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TRANSDERMAL DRUG DELIVERY USING ULTRASOUND: PENETRATION MECHANISMS AND APPLICATIONS

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ABSTRACT

Transdermal drug delivery (TDD) has advantages over oral drug delivery. However, the use of TDD is limited by the outermost layer of stratum corneum (SC) which exhibits low permeability. Recently, low frequency ultrasound mediated drug delivery has been studied as a method to enhance transdermal drug penetration. Several studies investigated the mechanisms of transdermal drug penetration with the help of ultrasound. In addition, cavitation is believed to be the predominant mechanism. Moreover, other studies discussed different ultrasound parameters and their effect on drug penetration through the skin. Several applications of TDD using ultrasound were approved on proteins, antibiotics, NSAIDs and other drugs loaded in nano carriers. This review presents the details of studies on ultrasound including ultrasound parameters and their effect on TDD, mechanisms of drug penetration due to ultrasound and various therapeutic applications using ultrasound.

Keywords: ultrasound, low frequency ultrasound, transdermal drug delivery (TDD), cavitation

INTRODUCTION:

Since they are convenient, oral medications are the most commonly used drug worldwide **[1]**. However, oral administration of medicines has several disadvantages such as the exposure to hepatic first pass effect, destruction of drug molecule in GI tract and other problems in connection with the disposal of orally taken drugs **[2]**. According to release various diseases, pharmaceutical companies produced many advanced and potent drugs. Numerous of these drugs cannot effectively work in the body due to biological barriers such as blood brain barrier **[3]**. Transdermal drug delivery (TDD) has many attractive advantages and is considered as an alternative to Oral and injection medications. For example, transdermal drug delivery can prevent gastrointestinal drug metabolism **[4]**, maintain a steady drug concentration due to its low hepatic first pass effect **[5, 6]** and provide sustained release of drugs for up to 7 days **[4]**. In spite of the different advantages of TDD as well as the efforts made by the researchers to develop this route of administration **[2]**, the low permeability of human skin remains the main reason that limits the use of TDD as a route of administration **[6]**. However, there are fewer than 20 drugs that can be currently delivered across the skin **[7]**. Scoplamine was introduced in 1981 as the first transdermal therapeutic system. In addition, other drugs like (Nitroglycerine, estradiol and Clonidine) have been developed to be delivered transdermally **[8]**. Few drugs like peptides and proteins are now successfully delivered across the skin for clinical applications **[9]**. The low permeability of the stratum corneum (SC) is the main challenge and barrier against development of transdermal drug delivery systems either topically or systemically **[10]**. SC is the outermost hard layer of the skin that consists of a continuous lipid layer with embedded dead cells from keratinocytes. Since the diffusion rate depends on the size, hydrophobicity and concentration of drugs across SC, skin can permeate only lipophilic drugs with small doses (<10 mg/day) and low molecular weight drugs (<500 Da) **[3]**. On the other hand, some current studies show that the whole epidermis, not just SC, is responsible for low skin permeability. This is very important for drugs that need to reach blood circulation like insulin **[11]**. Thereby, the application of TDD is very limited and just few low molecular weights of compounds can be delivered by skin **[4]**. Due to the fact stating that most drugs cannot permeate through the skin in the therapeutic quantity **[6]**, disordering the stratum corneum

structure of the skin is becoming necessary **[12]**. To overcome the skin barriers caused by SC, several approaches were proposed and developed **[13]** including chemical penetration enhancers or physical methods such as micro needles **[14]**, iontophoresis **[15]**, electroporation **[16]** and ultrasound (sonophoresis) **[17]**. Using external physical enhancers (e.g ultrasound) could be better than chemical penetration enhancers, as the external enhancers can be stopped and reused if needed. Thus, they control the rate of drug release **[8]**. In this review, we make an overview on previous studies that reported the effect of ultrasound on the drug uptake through the skin and the mechanisms of drug penetration with the help of US.

1. Ultrasound as an enhancer for percutaneous drug absorption:

According to the development of diagnostic ultrasound and its therapeutic benefits, ultrasound (US) has been introduced as a method to treat cancer. Ultrasound generates heat effectively and is used for hyperthermia treatment. It is found that high intensity focused ultrasound (HIFU) is very effective in the treatment of tumors **[18]**. Nowadays, using HIFU is becoming the commonly accepted method used clinically as a treatment modality enabling non-invasive tissue heating and ablation in the numerous applications of radiation therapy such as tumor therapy lithotripsy, ultrasound assisted lipoplasty and ultrasonic surgical instruments **[6]**. US is also used for the enhancement of drug uptake through skin. It is available as a technique called sonophoresis. Most studies showed that the low frequency sonophoresis (20-150 kHz) enhances transdermal absorption of many drugs in vitro **[10]**. Fellinger and Schmid were the first researchers who reported the successful treatment of polyarthritis in the hand and joints by using US combined with hydrocortisone in 1954 **[19, 20]**. In 2004, US FDA approved low frequency sonophoresis (LFS) for delivering the local anesthetic lidocaine **[19]**. Accordingly, the studies conducted on US for enhancing the transdermal transport of several drugs and vaccines are increasing exponentially in recent years **[11]**. The function of SC barrier is changed by Waveform produced from LF sonophoresis. Hence, increasing the percutaneous absorption of the drugs and enhancing their transdermal transport through the skin. US is non-invasive, painless and functions independently of the drug electrical characteristics **[2]**.

