induces an enhancement of only 1.5-fold. They further hypothesized that the observed inverse dependence of sonophoretic enhancement on ultrasound frequency occurs because cavitational effects, which are primarily responsible for sonophoresis, vary inversely with ultrasound frequency (82).

The effects of therapeutic ultrasound (1 MHz, 1.4 W/cm$^2$, continuous) with different types of chemical enhancers on the transdermal transport of corticosterone and four other model drugs dexamethasone, estradiol, lidocaine and testosterone was investigated by Johnson *et al.* (50). Typical enhancements induced by therapeutic ultrasound are ~10-fold (20). Thus such enhancement might be sufficient for local delivery of drugs like corticosterone, but not for systemic delivery of drugs (10, 11, 58).

As cavitation could be more easily and effectively generated at low frequencies, it was then found that any frequency lower than that corresponding to therapeutic ultrasound frequency (0.7-3 MHz) should be more effective in enhancing transdermal drug delivery. This is a direct consequence of reduced acoustic cavitation at high ultrasound frequencies, due to the fact that the time between the positive and negative acoustic pressures becomes too short. In addition, the number and size of cavitation bubbles generated decreases as the frequency increases (132,134). Thus, the use of low frequency ultrasound rather than therapeutic ultrasound was shown to be more effective in enhancing membrane permeability and hence transdermal transport and has been more successful in recent years (81,128). For example, Tachibana and Tachibana (126) and Tachibana (127) have reported that the use of low frequency ultrasound (48 KHz) enhanced transdermal transport of insulin across diabetic rat skin. Merino *et al.* (78) have investigated the ultrasound effects of low (20 KHz) and high (10 MHz) frequency for the transdermal transport of mannitol. They observed that only low frequency ultrasound resulted in significantly increased permeation as compared to high frequency. Tachibana and Tachibana have used low frequency ultrasound at 48 kHz for delivering lidocaine to hairless mice (128). From their studies it was observed that ultrasound with 2% lidocaine rapidly induces an anesthetic effect. Application of ultrasound at 20 kHz induced transdermal transport enhancements of up to 1000 times higher than those induced by therapeutic ultrasound for low molecular weight drugs and high molecular weight proteins (81,83). Following table (from ref. 83) provides different types of drugs and their corresponding penetration enhancements using low frequency ultrasound (20 kHz). Here, penetration enhancement has been defined as the ratio of skin permeability when ultrasound was applied ($P_{US}$) and the control skin permeability ($P_C$) (Table 1).

A detailed investigation of the dependence of permeability enhancement on frequency in the low frequency regime (20-100 kHz) has been reported by Tezel *et al.* (134). At a given intensity, the enhancement decreased with increasing ultrasound frequency. For example, the enhancement decreased dramatically from about 45-fold at a frequency of 19.6 kHz to negligible at a frequency of 93.4 kHz with a constant ultrasound intensity of 0.84 W/cm$^2$. Such a strong dependence of permeability enhancement on ultrasound frequency is an indicator of the role of cavitation in low frequency sonophoresis (91).

**Table 1** Drugs and their corresponding penetration enhancements using low frequency ultrasound (20 kHz)

| Compound | Hydrophilicity | MW (Da) | Penetration Enhancement ($P_{US}/P_C$) |
|---|---|---|---|
| Aldosterone | slightly hydrophilic | 360 | 1400 |
| Butanol | slightly hydrophilic | 74 | 29 |
| Corticosterone | slightly lipophilic | 346 | 80 |
| Estradiol | very lipophilic | 272 | 3 |
| Salicylic acid | Hydrophilic | 138 | 400 |
| Sucrose | Hydrophilic | 342 | 5000 |

Although lower frequencies induce higher enhancements, the transport at low-frequencies was found to be localized to certain areas termed as localized transport pathways (134). With an increase in ultrasound frequency, the transport was found to be more homogeneous. The optimum appears to be around 60 kHz where significant transport enhancements can be obtained with reasonable energy doses while simultaneously achieving reasonable homogeneity of the transport pathways (91).

The *in vivo* and the *in vitro* correlation of the effects of low frequency ultrasound on the percutaneous penetration of mannitol, a hydrophilic permeant, was investigated using three *in vitro* skin models and *in vivo* pig as the animal

model, in order to predict the effects of low frequency ultrasound *in vivo* on the transdermal delivery of hydrophilic permeants. The results of the study suggest that low frequency ultrasound represents a good method of enhancing the systemic absorption of hydrophilic permeants, while it does not significantly alter the vehicle-to-skin partition coefficient for the same class of permeants (130).

A more recent study by Tang *et al.* (131) identifies the critical type(s) and site(s) of cavitation that are responsible for skin permeabilization during low frequency sonophoresis using pig full-thickness skin and the effect of low frequency ultrasound (20 kHz) on the skin permeability was monitored by measuring the increase in the skin electrical conductance. An acoustic method, as well as chemical and physical dosimetry techniques, was utilized to monitor the cavitation activities. The study showed definitively that ultrasound-induced cavitation is the key mechanism via which low frequency ultrasound permeabilizes the skin. By selectively suppressing cavitation outside the skin using a high-viscosity coupling medium, they have further demonstrated that cavitation occurring outside the skin is responsible for the skin permeabilization effect, while internal cavitation (cavitation inside the skin) was not detected using the acoustic measurement method under the ultrasound conditions examined. From the acoustic measurement of the two types of cavitation activities (transient *vs.* stable), they indicated that transient cavitation plays the major role in low frequency ultrasound induced skin permeabilization. Through quantification of the transient cavitation activity at two specific locations of the low frequency ultrasound system, including comparing the dependence of these cavitation activities on ultrasound intensity with that of the skin permeabilization effect, they have demonstrated that transient cavitation occurring on, or in the vicinity of, the skin membrane is the central mechanism that is responsible for the observed enhancement of skin permeability by low frequency ultrasound (131).

Kushner *et al.* (56) recently demonstrated the existence of hypothesized localized transport regions (LTRs - localized regions of high permeability) responsible for the enhanced permeability during low frequency sonophoresis experimentally. They found an enhancement of higher than 80-fold of calcein permeability in the presence of LTRs. Also, an analysis based on porosity/tortuosity ratio suggests that trans-cellular transdermal transport pathways are present within the highly permeable and highly structurally perturbed LTRs (56).

– *Mode of application* (Pulse or continuous): Ultrasound waves can be applied continuously (continuous mode) or in a pulse (sequential, discontinuous) mode. In continuous mode, the ultrasound waves are persistent and thus the heat will be transferred to the body tissues resulting in greater heating effect (tissue heating). Whereas, the "pulsed" has the option of on/off cycles, each component of which can be varied so that the waves go in short pulses thereby altering the dose of ultrasound applied and thereby preventing the tissue heating (98). This heating effect may have a deleterious effect on sonophoresis. Tissue heating can become very painful, necessitating continuous motion of the ultrasound head, which diffuses the ultrasonic energy over a larger area. With pulsed-wave ultrasound, patients can tolerate a virtually stationary sound head, ensuring a more concentrated ultrasound dosage at the treatment site (6). Thus, the choice of pulsing mode is generally followed just to minimize the associated thermal effects. But, either form at low intensity will produce non-thermal effects. Experiments performed on hairless rats clearly demonstrate that the sonophoresis efficiency appeared to be highly dependent on the net exposure time and the ultrasound pulse "on" duration (14).

Boucaud *et al.* (12) have investigated the dependence of ultrasound-induced transdermal delivery of insulin *in vivo* to hairless rats using 20 kHz ultrasound applied over a range of pulse length. Change in blood glucose levels of the animals was monitored to assess insulin transport. The findings indicated that sonophoretic enhancement is dependent on length of ultrasound pulse that is consistent with a cavitation-based mechanism.

The effect of pulsed output ultrasound (1 MHz) with on/off ratios of 1:2, 1:4 and 1:9 on transdermal absorption of indomethacin from an ointment was studied in rats by Asano *et al.* (4). 1:2 pulsed output ultrasound appeared to be the most effective in improving the transdermal absorption. They have also found that with pulsed output it was possible to use higher intensities of ultrasound without increasing skin temperature or damaging skin.