1.1 Ultrasound parameters and their effect on transdermal drug penetration:

The main component of ultrasound (US) is transducer **[21]** which comprises of quartz crystals called piezoelectric. Acoustic waves are produced by oscillations which result from the transformation of electric energy into mechanical energy. Waves are divided into two parts. The first part of waves is reflected by the medium where they fall. Other waves penetrate into the medium in which they are propagated. Thus, the lost energy, which are formed during propagation, is converted into heat **[22]**. The limiting factor facing ultrasound mediated transdermal drug delivery system (TDDS) is the transducer which is often expensive, bulky and large in size. Therefore, small US transducers (Cymbal) have been produced, weighting only 3g, in order to make ultrasound portable and user friendly **[21]**. It has been found that the enhancement of skin permeability caused by sonophoresis depends on different ultrasound parameters such as frequency, intensity, duration of ultrasound application **[23]**, continuous or pulse mode, type of coupling agents and coupling technique **[24]**. On the other hand, Mitragotri et al determined the threshold by using in vitro measurements of the dependence of Sonophoresis enhancement on ultrasound parameters. They showed that the enhancement are directly proportional with ultrasound intensity and exposure times and it is independent of the duty cycle (continuous or pulsed mode) in some parameter studies. Once the threshold is crossed, the enhancement varies linearly with ultrasound energy density **[25]**.

Frequency:

Ultrasound (US) frequency is the number of oscillations that take place per unit time. It can be measured in units per second or Hertz (Hz) **[21]**. Most previous studies classified the US frequency into two main types; low frequency ultrasound (LFUS) (20-100 kHz) and therapeutic frequency ultrasound (1-3 MHz) **[2, 23 and 26]**. The size of US transducer crystal plays a key role in the frequency of an emitted wave. There is an inversely proportional relationship between acoustic wave and its frequency. Thus, the more the frequency, the less deeply the US penetrates into the skin **[22]**. Accordingly, sonophoresis works when low frequency ultrasound (100 kHz) promotes the skin permeability and then improves transdermal drug delivery (TDD) **[27]**. In the last days, low frequency US has been used in several medical applications. One of these applications is the enhancement of transdermal drug transport, whereas LFUS distorts lipid bilayer due to cavitation and then generates aqueous channels into the

stratum corneum (SC). The main factor that helps the LFUS to increase skin permeability is cavitation. Cavitation is described as the formation and collapse of gaseous cavities. This explanation is considered comprehensive for the working mechanism of sonophoresis **[2]**. In other words, the sonication using low-frequency ultrasound has been shown to create openings in the stratum corenum **[7]**. Aldwekat and Alarjah found that the skin permeability increases significantly when frequency of power decreases to 20 kHz after adjusting intensity and treating skin for 5-minute time **[2]**. Another study approved that low frequency ultrasound enhanced the percutaneous transport of water, caffeine and fentanyl through human and hairless rat skins in vitro **[5]**. In addition, low frequency ultrasound increases skin permeability of other several drugs such as insulin, lidocaine, tetanus toxoid antigen and low molecular weight heparin **[7]**. Mitragotri *et al* assessed the effect of application low frequency ultrasound (20kHz, 125mW/cm2,100msec pulses applied every second) on the transdermal transport for different drugs by using in vitro (human cadaver epidermis) as well as in vivo (hairless rat skin) **[28]**. They found that LFUS enhanced transdermal transport of several substances such as estradiol, salicylic acid, corticosterone, sucrose, aldosterone, water and butanol across human cadaver skin and salicylic acid across hairless rat skin. Merino et al confirms the much greater efficiency of low frequency sonophoresis as a method to enhance transdermal permeability, although most literatures agreed that LFUS (20-100 kHz) enhances transdermal drug delivery for many drugs and vehicles. Recently, there are many studies try to approve the effectiveness of middle Frequency US (100-200 kHz) and high frequency US (1-3 MHz) **[11]**. Some sonophoretic studies showed that sonophoresis has no effect on the skin permeability while other studies demonstrated moderate to significant effect of sonophoresis on the transdermal drug delivery enhancement. The variation in the permeability enhancement by therapeutic frequency US could be due to the differences of physicochemical properties of the studied molecules and carriers **[2]**. Mitragotri et al found that 1-MHz ultrasound enhances transdermal transport of estradiol across human cadaver skin in vitro by 13-fold, but the 3-MHz ultrasound at the same intensity induces an enhancement of only 1.5-fold. This is caused by cavitation effect **[4]**. Therapeutic ultrasound with frequency between 1-3 MHz and intensity in range of 1-

2 W/cm2 is the most commonly used US method **[29]**. This may occur due to the ability of higher frequency (>1MHz) to increase concentration of energy deposition in the SC **[30]**.