Cagnie *et al.* (20) have examined the influence of ultrasound on the transdermal delivery of ketoprofen in humans and compared the concentrations found after continuous and pulsed application. For this purpose, one group of persons was administered ketoprofen gel using continuous ultrasound (1 MHz, 1.5 W/cm$^2$, for 5 min). Second group received the same treatment but with pulsed ultrasound (100 Hz, 20% duty cycle). Biopsies of adipose tissue and synovial tissue were taken during surgery to evaluate the local penetration of the drug. Blood samples also were collected to determine whether ketoprofen entered the systemic circulation. The concentration of ketoprofen in fat tissue and synovial tissue was consistently higher in the group of people administered with pulse ultrasound as compared to the people administered with continuous ultrasound.

Benson *et al.* (8) found that pulsed-output ultrasound provided the most effective conditions in the technique of sonophoresis of lignocaine and prilocaine from EMLA (eutectic mixture of local anaesthetics) cream. Nevertheless, conflicting results have also been reported concerning its occurrence during pulsed ultrasound versus continuous

ultrasound. Nussbaum (98) has reported that the scale of cavitation depends on the ultrasound characteristics; bubble growth is limited by low-intensity, high frequency, and pulsed ultrasound. Mitragotri *et al.* (82) confirmed this statement. They found that the cavitation threshold increases as the mode of ultrasound application changes from continuous to pulsed. Sun and Liu (120), however, suggested that cavitation is more likely to occur when pulsed ultrasound is used, provided that the ultrasound intensity during the pulses exceeds the threshold of cavitation occurrence and the duration of the pulses is long enough for the cavitation to develop.

– *Intensity*: The intensity I is directly dependent on the acoustic energy (E) emitted and the speed of sound (c) in the medium as expressed by the following expression (45, 48),

$$I = cE \qquad \text{Eq. 1}$$

Energy, E itself dependent on the density of the propagation medium ρ, on the total pressure p and on the speed of sound (equal to the sum of the atmospheric pressure and the pressure created by the ultrasound wave). Therefore, the emitted energy can be expressed as in the following equation (52),

$$E = p^2/\rho c^2 \qquad \text{Eq. 2}$$

The minimum ultrasound intensity required for the onset of cavitation, referred to as cavitation threshold or threshold intensity, increases rapidly with ultrasound frequency (71,72,82). The ultrasound power intensities usually employed for transdermal drug delivery lie between 0.1 and 3 W/cm$^2$ with low frequency application (20-100 kHz) and 0.1 and 10 W/cm$^2$ with high frequency application (1-3 MHz) (69). Below the threshold intensity no detectable enhancement has been observed. Once the intensity exceeds this threshold, the enhancement increases strongly with the intensity until another threshold intensity, referred to as the decoupling intensity is reached. Beyond this intensity, the enhancement does not increase with further increase in the intensity due to acoustic decoupling. The threshold intensity for porcine skin increased from about 0.11 W/cm$^2$ at 19.6 kHz to more than 2 W/cm$^2$ at 93.4 kHz. The origin of this substantial increase in the threshold intensity with frequency may be attributed to cavitation.

The effect of ultrasound (1 MHz) on transdermal absorption of indomethacin from an ointment was studied in rats by Miyazaki *et al.* (92). Ultrasound energy was supplied for between 5 and 20 min at a range of intensities (0.25, 0.5, 0.75 and 1 W/ cm$^2$), energy levels commonly used for therapeutic purposes. For evaluating skin penetration of indomethacin, the change of plasma concentration was measured. The pronounced effect of ultrasound on the transdermal absorption of indomethacin was observed at all ultrasound energy levels studied. The intensity and the time of application were found to play an important role in the transdermal sonophoretic delivery system of indomethacin; 0.75 W/cm$^2$ appeared to be the most effective intensity in improving the transdermal absorption of indomethacin, while the 10 min ultrasound treatment was the most effective. Although the highest penetration was observed at an intensity of 0.75 W/cm$^2$, 0.5 W/cm$^2$ was preferred because intensities of less than this for 10 min application did not result in any significant skin temperature rise nor did it have any destructive effect on rat skin. Progressively more skin damage was noted as the intensity and the time of application of ultrasound increased.

– *Cavitation nuclei*: As the sonophoresis enhancement is mediated through cavitation, i.e. the formation and collapse of gaseous bubbles, it is expected that by providing the nuclei for cavitation - externally, the efficacy of sonophoresis could be significantly enhanced. The occurrence of cavitation in water is facilitated by the presence of dissolved gas (32,113). Terahara *et al.* (133) have used two porous resins, Diaion® HP20 and Diaion HP2MG (2MG), as cavitation nuclei. Resultant cavitation effect was measured from the pitting of aluminum foil. It has been found that 2MG showed a higher efficacy in enhancing cavitation compared with Diaion HP20. 2MG was also effective in enhancing transdermal mannitol transport. These results confirm that the addition of cavitation nuclei such as porous resins further increases the effect of low frequency ultrasound on skin permeability.

In addition to the above parameters, sonophoretic enhancement also depends on transducer geometry as well as the distance between the transducer and the skin. Detailed dependence of enhancement on these parameters has not yet been studied (91).

*Mechanisms behind sonophoresis*

Driving force for increased skin permeability of transdermal drug delivery

– *Acoustic cavitational bubble collapse effects*: Passing ultrasound waves causes cavitation which is the growth and explosive collapse of the microscopic bubbles of a few μm in diameter. When the bubbles collapse quasi adiabatically, it results mainly in the following 3 effects:
a) Generation of extreme conditions: Adiabatically when the bubbles collapse or implode, it results in the concentration of energy or causes in the generation of a short-lived, localized high-energy spot. This energy concentration has been measured in terms of temperature and pressure, which are extreme on a microsecond timescale (124). An asymmetrically collapsing bubble next to a wall has been shown in Fig. 4 (Photo from ref. 26). The maximal diameter of the bubble is about 1 mm (Fig. 4).
b) Mechanical effect: The adiabatic collapse of bubbles also incites the mechanical effect, microstreaming. This is caused

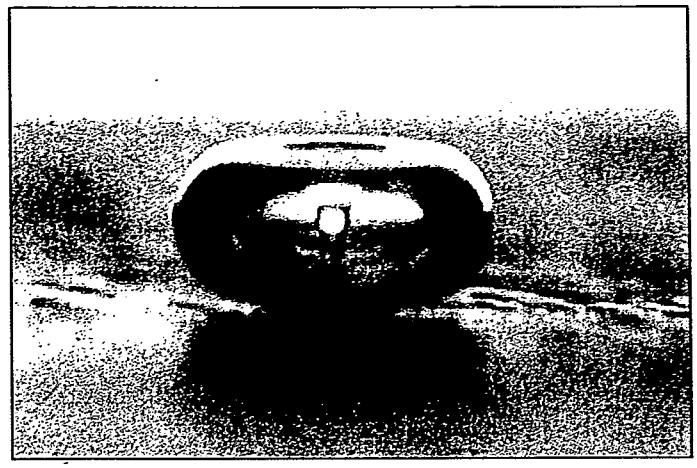

**Fig. 4** An asymmetrically collapsing cavitation bubble

by the unidirectional movement of fluids along cell membranes. Oscillation of cavitation bubbles might also contribute to microstreaming. Microstreaming may alter cell membrane structure, function and permeability (138) or porosity (129). The potential clinical value of this microstreaming has not been explored much (58).

c) Thermal effects: The thermal effect of ultrasound on the skin results from the transfer and conversion of mechanical energy generated by the vibration of a piezoelectric crystal in the sonophoresis probe which prompts the absorption of ultrasound by the skin (9). Absorption by the skin of this energy causes a temperature increase, which is directly related to the intensity of the sound wave (43). Prediction of the actual temperature increase produced by a particular sonophoretic profile is difficult, however, without a precise knowledge of the acoustic absorption coefficients, and of the conduction and convection properties, of the tissues involved. Furthermore, experimentally, there have been few studies quantifying the matter by which it has been modified has also been represented (43). Recently, most investigations have focused upon the use of US at a frequency of about 20 kHz, at a 10% duty cycle (0.1 on, 0.9 off), for periods from minutes to a few hours (83,88). While the results obtained implicate cavitational effects as a principal mechanism, the role of the accompanying thermal effect has not been deduced. Given that skin permeability can increase significantly with temperature (for example, the absorption of estradiol doubled when the temperature was increased by 10°C) (82), and that phase transitions of the intercellular lipids of the SC can occur at temperatures close to physiological (37,101), it is clearly possible that thermal changes can contribute to sonophoretically-enhanced transdermal transport (78).

Excessive thermal effects, seen in particular with higher ultrasound intensities, may damage the tissue (30). Machet *et al.* (68) have also demonstrated that the thermal effect of ultrasound was the principal explanation for the increase in the diffusion rate of digoxin. The increased skin permeability thus could be due to the amalgamated effects of all the above mentioned. For example, the above said effects results in a modification or disorganization of the stratum corneum in terms of increased fluidity combined with enlargement or widening of the intercellular space, which paves the way for drug passage. In addition, the above effects on keratinocytes or corneocytes cause temporary or permanent holes in driving the drug and vehicle by convection (14).

*Contribution of pressure induced by ultrasound for drug permeation (non-thermal or non-cavitational effect)*

Emission of the ultrasonic wave in the intercellular lipid accompanies a rise in the pressure. Considering the speed of sound in intercellular lipids at 1000 m/s (113), the pressure induced by the ultrasound was estimated as $5 \times 10^{-6}$ bar with 1 W/cm$^2$ and $5 \times 10^{-4}$ bar with 2 W/cm$^2$. Now, the question is whether this rise in pressure or the resultant pressure can cause the active drug ingredient to pass through mechanically. The relative contribution of this flow induced by the pressure, calculated for the diffusion of urea through a synthetic dialysis membrane represents 0.2% with 1 W/cm$^2$ and 2% with 100 W/cm$^2$. Thus, the pressure does not have a significant contribution to the increased percutaneous flow induced by ultrasound.

The non-thermal mechanical characteristics of ultrasound can also enhance drug diffusion by oscillating the cells at high speed, changing the resting potential of the cell membrane and potentially disrupting the cell membrane of some of the cells in the area (19). There may be some pushing and pulling of the cells with the propagation of the sound wave through heterogeneous tissues, but it is unlikely that radiation or streaming forces are sufficiently strong or

consistent enough to push drug molecules into the tissue.

From the ultrasonic parameters that have been discussed as above, if one identifies the dominant which induces sonophoresis to a greater extent, then, a better selection of ultrasound parameters and surrounding physicochemical conditions can be made. This will selectively enhance the favorable phenomena, thereby broadening the types of drugs that can be administered transdermally (82).

*Supporting evidence on the role of ultrasonic cavitation effects for transdermal drug delivery*

The role of cavitation in increasing percutaneous permeability due to ultrasound is well supported by a series of *in vitro* experiments: a) the importance of keeping dissolved gas in the medium to form nuclei of cavitation (82), and this gives indirect confirmation about the definite occurrence of cavitation as dissolved gases like entrapped air which contains both oxygen and nitrogen are present in stratum corneum, b) the possibility of permeating cell membranes *in vitro* is enhanced in the presence of artificial cavitation nuclei (39), c) demonstration of possible pores created by ultrasound on the skin surface (68), and within the SC (114,140), d) demonstration of multiple pits induced by bubble implosion on aluminium foil exposed to ultrasound and its correlation with intensity and skin conductivity (132). The possible occurrence and the consequences of cavitation in cells or tissues (22,100) and possible applications in therapy for destruction of cancers (47) and gene therapy (80) have also been studied. The promise of gene therapy lies in the potential to ameliorate or cure conditions that are resistant to conventional therapeutic approaches. Progress in vascular and all other fields of gene therapy has been hampered by concerns over the safety and practicality of recombinant viral vectors and the inefficiency of current non-viral transfection techniques. There is increasing evidence that exposure of eukaryotic cells to relatively modest ultrasound intensity, within the range emitted by diagnostic transducers, either alone or in combination with other non-viral techniques, can enhance transgene expression by up to several orders of magnitude over naked DNA alone. In combination with the flexibility and excellent clinical safety profile of therapeutic and diagnostic ultrasound, it has been suggested that the ultrasound-assisted gene delivery has great promise as a novel approach to improve the efficiency of many forms of non-viral gene delivery (96). Application of ultrasound for gene delivery to cells requires control of cavitation activity. Many studies have been performed using *in vitro* exposure systems, for which cavitation is virtually ubiquitous. *in vivo*, cavitation initiation and control is more difficult, but can be enhanced by cavitation nucleation agents, such as an ultrasound contrast agent. Sonoporation and ultrasonically enhanced gene delivery has been reported for a wide range of conditions including low frequency sonication (kilohertz frequencies), lithotripter shockwaves, HIFU (high intensity focused ultrasound), and even diagnostic ultrasound (megahertz frequencies). The use of ultrasound for non-viral gene delivery has been demonstrated for a robust array of *in vitro* and mammalian systems, which provides a fundamental basis and strong promise for development of new gene therapy methods for clinical medicine.

Indirect proof of cavitation was demonstrated in isolated epidermis *in vitro*, after incubating epidermis with fluorescein, resulting in bleaching of fluorescence probably due to the production of hydroxyl radicals generated by cavitation (82). The existence of dissolved gas deep in living tissue can allow the development of cavitation bubbles (42). Small cavities the size of a few microns, which could correspond to the size of cavitation bubbles on the surface of the stratum corneum, was shown *in vitro* using scanning electron microscopy (68). Scanning electron microscopy showed 1-3 mm holes on the surface of the stratum corneum after exposure to ultrasound (1.1 MHz, 1.5 W/cm$^2$). Such crater-like images of 5-15 mm were also reported in hairless mouse skin exposed to ultrasound *in vitro* (1 MHz, 4.3 W/cm$^2$) (44). Such images have also been shown when experimentally exposing aluminum foil to 20 kHz ultrasound (88,132) and the quantity of pits increased with intensity and reduction of the distance between the skin and the probe.

Yamashita *et al.* (141) have investigated the morphological changes induced in hairless mouse skin after ultrasound irradiation. The scanning electron microscopy examination of hairless mouse skin exposed to ultrasound demonstrated large craterlike pores of 100 μm diameter on the surface of the stratum corneum, corresponding to the size of the bubbles of cavitation, which has been shown in Fig. 5. No lesions were demonstrated after sonication using degassed water indicating that cavitation was the causative mechanism (Fig. 5).

*Sonophoretic enhancement - Differences among drugs*

After optimizing the cavitational parameters for an enhanced drug delivery, the question immediately comes into mind is whether all the drugs applied will be delivered to the same extent? Because, enhancements in the levels of drugs transported through the skin were only observed for particular drugs. This variation between drugs raised controversy about the use of sonophoresis for drug delivery. An explanation for the variation was recently offered based on the differences in physiochemical properties of drugs, for example, lipophilicity and molecular weight. Specifically, small lipophilic drugs, which rapidly diffuse through the skin under passive conditions, do not show enhanced transport after application of ultrasound (84).

The sonophoretic enhancement of transdermal drug transport has been quantitatively predicted based on the knowledge of two physiochemical properties of the drug:

passive skin permeability, $P^P$ and octanol–water partition coefficient, $K_{o/w}$, using the following equation (84):

$$e \sim K_{o/w}^{0.75} / (4 \times 10^4) P^P \quad \text{Eq. 3}$$

where '*e*' is the relative sonophoretic transdermal transport enhancement defined as, [(sonophoretic permeability / passive permeability) - 1].

Based on this equation it can be inferred that this technology is most useful in transporting drugs of a high molecular weight and hydrophilic drugs. Experimental results indicate that the slower the diffusion of a permeant through the lipid bilayers of the SC, the more effective is ultrasound in enhancing its transport, i.e. the drugs passively diffusing through the skin at a slow rate are most enhanced by the application of ultrasound (82).

Whereas, recently Katz *et al.* (51) examined the speed of onset of cutaneous anesthesia by eutectic mixture of local anesthetics (EMLA) cream after brief (approximately 10-S) pretreatment of the underlying skin with low frequency (55 kHz) ultrasound, in human subjects. After ultrasound pretreatment and then 5, 10 or 15 min after EMLA cream application, pain scores and overall preference were statistically indistinguishable from EMLA cream application for 60 min (without ultrasound pretreatment). There were no significant adverse effects and found that low frequency ultrasound pretreatment appears to be safe and effective in producing rapid onset of EMLA cream in this model, with results as early as 5 min.

*Role of cavitational effects on the stability of administered drugs*

It is obvious and highly reasonable to expect the same cavitation and related effects which are responsible in increasing the skin permeability could have effect on the applied drug itself, for example degradation. Eventual degradation of drugs to ultrasound was studied *in vitro* and showed absence of degradation for oligodeoxynucleotides (74), insulin (12), fentanyl and caffeine (13). The persistence of biological activity of insulin and low molecular weight heparin *in vivo* is also an evidence for the absence of degradation (82,90).