Intensity:

The intensity is another factor to be considered upon evaluating the sonophoresis **[2]**. Ultrasound intensity (acoustic intensity) is defined as average of the net flow of acoustic energy (the concentration of energy) through a unit area. Along with the propagation of the ultrasound wave, the intensity decreases **[21]**. In addition, the power of reading which is delivered by sinocators can be converted to intensity by dividing the power with the transducer tip area **[31]**. Thus, the intensity depends mainly on the acoustic energy (E) as well as the speed of sound (C) in the medium. Therefore, the intensity can be calculated by this rule: I=CE. Energy (E) depends on the number of propagated waves in the medium, on the total pressure and on the speed of sound. Intensities lie mostly between 0.5 and 2 W/cm^2 **[22]**. Meshali *et al* found increasing ultrasound intensity from 0.5 to 3.0 W/cm2 led to a proportional increase in drug transport **[32]**. Threshold value of US energy is the minimum energy which can make reversible structural changes in SC of the skin and this also leads to a change in skin permeability and conductivity. Some parameters such as frequency might change the threshold value **[21]**. The threshold intensity is defined as the intensity below which no enhancement could be achieved. Threshold intensity increases by increasing frequency **[2]**.

Application time:

Higher ultrasound application time means higher supplied energy will cross the medium [21]. However, Simonin found that applying ultrasound power for 3 min enhanced skin permeability significantly. On the other hand, 5-minute time enhanced diclofenac sodium (DS) permeability through EpiDerm significantly **[33]**. Thus, the application time factor is uneasy to study **[2]**. In order to optimize the application time parameter, other ultrasound parameters such as intensity, frequency and thermal effect should be considered. Many studies were done to optimize the ultrasound application time from 1 min to 4 h **[21]**. The study of Aldwekat and Alarjah was interested into the application time factor as a measure to be considered, they showed that irradiating the EpiDerm using the physiotherapeutic ultrasound 1 MHz for 10 min gave very similar effect to the 20 min treatment. The explanation of these results was

physiotherapeutic US exerts its effect through the stable type of cavitation which is the accumulation of the dissolved air into stable tiny bubbles. These tend to escape the media through the surface and after the first 10 minutes, a degassing effect of the solution has taken place and no more dissolved air was available to produce any further damage to the skin culture. In addition, they also demonstrated that the defective region in EpiDerm tissue enlarged upon increasing the sonication to 5 min **[2]**. Huang *et al* used the constant frequency (20 kHz) as a pretreatment; they treated skin by diclofenac with probe sonicotor at different time periods. In this study, it was clear that when the ultrasound application time increased, then DF skin permeation also increased. However, there are no enough studies that approve the strong relation between application time and skin permeation for different drugs. Hence, it is recommended researches focus their efforts on the ultrasound application time and its effect on transdermal drug delivery **[17]**.

Mode (duty cycle):

There are several advantages of pulsed mode ultrasound over continuous mode US. Continuous mode US has a tissue heating effect while pulsed mode US does not have. Heating effect can harm tissue and become very painful **[29]**. Moreover, cavitation occurs more when pulsed mode ultrasound used. That occurs when US intensity during pulses is higher than the threshold of cavitation occurrence. In addition, duration of pulses must be long enough in order to cavitation can develop **[34]**. However, according to Mitragotri *et al* changing the US mode from continuous mode to pulsed mode leads to increase cavitation threshold **[29]**. When Boucaud *et al* performed experiments with human skin, they found that Discontinuous mode US (1h, 10% duty cycle) was more effective than short continuous US. Thus, the mean area under the curve (AUC) value was four times more with pulsed mode than control **[5]**. On the other hand, Cagnie et al found that the pulsed mode US enhanced the ketoprofen permeation across the skin more than the continuous US mode. Therefore, the concentration of ketoprofen in plasma, fat tissue and synovial tissue in the group of pulsed mode US is higher than in the group of continuous US, although a statistical difference was not found between two groups treated by pulsed mode and another treated by continuous mode US **[29]**. Herwadkar *et al* compared the effect of pulsed mode ultrasound (50% duty cycle: 5s on 5s off) with continuous US mode (100% duty cycle)

on the skin permeation and intradermal accumulation of ketoprofen. This study found that the permeation of ketoprofen after continuous US treatment is similar after pulsed ultrasound treatment **[23]**. Similarly, Mitragotri *et al*. found that values of the skin conductivity are independent of the US mode **[25]**. However, the intradermal accumulation of ketoprofen was enhanced only by continuous US treatment. First explanation for these results was proposed by Herwadkar *et al*. as the continuous US treatment may increase temperature of coupling medium faster than pulsed treatment. The second explanation could be according to the pulsed US mode used with SLS surfactant in the coupling medium. Pulsed US mode applied with the present of SLS may cause some of cavitation bubbles to dissolve back in solution. This will lead to lower cavitation and lower permeability to deep layers of skin **[23]**. Aldwekat and Alarjah compared between the effect of pulsed US mode and the control. They found the permeation obtained using the pulsed US mode was insignificantly different from the control. The explanation for that at the pulsed mode the time of pulse is not long enough to generate the cavitation bubbles and to continue for its powerful collapse on the surface of SC in comparison to continuous US mode **[2]**.