*Role of cavitational effects on the skin*

An increasing utility of ultrasound in medicine, in specific in the transdermal transport of various drugs as well as in transdermal extraction of various drugs have caused a much concern directed to the issues of ultrasound bioeffects and safety (58). The world federation for ultrasound in Medicine and Biology (WFUMB) (151) had issued several publications related to safety of ultrasound bioeffects, addressing specifically thermal bioeffects and non-thermal bioeffects in an attempt to reach an international consensus to adopt a policy on safety guidelines (58). The use of ultrasound as an aid to increasing skin permeability is based on its non-thermal bioeffects, mostly cavitation. In view of this much attention should be paid to the issue of ultrasound affecting the structure of the skin; is it a reversible change? What is the role of free radicals that are generated during the

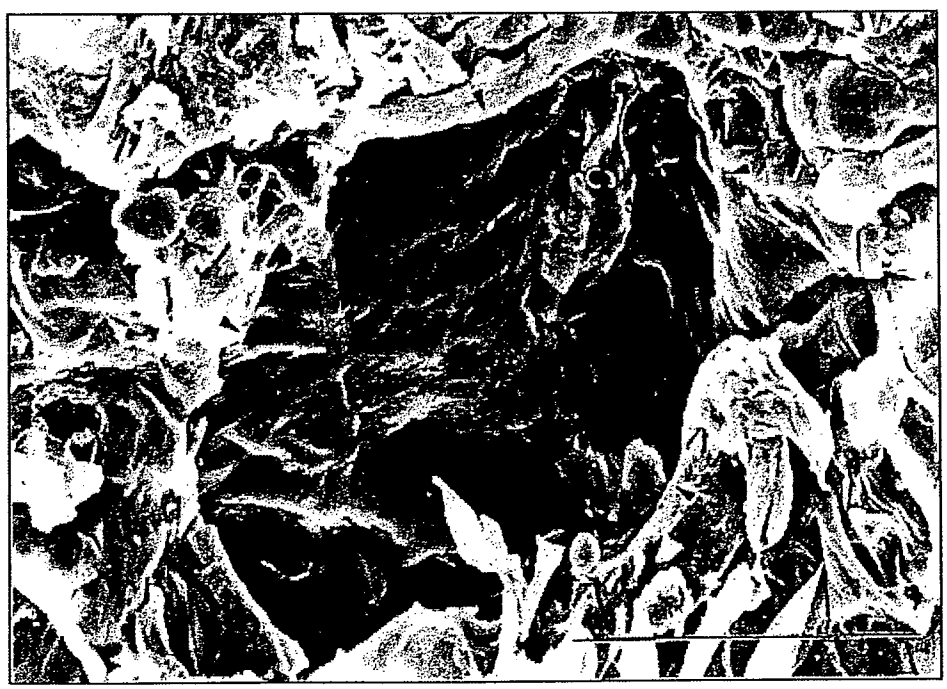

**Fig. 5** Craterlike pores of hairless mouse skin after 5 min of ultrasound exposure (Bar 50 μm)

cavitation process within the skin (58)?

Studies have been carried out to determine the safety of low frequency (20 kHz) sonophoresis on human and rat skin by evaluating their structural modifications after ultrasound exposure. Skin samples were observed under optical and electron microscopy to detect any structural changes. The skin samples exposed to ultrasound intensities lower than 2.5 W/cm$^2$ showed no modification (15). However it was found that at intensities higher than 2.5 W/cm$^2$, it caused a slight and transient erythema whereas severe skin lesions (dermal and muscle necrosis) were observed 24 hr later (54,66,93).

The application of 20 kHz ultrasound at an intensity of 3 W/cm$^2$ was shown to enhance the transdermal transport of interstitial fluid across hairless rat skin (21). In this study, ($H_2O$)-H-3 was used as a tracer which was injected intravenously. A measurable amount of water (>1 ml) was extracted without producing any histologic evidence of injury, even after repeated exposures. Mitragotri *et al.* (83) have shown that the barrier properties of the skin can be modified using low frequency ultrasound (20 kHz) to enhance the efficiency of transdermal drug delivery. Improvement of as much as 1000-fold was achieved in the delivery of hydrophilic and/or large compounds without long-term damage to the barrier properties of the skin (81). Whereas, the effect of an ultrasound (1 MHz) intensities (0.25, 0.5, 0.75 and 1 W/cm$^2$), on transdermal absorption of indomethacin from an ointment studied in rats by Miyazaki *et al.* (92) confirms that 0.5 W/cm$^2$ is the preferred intensity. Although, an intensity of 0.75 W/cm$^2$ leads to the highest penetration but it results in an increase in skin temperature significantly and hence destructive effect on skin. Progressively more skin damage was also noted as the intensity and the time of application of ultrasound increased. Thus, to develop a useful tool based on ultrasound technology, further intensive research focusing on safety issues is required to evaluate limiting ultrasound parameters for safe exposure (58).

*Ultrasound enhanced interstitial fluid extraction (for glucose monitoring)*

Ultrasound permeation of the skin can also be used for glucose monitoring in a home setting. There are several studies on the transdermal extraction of interstitial fluid - enhanced by ultrasound which offers a potential minimally invasive method of obtaining a fluid sample for at-home blood glucose monitoring (21,23,55,112). Ultrasound application led to *in vitro* transdermal extraction of with permeabilities several orders of magnitude higher than those obtained with passive diffusion across skin. For example, passive skin permeability of glucose was about 0.0003 cm/hr, compared with 0.17 cm/hr after ultrasound application (an increase of 570-fold) (55). A device is currently being developed (150) that permeates skin (for up to 24 hr) and then uses a sensor/patch to continuously extract interstitial fluid and monitor blood glucose levels. The continuous non-invasive monitoring of blood glucose may significantly improve patient compliance to frequent glucose testing, which has been shown to reduce severe complications related to diabetes.

*Novel portable ultrasound transdermal delivery system – Cymbal transducer design*

Although a commercial sonicator has been an excellent device for demonstrating drug delivery, the major drawback so far in exploiting the commercial ultrasound device for non-invasive drug delivery is the large size and weight of the ultrasound device. In addition, they require power from a standard outlet with the converter (ultrasonic probe) approximately 20 cm in length and weighing almost a kilogram. For practical application related to portable and low-profile (smaller and light-weight) transdermal drug-delivery system, novel transducer design is the main criteria without compromising on frequency and intensity so that it operates very similar to commercial sonicator.

Recently, cymbal array (f = 20 kHz) design with a light-weight (<22 g), low-profile (37 x 37 x 7 mm$^3$) has been used to generate ultrasound and for transdermally enhancing the delivery of insulin (63). Advantage of using this cymbal array design is that the standard array covers a 37 x 37 mm$^2$ area, whereas the probe tip on a sonicator covers only 10 mm diameter (70). Additional advantage that has been demonstrated with this design was that with short ultrasound exposure time of 5 min, transdermal delivery of insulin reduced the glucose to a significant level. This gives an indication that ultrasound exposure times do not need to be long to deliver a clinically significant insulin dose to reduce a high blood glucose level. These results are further supported by smith *et al.* (119) who have used cymbal array for increasing the transport of insulin.

*Synergistic effects of ultrasound and other enhancers*

Sonophoresis has also been shown to operate in synergy with other enhancers of transdermal drug transport, including chemicals, electroporation and iontophoresis (85). Understanding the synergistic relationship that exists between various enhancers and selecting the right combination represents a large opportunity to develop potent and safe methods to enhance transdermal drug delivery. The effects of combination of enhancers including a) polyethylene glycol 200 dilaurate (PEG), b) isopropyl myristate (IM), c) glycerol trioleate (GT), d) 50% EtOH saturated with linoleic acid (LA/EtOH), and therapeutic ultrasound (1 MHz, 1.4 W/cm$^2$, continuous) on transdermal drug transport of corticosterone have been investigated. LA/EtOH was found to be the most effective of these enhancers, increasing the corticosterone flux from the saturated solutions by up to 13000-fold. Similar enhancements have been obtained with LA/EtOH, with and

without ultrasound for four other model drugs, dexamethasone, estradiol, lidocaine and testosterone (50).