Distance of ultrasound transducer:

One of the important factors can affect on the transdermal drug permeation is the distance between US transducer (horn) and the skin. If the distance increases between the wave source and skin, the intensity of the waves will be attenuated in the skin medium **[21]**. Increasing distances between US horn and skin reduces cavitation effects hence decreasing drug permeability **[23]**. Several studies investigated the effect of the distance between US horn and skin on the US assisted transdermal delivery of ketoprofen; distances between horn and skin were investigated in range from zero to 4 cm with 0.3 cm to 1 cm distances **[35]**. Permeation of ketoprofen after application US (50% duty cycle, 1% SLS coupling medium) decreased significantly when the distance between US horn and skin increased from 0.3 to 0.6 cm **[23, 36]**. Huang et al 2015 studied the effect of probe-to-skin distance on the transdermal permeation of diclofenac (DF). They maintained the distance at 0.5 cm and 1 cm between skin and US horn. When the distance between the skin and probe increased, The DF release and permeation into skin decreased. However, this permeation enhancement effect was not statistically significant **[37]**.

Contrast agents and skin penetration:

Cavitation in high frequency Ultrasound should be induced to lower the energy threshold of tissue damage in US surgery by using US contrast agent (UCA) which is also used to reduce the blood brain barrier (BBB) with low intensity US. Contrast agents are composed from engineered micro bubbles and have been studied to be carriers for targeted drug delivery **[38]**. Nowadays, there are different approaches to enhance the transdermal drug delivery. One of the most distinguished approaches is the utilization of the contrast agents for increasing cavitation hence increasing drug permeation across the skin. Actively-induced cavitation with low-intensity ultrasound (less than ~1 MPa) can disorder the lipid bilayers in the SC and form aqueous channels by stable cavitation **[39]**. Park *et al* used ultrasound contrast agent (0.1% Definity ®) instead of low frequency US to promote cavitation formation hence enhancing drug delivery. They aimed to deliver 70% glycerol with 0.1% Definity® into porcine skin samples by using therapeutic US (1MHz, pressure 600 kPa, and 10% duty cycle for 60 min). It is found that the use of UCA can be effective in TDD, in which relative contrast (RC) after sonophoresis with UCA was approximately 80% higher than RC after sonophoresis without UCA. Moreover, the variance of RC was also reduced by more than 50% with the addition of UCA **[38]**. In another study, US contrast agents such as SonoVue® and Definity® were used to study the feasibility of controlled cavitation using UCAs at high frequency (2.47 MHz and 1.12 MHz) through in vivo experiments in a rat model. The target drug used was fluorescein isothiocyanate (FITC)-dextran with 0.1% UCA to be delivered across rat skin. They found that sonophoresis method significantly improved drug penetration compared with the traditional sonophoresis method with 4 kD, 20 kD and 150 kD FITC-dextrans at 1.12 MHz, and with 4 kD and 20 kD FITC-dextrans at 2.47 MHz Thereby sonophoresis with UCAs could be more effective in TDD **[40]**.

1.2 Other external factors affecting ultrasound mediated TDD:

In addition to ultrasound exposure parameters such as frequency and intensity, it is also important to distinguish between ultrasound waves being applied prior to placement of the drug carrier on the skin (pretreatment sonophoresis) **[31, 63]** or after placement (simultaneous sonophoresis) **[31, 62]**. One of the most important factors may play a role in TDD is the treated area, whereas the treatment area should not be twice more than the effective radiating area

of the ultrasound. In addition, drug formulation is another important factor which plays an important role in transdermal drug permeation. Gel based preparations are the best choice, since gel has good transmissivity of US **[29]**.

2. Mechanism of penetration using ultrasound:

Until these days, the mechanism of sonophoresis enhanced drug delivery is not understood **[12]**. It was suggested several mechanisms that explain the way of drug permeation across skin by ultrasound. These mechanisms divided into three main types: thermal effects (heating of coupling medium) **[5]**, non-thermal; cavitational effect and non cavitational effect (acoustic streaming) **[3, 6]**.

2.1 Thermal effects:

The transfer and conversion of mechanical energy produced by piezoelectric crystal in the US horn lead to formation of thermal effect of US **[41]**. The acoustic waves are propagated through the medium. During propagation the waves will be attenuated due to scattering or absorption by the medium **[2]**. In addition, because the tissue is an inhomogeneous system, US waves can be reflected from the propagation path. When the density of the inhomogeneities increases, the acoustic wave might scatter until it is completely absorbed by tissue **[3]**. The beam absorption causes an increase in medium temperature hence converting US energy into heat **[2]**. This temperature increase caused by US beam absorption has a direct relationship with the intensity of sound wave **[41]**. The collapsed transient cavitation bubbles contribute also in increasing the medium temperature **[2]**. The heat generated in the tissue has linear relationship with the absorption coefficient of the tissue, intensity and frequency of US waves **[3]**. If the medium's absorption coefficient is high, then the temperature increase is high thus increasing the thermal effect. The use of US on the brain is still considered a great challenge, since the bone tissue has high US absorption coefficients while the muscle has a low absorption coefficient. In addition, the absorption coefficient of medium (material or tissue) increases by increasing US frequency. This will lead to increase tissue temperature for the same US intensity **[6]**. On the other hand, the impact of temperature increases on the donor compartment (tissue or medium) is directly proportional with US frequency, whereas previously observed an increase in temperature of donor compartment when experiments were performed with high frequency US **[5]**. The tissue could be ablated by thermal effect when it is exposed to high US densities **[3]**. There are few

studies that predict of the actual temperature increase produced by a particular sonophoretic profile in the skin. This prediction is very difficult without accurate data about US absorption coefficient, conduction and convection properties of the studied tissues **[41]**. The effect of skin heating on the enhancement of drug absorption was investigated. It is found that transdermal digoxin transport was enhanced by heating the skin surface and that was similar to the enhancement observed with high frequency US **[5]**. The absorption of estradiol doubled when the temperature increased by 10 ˚C **[41]**. No enhancement of transdermal transport of mannitol, estradiol or hydrocortisone was observed when the heating of the skin has been suppressed. Boucaud et al found the LFUS increases donor compartment temperature from 5-7 °C. However, the flux enhancement of caffeine through hairless rat skin samples was 75-fold **[5]**. This value suggests that the ultrasound-enhancement was induced by factors other than heating of the donor compartment. Thermal effects can enhance the drug diffusion topically across SC by several ways: heating caused by US increases the kinetic energy of the drug molecules, promotes dilation some point in the skin like hair follicles and sweet glands and increases the blood flow (circulation) in the sonicated area **[29]**. Aldwekat *et al* minimized the thermal contribution when they studied the permeation improvement across EpiDerm™ upon insonation, since some results gave negative enhancement due to thermal effect. This is explained by the thermal effect may promote the reverse diffusion of the drug molecules to the gel vehicle rather than to pass through the skin. This finding could support the thought saying that the effect of US on the enhancement drug permeation might be due to the other US mechanisms **[2]**.

2.2 Acoustic cavitation:

The US mechanism in enhancement of transdermal delivery is not yet known **[23]**. However, it is well known that US enhances Transdermal drug delivery by activating bubbles (acoustic cavitation) **[42]**. Acoustic cavitation is considered the main mechanism in US assisted TDD **[26]**. Cavitation is defined as the formation of micro bubbles within coupling medium or within the skin in insonated medium **[5]**. These bubbles are generated by changes in ultrasound-induced pressure in the medium **[6]**. Due to exposure these bubbles to intensive sound, implosive collapse of the bubbles occurs **[43]**. US frequency and intensity are the main factors that control the cavitation thresholds, in

which low US frequency and high intensities lead to rapid cavitation **[12]**. The bubbles can perturb the structure of stratum corneum by the high pressure or approaching the skin surface without penetrating the SC resulting in stronger perturbation and collapse at the SC surface itself asymmetrically. Thus, producing high velocity liquid micro jet which impinge on the stratum corneum surface in a velocity of about 100 m/s **[2]**. The cavitation disorders the lipid bilayers in the skin, which in turn leads to formation of aqueous channels in the skin. These channels promote drug transfer through SC **[5, 28 and 39]**. The acoustic cavitation can be classified into stable and inertial cavitation **[42]**. Inertial cavitation can have a diameter up to 150 lm each **[2, 33]**. These bubbles grow rapidly and when they reach a certain diameter, they collapse **[6]**. The collapse of the micro bubbles induces shock waves for a short time which produce high temperature, huge pressure, severe fluid flow effects (i.e. micro-jetting, efficient mixing and agitation) **[44]** and a secondary effect in generating reactive species such as free radicals which can induce chemical transformations **[3]**. Accordingly, structural changes in the surrounding tissue occur from non-symmetrical bubble collapse which in turn exhibits convection **[6]**. On the other hand, stable cavitation was defined by liu and Hsieh as "radius of bubbles oscillates around an equilibrium value over a considerable number of acoustic cycles without the generation of bubble collapse" **[42]**. Cavitation produced by US can affect the tissues and biological system. Cavitation can cause different effects on biological system such as mechanical stress, increasing temperature and increasing chemical reactivity, affect drug transport **[29]**, white noise, sonochemical reactions, erosion of hard materials, rupture of living cells and emission of light (sonoluminescence) **[43]**. There are several studies which found the relation between the amount of acoustic cavitation and the US assisted transdermal drug delivery achieved **[11]**. In the study of Bhatnagar et al, the inertial cavitation was found to be the main mechanism of enhancing drug transport of US in agar gel, in which inertial cavitation has greater effect than acoustic radiation force or small levels of heating **[45]**. Feiszthuber et al investigated the enhancing delivery of insulin by applicability of cavitation-mediated transport. In this study, different US exposure regimes were used in agar and porcine skin and then the level of cavitation activity were quantified over these US profiles. The results suggest that the

enhancement of delivery is dominated by the level of cavitation activity as detected via passive cavitation detector (PCD) transducer rather than by the pressure amplitude used or by the presence or absence of artificial cavitation nuclei **[11]**. Most of the studies which investigate the US assisted transdermal vaccine delivery demonstrated that the inertial cavitation is effective for enhancing skin permeability to the drug and vaccines of around 40-70 kDa **[13]**.