Kost *et al.* (54) have found that the combination of electroporation with ultrasound produced a synergistic interaction and have suggested that this may be caused by ultrasound disorganising stratum corneum lipids to an extent where they were more susceptible to the effects of electroporation. The combination of low frequency ultrasound and iontophoresis also increased the flux of heparin across pig skin above that observed for each of the techniques alone (59). Despite these studies, however, the combination technologies will lag behind the development of individual technologies until safety and efficacy evaluations and validations *in vivo* and in human volunteers can be convincingly demonstrated (25).

*Ultrasound enhanced delivery through cornea*

The successful results of ultrasound-enhanced transdermal drug delivery has also motivated on the investigation of the use of ultrasound to enhance drug delivery through the cornea (144). It has been found that the application of 20-kHz at intensity of 14 W/cm$^2$ resulted in a 4-fold increase in the corneal permeability of atenolol, carteolol, timolol and betaxolol drugs (143). Ultrasound application at medium frequencies (470-880 kHz) and intensities of 0.2-0.3 W/cm$^2$ has also been used for transcorneal drug delivery, to improve treatment of corneal inflammation, wounds, and retinal dystrophy (97,136).

## SUMMARY AND OUTLOOK

Transdermal transport of drugs can be temporarily enhanced and controlled by exploiting the cavitational collapse effects of ultrasound. The most exciting results obtained from various experiments confirm that this method can give about 1000-fold better penetration compared to simple topical application. Sonophoresis is undergoing a renaissance and a significant amount of progress has been made towards this technologically attractive and more promising process. Thus, it has changed from a poorly understood magical treatment to a highly specialized mechanism, but, now that requires the utmost scientific accuracy to be effective which could lead to the development and availability of practical devices based on this technology. Also, the ultimate goal of sonophoresis is to enhance transdermal transport of a *broad variety of drugs*, including high molecular weight proteins which are again possible by the optimization of all the possible parameters of ultrasound and improved understanding of the cavitation events which are occurring on the skin. The recent FDA approval of the use of low frequency portable ultrasound device for skin permeabilisation and the development of low frequency low-profile transducers for sonophoresis has further given a strong hope on this field. Technological change, nonetheless, is transforming the landscape of ultrasound and we can believe that further breakthroughs are on the horizon.

**Acknowledgements** – One of the authors M.Sivakumar thanks the JSPS Postdoctoral Fellowship program for foreign researchers for awarding the fellowship (2003-05).

## REFERENCES

1. Albery, W.J. and Hadgraft, J., Percutaneous absorption: theoretical description. *J. Pharm. Pharmacol.* 1979, **31**: 129-139.
2. Antich, T.J., Phonophoresis. *J Orth. Sports Phys. Ther.* 1982, **4**: 99.
3. Apfel, R.E., Acoustic cavitation - A possible consequence of biomedical uses of ultrasound. *Br. J. Cancer* 1982, **45**(Suppl.): 140-146.
4. Asano, J., Suisha, F., Takada, M., Kawasaki, N. and Miyazaki, S., Effect of pulsed output ultrasound on the transdermal absorption of indomethacin from an ointment in rats. *Biol. Pharm. Bull.* 1997, **20**: 288-291.
5. Atchley, A.A. and Crum, L.A., Acoustic cavitation in bubble dynamics. In: *Ultrasound: its Chemical, Physical and Biological Effects*, Suslick, K.S. (ed.), VCH, New York, 1988.
6. Bare, A., McAnaw, M. and Pritchard, A., Phonophoretic delivery of 10 % hydrocortisone through the epidermis of humans as determined by serum cortisol concentrations. *Phys. Ther.* 1996, **76**: 738-749.
7. Barry, B.W., Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur. J. Pharm. Sci.* 2001, **14**(2): 101-114.
8. Benson, H.A.E., McElnay, J.C. and Harland, R., Phonophoresis of lignocaine and prilocaine from EMLA cream. *Int. J. Pharm.* 1988, **44**: 65-69.
9. Benwell, A.D. and Bly, S.H.P., Sources and applications of ultrasound. In: *Ultrasound: Medical Application, Biological Effects and Hazard Potentials*, Repacholi, M.H. Grandolfo, M. and Rindi, A. (eds.), Plenum Press, New York, 1987, pp. 29-47.
10. Bommannan, D., Menor, G.K., Okuyama, H., Elias, P.M. and Guy R.H., Sonophoresis. II. Examination of the mechanism(s) of ultrasound-enhanced transdermal drug delivery. *Pharm. Res.* 1992, **9**: 1043-1047.
11. Bommannan, D., Okuyama, H., Stauffer, P. and Guy, R.H., Sonophoresis. I., The use of high frequency ultrasound to enhance transdermal drug delivery. *Pharm. Res.* 1992, **9**: 559-564.
12. Boucaud, A., Garrigue, M.A., Machet, L., Vaillant, L. and Patat, F., Effect of sonication parameters on transdermal delivery of insulin to hairless rats. *J. Control Release* 2002, **81**: 113-119.
13. Boucaud, A., Machet, L., Arbeille, B., Machet, M.C., Soumac, M., Mavon, A., Patat, F. and Vaillant, L., *In vitro* study of low-frequency ultrasound-enhanced transdermal transport of fentanyl and caffeine across human and hairless rat skin. *Int. J. Pharm.* 2001, **228**: 69-77.
14. Boucaud, A., Machet, L., Garrigue, M.A., Vaillant, L., Patat, F., A practical use of low frequency ultrasound for rapid and reproducible transdermal delivery of insulin, Presented at the IEEE Ultrasonics Symposium, Atlanta, USA, 2001.
15. Boucaud, A., Montharu, J., Machet, L., Arbeille, B., Machet, M.C., Patat, F. and Vaillant, L., Clinical, histologic and electron microscopy study of skin exposed to low-frequency ultrasound. *Anat. Rec.* 2001, **264**(1): 114-119.
16. Bramson, J., Dayball, K., Evelegh, C., Wan, Y.H., Page, D. and Smith, A., Enabling topical immunization via microporation: a novel method for pain-free and needle-free delivery of adenovirus-based vaccines. *Gene Ther.* 2003, **10**: 251-260.
17. Bremseth, D.L. and Pass, F., Delivery of insulin by jet injection: recent observations. *Diab. Technol. Ther.* 2001, **3**: 225-232.
18. Burnette, R.R., Iontophoresis. In: *Transdermal Therapeutic Systems*, Hadgraft, J. and Guy, R. (eds.), Marcel Dekker, New York, 1989, p.