The effect of ultrasound frequency on acoustic cavitation:

Acoustic cavitation has inverse proportional relationship with US frequency. It showed that low frequency US enhances both stable and transient cavitation while at therapeutic frequencies only stable cavitation takes place, since the time required to form a full gas bubble is less than the time between negative and positive US pressure at the High frequency **[2]**. Several researchers found that low frequency US (20 kHz-100 kHz) can enhance acoustic cavitation in SC which in turn increases skin permeability hence enhancing transdermal drug delivery **[3]**. There are two methods to optimize the efficiency of ultrasound in TDD and increasing cavitation activity. The first method includes the addition of coalescence inhibiting solutes such as polymers and surfactants. The other approach implies the use a combination of two or more transducers with different frequencies or what so called "Dual Frequency" **[44]**. Most of published studies approved that dual frequency has a positive effect on the skin permeability. Schoellhammer et al investigated the ability to enhance skin permeability without increasing US intensity and treatment time. For this purpose, they used simultaneous application of two distinct ultrasound frequencies in the range (20 kHz to 3MHz) as a pretreatment. The results from aluminum foil pitting experiments showed the use of two frequencies instead of one low frequency increased cavitational activity significantly. In addition, in vitro tests with porcine skin using simultaneous two frequencies (low and high) enhanced significantly glucose (180 Da) and inulin (5000 Da) transdermal flux experiments **[46]**. Liu and Hsieh designed dual-frequency ultrasonic waves with single piezoelectric elements thus enhancing acoustic cavitation. Macroscopic bubbles were detected optically. The results confirmed that single-element dual-frequency ultrasound stimulation can enhance acoustic cavitation. Dual frequency can generate bubble five times higher than single frequency **[42]**. In addition, it can increase hydrogen peroxide production up to an

increase of one-fold. In another study, Schoellhammer et al used both high frequency sonophoresis (1MHz) and low frequency sonophoresis (20 kHz) to increase the localized transport regions (LTRs) and decrease the treatment time. The results demonstrated that LTR formation was more than 27% of the treated skin area when multiple frequencies were used. LTR reached 5%-10% using low frequency sonophoresis. High-frequency horn used in this study may add additional bubbles that can grow and collapse under low frequency horn **[47]**. Although most investigations demonstrated only the positive effects of dual- or multi-frequency fields, there are some studies that disapprove this hypothesis and show the destructive effect of the Dual frequency under certain conditions **[44]**.

2.3 Acoustic streaming (convection):

Acoustic streaming is defined as the fluid movement in the direction of US waves. This movement is due to the direction of the beam away from transducer and energy transfer from the US wave to the fluid **[48]**. Micro streaming considered the development of fluid high velocities in small scale eddying as a consequence of ultrasonic wave scatterings and reflections. The main cause for acoustic streaming is Ultrasound reflections and other distortions that are generated during wave propagations. In addition, oscillations of cavitational bubbles may enhance the generation of acoustic streaming **[6]**. The velocity of acoustic streaming is determined by fluid properties, including acoustic attenuation, viscosity and sound velocity. In addition, it is determined by temporal average intensity, sound frequency; transmit aperture size and the pressure amplitude **[48, 49]**, in which increasing fluid viscosity decreases acoustic streaming velocity **[50]** while frequency decreasing transmit aperture size and increasing pressure amplitude increase the velocity **[51]**.

3. Ultrasound applications in enhancement Transdermal Drug Delivery:

During the last years, ultrasound has been widely investigated as an enhancement method for TDD. **Table 1** summarizes some studies that investigate the effect of US on the TDD. Fellinger and schmid were the first researchers who used Ultrasound (phonophoresis) to enhance transdermal hydrocortisone delivery in digital polyarthritis cases **[20, 29]**. Anti-inflammatory medications have been delivered successfully to treat inflamed subcutaneous tissues. Recently, there is increase in the investigations that concentrate on the delivering of non-steroidal ant-inflammatory drugs (NSAIDs) across the

skin not only topically but also systemically. However, these studies were done on either animal models or clinically **[29]**. Ketoprofen is one of NSAIDs that were tried to be delivered systemically and topically. Cagnie et al examined the influence of ultrasound on the transdermal delivery of ketoprofen (Fastum gel) in humans using clinically relevant settings and media. They assessed local absorption and distribution of ketoprofen after phonophoresis in relation to plasma level as well as compare the concentrations found after continuous, pulsed and sham ultrasound application. The concentration of ketoprofen in plasma is very low and this result is corresponding with other previous studies. On the other hand, the concentration of ketoprofen in group treated by pulsed phonophoresis (1 MHz, 1.5 W/cm2, for 5 minutes) is higher than in group treated by continuous phonophoresis (100 Hz, 20% duty cycle). It is found also that the concentration of ketoprofen in synovial tissue is higher than in adipose tissue. That is attributed to the ability of ketoprofen to bind with proteins that present in synovial tissues. In addition, synovial tissues are highly vascularized **[29]**. In the study of Herwadkar et al, authors assessed the delivery of ketoprofen into and across the skin in vitro by using LF sonophoresis with optimized parameters (2 min application, 100% duty cycle, 0.3 cm distance between horn and skin, 1% SLS as coupling medium). They found that in comparison to passive delivery the application of US enhanced permeation of ketoprofen significantly **[23]**. Yi Han *et al* tried to solve the problem of a lower local effective TB drug concentration. The anti TB drugs were applied on the lesion with using acoustic waves. The results demonstrated that this method can quickly and directly deliver the anti-TB drugs to the lesions and achieve high local plasma concentrations. The transdermal ultrasound method was very effective compared with the oral administration of TB drugs only. In addition, this method solves the problem of low local drug concentration as well as the general anesthesia accompanied to surgery **[17]**.

3.1 Transdermal protein delivery using ultrasound:

Due to their low skin permeability, no proteins and peptides are available as a transdermal formulation for clinical applications. Although transdermal drug delivery of proteins has many advantages for several reasons. proteins can be degraded in GIT, the clearance of some proteins such as interferon is very fast, transdermal method is easier to use than injections **[4]**, avoiding

hepatic first-pass metabolism, it is present large surface area for absorption (approximately 2 m2) and has relatively low proteolytic activity. In addition, skin is one of the most easily accessible organs of the body **[6]**. However, one of difficulties with transdermal drug delivery is the uncertainty in delivered dose compared to hypodermic needle injection **[11]**. Using LFUS in in-vitro enhancement transdermal delivery of proteins including insulin, interferon and erythropoietin has been widely investigated **[3]**. Lee *et al* assessed ultrasound-mediated transdermal drug delivery to enhance delivery of insulin in vivo using rabbits and determined if an ultrasound device based on the low-profile cymbal transducer array could be used for transdermal insulin delivery. Lee et al found that compared to two control groups which were saline with US and Insulin without US``1, the blood glucose level of Ultrasound-insulin group decreased significantly **[9]**. Park *et al* tested the delivery of insulin on pigs by using lightweight cymbal transducer at optimized parameters 20 kHz with an Isptp =100 mW/cm2 at a 20% duty cycle for 60 min. The insulin level decreased to -72+-5 mg/dl at 60 min and continued to decrease to -91+-23 mg/dl in 90 min in US-Insulin treated group. Thereby, this decrease was significant comparing to control untreated group. This study demonstrates promising pre-clinical results to use ultrasound for enhancing transdermal insulin transport in human **[52]**. Smith *et al* tested in vitro transport of insulin across human skin by using two types of lightweight cymbal transducer arrays which are: a stack array with intensity (ISPTP) of 15.4 ± 0.6 mW/cm2 and a standard array with intensity (ISPTP) of 173.7 ± 1.2 mW/cm2 at 20 kHz. The results showed that using Humalog insulin with standard array demonstrate 4-fold increase in the group treated by sonophoresis over that found in the control group **[53]**.

3.2 Enhancement of TDD using ultrasound with chemical enhancers and Nano carriers:

Surfactant can improve drug transport across skin via their interaction with polar group of lipid bilayers in the skin thereby disrupting hydration spheres. Investigations showed using ultrasound in combination with surfactant enhances transdermal drug transport more than using ultrasound alone. ultrasound can increase the depth of delivery and dispersion the surfactant in the skin. On the other hand, surfactant and ultrasound can be used as a synergic method in TDD in diabetic patient, since surfactant with cavitational effect result from ultrasound may