247.
19. Byl, N.N., The use of ultrasound as an enhancer for transcutaneous drug-delivery-phonophoresis. *Phys. Ther.* 1995, **75**: 539-553.
20. Cagnie, B., Vinck, E., Rimbaut, S. and Vanderstraeten, G., Phonophoresis versus topical application of ketoprofen: Comparison between tissue and plasma levels. *Phys. Ther.* 2003, **83**(8): 707-712.
21. Cantrell, J.T., McArthur, M.J. and Pishko, M.V., Transdermal extraction of interstitial fluid by low-frequency ultrasound quantified with ($H_2O$)-H-3 as a tracer molecule. *J. Pharm. Sci.* 2000, **89**(9): 1170-1179.
22. Carstensen, E.L., Gracewski, S. and Dalecki, D., The search for cavitation *in vivo*. *Ultrasound Med. Biol.* 2000, **26**: 1377-1385.
23. Chuang, H., Taylor, E. and Davison, T.W., Clinical evaluation of a continuous minimally invasive glucose flux sensor placed over ultrasonically permeated skin. *Diab. Technol. Ther.* 2004, **6**(1): 21-30.
24. Cornwell, P.A. and Barry, B.W., Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. *J. Pharm. Pharmacol.* 1994, **46**(4): 261-269.
25. Cross, S.E. and Roberts, M.S., Physical enhancement of transdermal drug application: is delivery, technology keeping up with pharmaceutical development? *Curr. Drug Delivery* 2004, **1**: 81-92.
26. Crum, L.A., Surface oscillations and jet development in pulsating bubbles. *J. Phys.* 1979, **40**: 285-288.
27. Daniels, R., Strategies for skin penetration enhancement, Skin Care Forum, 2004, **37** *http://www.scf-online.com/english/37_e/skinpenetration37_e.htm*
28. Doukas, A.G. and Kollias, N., Transdermal drug delivery with a pressure wave. *Adv. Drug Deliver Rev.* 2004, **56**(5): 559-579.
29. Duck, F.A., Baker, A.C. and Staritt, H.C., *Ultrasound in Medicine*, Institute of Physics Publ., Bristol, 1998.
30. Dyson, M., Mechanisms involved in therapeutic ultrasound. *Physiotherapy* 1987, **73**: 116-120.
31. Edmonds, P.D. and Sancier, K.M., Evidence for free radical production by ultrasonic cavitation in biological media. *Ultrasound Med. Biol.* 1983, **9**: 635-639.
32. Esche, R., Untersuchung der Schwingungskavitation in Flüssigkeiten. *Acustica* 1952, **2**: 208-218.
33. Everbach, E.C., Makin, I.R., Azadniv, M. and Meltzer, R.S., Correlation of ultrasound-induced hemolysis with cavitation detector output *in vitro*. *Ultrasound Med. Biol.* 1997, **23**: 619-624.
34. Fellinger, K. and Schmidt, J., *Klinik and Therapies des Chromischen Gelenkreumatismus*. Maudrich Vienna, Austria, 1954, 549.
35. Fernandes, D., Maximising skin care with the use of advanced skin penetration techniques, 6th Internet World Congress for Biomedical Sciences, 2000, February 14-25.
36. Flynn, H.G., Generation of transient cavities in liquids by microsecond pulses of ultrasound. *J. Acoust. Soc. Am.* 1982, **72**: 1926-1932.
37. Gay, C.L., Guy, R.H., Golden, G.M., Mak, V.H. and Francoeur, M.L., Characterization of low-temperature (i.e., <65 degrees C) lipid transitions in human stratum corneum. *J. Invest. Dermatol.* 1994, **103**: 233-239.
38. Gebhart, S., Faupel, M., Fowler, R., Kapsner, C., Lincoln, D., McGee, V., Pasqua, J., Steed, L., Wangsness, M., Xu, F. and Vanstory, M., Glucose sensing in transdermal body fluid collected under continuous vacuum pressure via micropores in the stratum corneum. *Diab. Technol. Ther.* 2003, **5**(2): 159-166.
39. Greenleaf, W.J, Bolander, M.E., Sarkar, G., Goldring, M.B. and Greeleaf, J.F., Artificial cavitation nuclei significantly enhance acoustically induced cell transfection. *Ultrasound Med. Biol.* 1998, **24**: 587-595.
40. Guy, R.H. and Hadgraft, J., Selection of drug candidates for transdermal drug delivery. In: *Transdermal Drug Delivery, Developmental Issues and Research Initiatives*, Hadgraft, J. and Guy, R.H. (eds.), Marcel Dekker, New York 1989, pp. 59-81.
41. Guy, R.H. and Hadgraft, J., Principles of skin permeability relevant to chemical exposure. In: *Dermal and Ocular Toxicology: Fundamentals and Methods*, Hobson, D.W. (ed), CRC Press, Boca-Raton, FL, 1991, pp. 221-246.
42. ter Haar, G.R. and Daniels, S., Evidence for ultrasonically induced cavitation *in vivo*. *Phys. Med. Biol.* 1981, **26**: 1145-1149.
43. ter Haar, G.R., Biological effects of ultrasound in clinical applications. In: *Ultrasound its Chemical, Physical and Biological Effect*, Suslick, K.S. (ed.), VCH, New York, 1988, pp. 305-320.
44. Hikima, T., Hirai, Y. and Tojo, K., Effect of ultrasound application on skin metabolism of prednisolone 21-acetate. *Pharm. Res.* 1998, **15**: 1680-1683.
45. Hill, C.R., *Physical Principles of Medical Ultrasonics*, Ellis Horwood, Chichester, 1986.
46. Hingson, R.A. and Figge, F.H., A survey of the development of jet injection in parenteral therapy. *Curr. Res. Anesth. Analg.* 1952, **31**: 361-366.
47. Huber, P.E. and Debus, J., Tumour cytotoxicity *in vivo* and radical formation *in vitro* depend on the shock wave-induced cavitation. *Radiat. Res.* 2001, **156**: 301-309.
48. Hussey, M., *Basic Physics and Technology of Medical Diagnostic Ultrasound*, MacMillan and Sons, London, 1985.
49. Jacques, S.L., McAuliffe, D.J., Irvin, B.S., Blank, I.H. and Parrish, J.A., Controlled removal of human stratum-corneum by pulsed laser. *J. Invest. Dermatol.* 1987, **88**: 88-93.
50. Johnson, M.E., Mitragotri, S., Patel, A., Blankschtein, D. and Langer, R., Synergistic effects of chemical enhancers and therapeutic ultrasound on transdermal drug delivery. *J. Pharm. Sci.* 1996, **85**(7): 670-679.
51. Katz, N.P., Shapiro, D.E., Herrmann, T.E., Kost, J. and Custer, L.M., Rapid onset of cutaneous anesthesia with EMLA cream - After pretreatment with a new ultrasound-emitting device. *Anesth. Analg.* 2004, **98**: 371-376.
52. Kinsler, L.E., Frey, A.R., Coppens, A.B. and Sanders, J.V., *Fundamentals of Acoustics*, John Wiley & Sons, 3d ed., 1982.
53. Kodama, T., Hamblin, M.R. and Doukas, A.G., Cytoplasmic molecular delivery with shock waves: importance of impulse. *Biophys. J.* 2000, **79**(4): 1821-1832.
54. Kost, J., Pliquett, U., Mitragotri, S., Yamamoto, A., Langer, R. and Weaver, J.C.J., Synergistic effect of electric field and ultrasound on transdermal transport. *Pharm. Res.* 1996, **13**: 633-638.
55. Kost, J., Mitragotri, S., Gabbay, R.A., Pishko, M. and Langer, R., Transdermal monitoring of glucose and other analytes using ultrasound. *Nat. Med.* 2000, **6**(3): 347-350.
56. Kushner, J., Blankschtein, D. and Langer, R., Experimental demonstration of the existence of highly permeable localize transport regions in low-frequency sonophoresis. *J. Pharm. Sci.* 2004, **93**(11): 2733-2745.
57. Langer, R., Biomaterials in drug delivery and tissue engineering: one laboratory's experience. *Acc. Chem. Res.* 2000, **33**: 94-101.
58. Lavon, I. and Kost, J., Ultrasound and transdermal drug delivery. *Drug Discov Today* 2004, **9**(15): 670-676.
59. Le, L., Kost, J. and Mitragotri, S., Combined effect of low-frequency ultrasound and iontophoresis: Applications for transdermal heparin delivery. *Pharm. Res.* 2000, **17**(9): 1151-1154.
60. Lee, S., Kollias, N., McAuliffe, D.J., Flotte, T.J. and Doukas, A.G., Topical drug delivery in humans with a single photomechanical wave. *Pharm. Res.* 1999, **16**: 1717-1721.
61. Lee, S., McAuliffe, D.J., Mulholland, S.E. and Doukas, A.G., Photomechanical transdermal delivery of insulin *in vivo*. *Lasers Surg. Med.* 2001, **28**: 282-285.
62. Lee, S., McAuliffe, D.J., Kollias, N., Flotte, T.J. and Doukas, A.G., Photomechanical delivery of 100-nm microspheres through the stratum corneum: Implications for transdermal drug delivery. *Lasers Surg. Med.* 2002, **31**: 207-210.

63. Lee, S.J., Newnham, R.E. and Smith, N.B., Short ultrasound exposure times for noninvasive insulin delivery in rats using the lightweight cymbal array. *IEEE T. Ultrason. Ferr.* 2004, **51**(2): 176-180.
64. Lee, W.R., Shen, S.C., Lai, H.H., Hu, C.H. and Fang, J.Y., Transdermal drug delivery enhanced and controlled by erbium: YAG laser: a comparative study of lipophilic and hydrophilic drugs. *J. Control Release* 2001, **75**: 155-166.
65. Leighton, T., *The Acoustic Bubble*, Acad. Press, San Diego, 1997.
66. Levy, D., Kost, J., Meshulam, Y. and Langer, R., Effect of ultrasound on transdermal drug delivery to rats and guinea-pigs. *J. Clin. Invest.* 1989, **83**: 2074-2078.
67. Liu, J., Lewis, T. and Prausnitz, M., Non-invasive assessment and control of ultrasound-mediated membrane permeabilization. *Pharm. Res.* 1998, **15**(6): 918-924.
68. Machet, L., Cochelin, N., Patat, F., Arbeille, B., Machet, M.C., Lorette, G. and Vaillant, L., *in vitro* phonophoresis of mannitol, oestradiol and hydrocortisone across human and hairless mouse skin. *Int. J. Pharm.* 1998, **165**: 169-174.
69. Machet, L. and Boucaud, A., Phonophoresis: efficiency, mechanisms and skin tolerance. *Int. J. Pharm.* 2002, **243**: 1-15.
70. Maione, E., Shung, K.K., Meyer, R.J., Hughes, J.W., Newnham, R.E. and Smith, N.B., Transducer design for a portable ultrasound enhanced transdermal drug-delivery system. *IEEE T. Ultrason. Ferr.* 2002, **49**(10): 1430-1436.
71. Mason, T.J. and Lorimer, J.P., Sonochemistry - Theory, applications and uses of ultrasound in chemistry. Ellis Horwood, New York, 1988.
72. Mason, T.J., *Practical Sonochemistry*, Ellis Horwood, New York, 1991.
73. Mason, T.J., *Sonochemistry*, Oxford University Press, Oxford, 1999.
74. Meidan, V.M., Walmsley, A.D. and Irwin, W.J., Phonophoresis - Is it a reality. *Int. J. Pharm.* 1995, **118**: 129-149.
75. Menczel, E., Skin delipidization and percutaneous absorption. In: *Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery*, Bronaugh, R.L. and Maibach, H.I. (eds.), Marcel Dekker, New York, 1985, pp. 231-242.
76. Menon, G.K. and Elias, P.M., Morphologic basis for a pore-pathway in mammalian stratum corneum. *Skin Pharmacol.* 1997, **10**: 235-246.
77. Menon, G.K., Kollias, N. and Doukas, A.G., Ultrastructural evidence of stratum corneum permeabilisation induced by photomechanical waves. *J. Invest. Dermatol.* 2003, **121**(1): 104-109.
78. Merino, G., Kalia, Y.N., Delgado-Charro, M.B., Potts, R.O. and Guy, R.H., Frequency and thermal effects on the enhancement of transdermal transport by sonophoresis. *J. Control Release* 2003, **88**(1): 85-94.
79. Miller, M.W., Miller, D.L. and Brayman, A.A., A review of *in vitro* bioeffects of inertial ultrasonic from a mechanistic perspective. *Ultrasound Med. Biol.* 1996, **22**: 1131-1154.
80. Miller, D.L., Pislaru, S.V. and Greenleaf, J.E., Sonoporation: mechanical DNA delivery by ultrasonic cavitation. *Somat. Cell Mol. Genet.* 2002, **27**(1-6): 115-134.
81. Mitragotri, S., Blankschtein, D. and Langer, R., Ultrasound-mediated transdermal protein delivery. *Science* 1995, **269**: 850-853.
82. Mitragotri, S., Edwards, D.A., Blankschtein, D. and Langer, R., Mechanistic study of ultrasonically-enhanced transdermal drug-delivery. *J. Pharm. Sci.* 1995, **84**: 697-706.
83. Mitragotri, S., Blankschtein, D. and Langer, R., Transdermal drug delivery using low-frequency sonophoresis. *Pharm Res.* 1996, **13**: 411-420.
84. Mitragotri, S., Blankschtein, D. and Langer, R., An explanation for the variation of the sonophoretic transdermal transport enhancement from drug to drug. *J. Pharm. Sci.* 1997, **86**: 1190-1192.
85. Mitragotri, S., Synergistic effect of enhancers for transdermal drug delivery. *Pharm. Res.* 2000, **17**: 1354-1359.
86. Mitragotri, S., Coleman, M., Kost, J. and Langer, R., Analysis of ultrasonically extracted interstitial fluid as a predictor of blood glucose levels. *J. Appl. Physiol.* 2000, **89**: 961-966.
87. Mitragotri, S., Coleman, M., Kost, J. and Langer, R., Transdermal extraction of analytes using low-frequency ultrasound. *Pharm. Res.* 2000, **17**(4): 466-470.
88. Mitragotri, S., Farrell, J., Tang, H., Terahara, T., Kost, J. and Langer, R., Determination of threshold energy dose for ultrasound-induced transdermal drug transport. *J. Control. Release* 2000, **63**: 41-52.
89. Mitragotri, S. and Kost, J., Low frequency sonophoresis: a noninvasive method for drug delivery and diagnostics. *Biotechnol. Prog.* 2000, **16**: 488-492.
90. Mitragotri, S. and Kost, J., Transdermal delivery of heparin and low-molecular weight heparin using low-frequency ultrasound. *Pharm. Res.* 2001, **18**: 1151-1156.
91. Mitragotri, S. and Kost, J., Low-frequency sonophoresis - A review. *Adv Drug Deliver Rev.* 2004, **56**: 589-601.
92. Miyazaki, S., Mizuoka, H., Kohata, Y. and Takada, M., External control of drug release and penetration. VI. Enhancing effect of ultrasound on the transdermal absorption of indomethacin from an ointment in rats. *Chem. Pharm. Bull.* 1992, **40**(10): 2826-2830.
93. Monti, D., Saettone, M.F., Giannaccini, B. and Galli-Angeli, D., Enhancement of transdermal penetration of dapiprazole through hairless mouse skin. *J. Control Release* 1995, **33**: 71-77.
94. Murthy, S.N., Magnetophoresis: an approach to enhance transdermal drug diffusion. *Pharmazie* 1999, **54**: 377-379.
95. Nelson, J.S., McCullough, J.L., Glenn, T.C., Wright, W.H., Liaw, L.H. and Jacques, S.L., Midinfrared laser ablation of stratum-corneum enhances *in vitro* percutaneous transport of drugs. *J. Invest. Dermatol.* 1991, **97**(5): 874-879.
96. Newman, C.M., Lawrie, A., Brisken, A.F. and Cumberland, D.C., Ultrasound gene therapy: on the road from concept to reality. *Echocardiogr. J. Card.* 2001, **18**(4): 339-347.
97. Nuritdinov, V.A., Phonophoresis and cavitation. Phonophoresis and cavitation. *Vestn. Thalmol.* 1981, **1**: 56-58.
98. Nussbaum, E., Therapeutic ultrasound. In: *Physical Agents: Theory and Practice*, Behrens, B. and Michlovitz, S. (eds.), F.A. Davis, Philadelphia, 1996, pp. 81-117.
99. Pliquett, U. and Weaver, J.C., Electroporation of human skin; Simultaneous measurement of changes in the transport of two fluorescent molecules and in the passive electrical properties. *Bioelectrochem. Bioenerg.*, 1996, **39**: 1-12.
100. Poliachik, S.L., Chandler, W.L., Mourad, P.D., Ollos, R.J. and Crum, L.A., Activation, aggregation and adhesion of platelets exposed to high-intensity focused ultrasound. *Ultrasound Med. Biol.* 2001, **27**: 1567-1576.
101. Potts, R.O., Physical characterization of the stratum corneum: the relationship of mechanical and barrier properties to lipid and protein structure. In: *Transdermal Drug Delivery. Developmental Issues and Research Initiatives*, Hadgraft, J. and Guy, R.H. (eds.), Marcel Dekker, New York, 1989, pp. 23-57.
102. Potts, R.O. and Guy, R.H., Predicitng skin permeability. *Pharm. Res.* 1991, **9**: 663-669.
103. Potts, R.O. and Guy, R.H., A predictive algorithm for skin permeability: the effects of molecular size and hydrogen bond activity. *Pharm. Res.* 1995, **11**: 1628-1633.
104. Prausnitz, M.R., Bose, V.G., Langer, R. and Weaver, J.C., Electroporation of mammalian skin - A mechanism to enhance transdermal drug delivery. *Proc. Natl. Acad. Sci. USA* 1993, **90**: 10504-10508.
105. Prausnitz, M.R., Lee, C.S., Liu, C.H., Pang, J.C., Singh, T.P., Langer, R. and Weaver, J.C., Transdermal transport efficiency during skin electroporation and iontophoresis. *J. Control Release* 1996, **38**: 205-217.
106. Prausnitz, M.R., Mitragotri, S. and Langer, R., Current status and future potential of transdermal drug delivery. *Nat. Rev. Drug Discov.* 2004, **3**: 115-124.

107. Price, N.M., Schmitt, L.G., McGuire, J., Shaw, J.E. and Trobough, G., Transdermal scopolamine in the prevention of motion sickness at sea. *Clin. Pharmacol. Ther.* 1981, **29**: 414-419.
108. Roberts, M.S., Lai, P.M., Cross, S.E. and Yoshida, N.H., Solute structure as a determinant of iontophoretic transport. In: *Mechanisms of Transdermal Delivery*, Potts, P.O. and Guy, R.H. (eds.), Marcel Dekker, New York, 1997, pp. 291-349.
109. Santini, J.T., Cima, M.J. and Langer, R., A controlled-release microchip. *Nature* 1999, **397**: 335-338.
110. Scheuplein, R.J., Mechanism of percutaneous absorption: I. Routes of penetration and the influence of solubility. *J. Invest. Dermatol.* 1965, **29**: 131-149.
111. Scheuplein, R.J., Mechanism of percutaneous absorption: II. Transient diffusion and the relative importance of various routes of skin penetration. *J. Invest. Dermatol.* 1967, **48**: 79-88.
112. Sieg, A., Guy, R.H. and Delgado-Charro, M.B., Noninvasive and minimally invasive methods for transdermal glucose monitoring. *Diab. Technol. Ther.* 2005, **7**(1): 174-197.
113. Simonin, J.P., On the mechanisms of *in vitro* and *in vivo* phonophoresis. *J. Control Release* 1995, **33**: 125-141.
114. Singer, A.J., Homan, C.S., Church, A.L. and McClain, S.A., Low-frequency sonophoresis: Pathologic and thermal effects in dogs. *Acad. Emerg. Med.* 1998, **5**: 35-40.
115. Sintov, A.C., Krymberk, I., Daniel, D., Hannan, T., Sohn, Z. and Levin, G., Radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. *J. Control Release* 2003, **89**: 311-320.
116. Sivakumar, M., Senthilkumar, P. and Pandit, A.B., Ultrasound enhanced PTC conversion of benzamide to benzonitrile. *Synthetic Commun.* 2001, **31**(17): 2583-2587.
117. Sivakumar, M., Senthilkumar, P., Majumdar, S. and Pandit, A.B., Ultrasound mediated alkaline hydrolysis of methyl benzoate - reinvestigation with crucial parameters, *Ultrason. Sonochem.* 2002, 9(1): 25-30.
118. Smith, E.W. and Maibach, H.I., *Percutaneous Penetration Enhancers*, CRC Press, Boca Raton, FL, 1995.
119. Smith, N.B., Lee, S. and Shung, K.K., Ultrasound-mediated transdermal *in vivo* transport of insulin with low-profile cymbal arrays, *Ultrasound Med. Biol.* 2003, 29(8): 1205-1210.
120. Sun, Y. and Liu, J., Transdermal drug delivery by phonphoresis: basics, mechanisms and techniques of application. In: *Drug Permeation Enhancement*, Hseih, D.S. (ed.), Marcel Dekker, New York, 1994, pp. 303-321.
121. Sundaram, J., Mellein, B.R. and Mitragotri, S., An experimental and theoretical analysis of ultrasound-induced permeabilization of cell membranes. *Biophys J.* 2003, **84**: 3087-3101.
122. Suslick, K.S., Einhorn, J., Luche, J.-L., In: *Ultrasound, its Chemical, Physical and Biological Effect*, VCH, Weinheim, 1988.
123. Suslick, K.S., *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., J. Wiley & Sons, New York, 1998, **26**: pp. 517-541.
124. Suslick, K.S., Didenko, Y., Fang, M., Hyeon, T., Kolbeck, K.J. and McNamara, W.B., Acoustic cavitation and its chemical consequences. *Phil. Trans. Roy. Soc. A.* 1999, **357**: 335-353.
125. Suslick, K.S., McNamara III, W.B. and Didenko, Y., Hot spot conditions during multi-bubble cavitation. In: *Sonochemistry and Sonoluminescence*, Crum, L.A., Mason, T.J., Reisse, J. and Suslick, K.S. (eds.), Kluwer Publ., Dordrecht, Netherlands, 1999, pp. 191-204.
126. Tachibana, K. and Tachibana, S., Transdermal delivery of insulin by ultrasonic vibration. *J. Pharm. Pharmacol.* 1991, **43**(4): 270-271.
127. Tachibana, K., Transdermal delivery of insulin to alloxan-diabetic rabbits by ultrasound exposure. *Pharm. Res.* 1992, **9**(7): 952-954.
128. Tachibana, K. and Tachibana, S., Use of ultrasound to enhance the local-anesthetic effect of topically applied aqueous lidocaine. *Anesthesiology* 1993, **78**: 1091-1096.
129. Tachibana, K., Uchida, T., Ogawa, K., Yamashita, N. and Tamura, K., Induction of cell-membrane porosity by ultrasound. *Lancet* 1999, **353**(9162): 1409-1409.
130. Tang, H., Blankschtein, D. and Langer, R., Effects of low-frequency ultrasound on the transdermal permeation of mannitol: Comparative studies with *in vivo* and *in vitro* skin. *J. Pharm. Sci.* 2002, **91**(8): 1776-1794.
131. Tang, H., Wang, C.C.J., Blankschtein, D. and Langer, R., An investigation of the role of cavitation in low-frequency ultrasound-mediated transdermal drug transport, *Pharm. Res.* 2002, **19**(8): 1160-1169.
132. Terahara, T., Mitragotri, S., Kost, J. and Langer, R., Dependence of low-frequency sonophoresis on ultrasound parameters; distance of the horn and intensity. *Int. J. Pharm.* 2002, **235**: 35-42.
133. Terahara, T., Mitragotri, S. and Langer, R., Porous resins as a cavitation enhancer for low-frequency sonophoresis. *J. Pharm. Sci.* 2002, **91**: 753-759.
134. Tezel, A., Sens, A., Tuchscherer, J. and Mitragotri, S., Frequency dependence of sonophoresis. *Pharm. Res.* 2001, **18**: 1694-1700.
135. Tezel, A., Sens, A. and Mitragotri, S., Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy. *J. Pharm. Sci.* 2002, **91**(2): 444-453.
136. Tsok, R.M., Gereliuk, I.P., Tsok, O.B. and Kaminskii, I.M., The effect of ultrasonic oscillations of different frequencies on radionuclide accumulation in the eye tissues. *Ofthalmol Zh.* 1990, **1**: 46-49.
137. Walters, K.A., Penetration enhancers and their use in transdermal therapeutic systems. In: *Transdermal Drug Delivery, Developmental Issues and Research Initiatives*, Hadgraft, J. and Guy, R.H. (eds.), Marcel Dekker, New York 1989, pp. 197-246.
138. Williams, A.R., Production and transmission of ultrasound. *Physiotherapy* 1987, **73**: 113-116.
139. Williams, A.C. and Barry, B.W., Penetration enhancers, *Adv Drug Deliver Rev.* 2004, **56**(5): 603-618.
140. Wu, J., Chappelow, J., Yang, J. and Weimann, L., Defects generated in human stratum corneum specimens by ultrasound. *Ultrasound Med. Biol.* 1998, **24**: 705-710.
141. Yamashita, N., Tachibana, K., Ogawa, K., Tsujita, N. and Tmita, A., Scanning electron microscopic evaluation of the skin surface after ultrasound exposure. *Anat. Rec.* 1997, **247**: 455-461.
142. Zarnitsyn, V.G., Prausnitz, A.R. and Chizmadzhev, Y.A., Physical methods of nucleic acid delivery into cells and tissues. *Biol. Membr.* 2004, **21**(5): 355-373.
143. Zderic, V., Vaezy, S., Martin, R.W. and Clark, J.I., Ocular drug delivery using 20-kHz ultrasound. *Ultrasound Med Biol.* 2002, **28**(6): 823-829.
144. Zderic, V., Clark, J.I., Martin, R.W. and Vaezy, S., Ultrasound-enhanced transcorneal drug delivery. *Cornea* 2004, **23**(8): 804-811.
145. Zhang, I., Shung, K.K. and Edwards, D.A., Hydrogels with enhanced mass transfer for transdermal drug delivery. *J. Pharm. Sci.* 1996, **85**: 1312-1316.
146. Electronic Orange Book. Food and Drug Administration *www.fda.gov/cder/ob*
147. *http://www.3m.com/us/healthcare/manufacturers/dds/jhtml/patch_anatomy.jhtml*
148. *http://www.drugdeliverytech.com/cgi-bin/articles.cgi? idArticle=143*
149. *http://www.gf-lifestyles.com/transdermal_enhancement.html*
150. *http://www.sontra.com* (Symphony™ Diabetes Management System, Sontra, Franklin, MA, USA)
151. *http://www.transpharma-medical.com/product_apps_resources001130.html* and *http://www.wfumb.org*