inhibit bacterial growth at the surface of the skin **[27]**. Mitragotri *et al* used sodium lauryl sulfate (SLS) and low frequency ultrasound at 10 W/0.1 s ON and 0.9 s OFF for 90 min with each other and separately to enhance mannitol permeability through the skin; the study showed SLS and low-frequency ultrasound synergistically enhance transdermal drug transport. Whereas a 90-min application of ultrasound from a 1% SLS solution induced about a 200-fold increase in the skin permeability to mannitol, some studies claimed that using US-SLS may increase pore radii in the treated skin and in untreated skin **[54]**. Huang *et al* used SLS in different concentrations (1% and 2%) with sonophoresis to enhance transdermal diclofenac (DF) transport. They found that the DF skin permeation increased with increasing the SLS concentrations. They claimed that SLS increases pore of radii of sonophoresis treated skin. Similarly, sonophoresis causes imperfection in SC and this increases DF permeability through the skin. As a result, Sonophoresis with SLS causes more skin perturbation in comparison to sonophoresis alone **[37]**. Paliwal *et al*. have demonstrated increased occurrence imperfections in stratum corneum after ultrasound treatment. Addition of 1% SLS resulted in an increase in dimensions of lacunar regions thereby increasing the uptake of quantum dots in skin. It is also claimed that SLS may be responsible for increasing pore radii in the non-LTR regions of sonophoresis-treated skin **[55]**. Synergistic effect of ultrasound (1 MHz, 2 W/cm2) with several of chemical enhancers formulations such as polyethylene glycol 200 dilaurate (PEG), isopropyl myristate (IM), glycerol trioleate (GT), ethanol/pH 7.4 phosphate buffered saline in a one-to-one ratio (50% EtOH), 50% EtOH saturated with linoleic acid (LA/EtOH) and phosphate buffered saline (PBS) was studied by Johnson et al. to enhance the corticosterone permeation across the skin. A combination of LA/EtOH with ultrasound increased corticosterone flux by up to 13,000-fold, relative to the passive flux from PBS. This result is higher enhanced than that enhanced by US alone or chemical enhancer alone **[56]**. The effect of using 1% SLS as a coupling agent with sonophoresis was studied by Herwadkar et al to enhance transdermal permeation and intradermal accumulation of ketoprofen across the skin. Sonophoresis-SLS treatment resulted in 6.5-fold enhancement of permeation and 5-fold enhancement in skin accumulation over passive delivery **[23]**. In the study of Lopenz et al, authors applied US/SLS simultaneously as a pretreatment to enhance penetration of

rigid nanoparticles. They used different nanoparticles with different surface groups (cationic, anionic carboxylic acid and neutral amine). In addition, inductively-coupled plasma mass spectrometry and confocal microscopy were used to evaluate penetration for these nanoparticles through US/SLS treated and untreated skin. They found that nanoparticles applied in the treated skin fully penetrate the skin while 0.01% of nanoparticles penetrate the dermis of untreated skin **[57]**. Dendrimer-coupled sonophoresis is one of the novel techniques to enhance the skin permeation of active molecules. Authors tried to enhance permeation of DF through the skin by using novel TDDS comprising a polyamidomin dendrimer with sonophoresis. This study reported a novel permeation-enhancement technique based on dendrimer-coupled sonophoresis for the transdermal drug delivery of DF. The TDDS was developed using a Plackett–Burman statistical design. The effect of all independent variables on permeation enhancement was found to be statistically significant. Dendrimer-coupled sonophoresis delivery increased DF permeation by 16.5-fold. Manikkath *et al*. used low frequency US and PAMAM dendrimer as combination to enhance the skin permeation of ketoprofen. Authors applied dendrimer to skin with the drug, before the drug and as a complex of drug-dendrimer. This study showed that using PAMAM dendrimer with US produce drug levels similar to oral administration of ketoprofen **[58]**. Manikkath et al. studied individual and combined effects of Arginine terminated peptide dendrimers and low frequency ultrasound on the transdermal permeation of ketoprofen. They found that compared to passive diffusion of drug alone in vitro and in vivo, both peptide dendrimer and ultrasound worked in synergy. In vivo studies demonstrated that dendrimer and ultrasound-assisted permeation of drug achieved much higher plasma concentration of drug compared to passive diffusion. This study demonstrates that arginine terminated peptide dendrimers combined with sonophoresis can effectively improve the transdermal permeation of ketoprofen **[26]**. Drug transport through skin can be enhanced by the incorporation of lipid-based nano carriers and sonophoresis. This combination is one of the most currently studied branches **(Seah and Teo, 2018)**. Beker *et al* (2005) demonstrated that application of ultrasound treatment before topical anesthetic decreases the patient feeling in pain of IV cannulation. SonoPrep was used as ultrasound pretreatment with therapeutic frequency 53-

56 kHz. This treatment is followed by 5 minutes of 4% liposomal lidocaine cream, standard-care IV cannulation or standard care alone. The pain of patients was rated by a visual analog scale. The site of IV cannulation was assessed after IV insertion an after 20-36 hours. The results demonstrated significant less pain. Thereby the application of Ultrasound device to skin for 15 seconds followed by 5 min of 4% liposomal lidocaine cream significantly reduced patients' perception of the pain of an IV start when compared with standard care **[27]**. Bhatnagar *et al* proposed a system for vaccine delivery across the skin. They used nano-cavitation nuclei not only to enhance skin permeability but also to mediate the active transport of the therapeutics across the SC without depletion. Authors utilized nanocups to enhance ultrasound-mediated transdermal vaccine delivery, whereas vaccine is embedded alongside and a model vaccine in a hydrogel formulation can serve as a novel dosage form. This model enables simultaneous application of US mediated cavitation. Authors found that application of OVA and nano-cubs simultaneously with exposing to US for 90 leads to transport vaccine to depths 550 micrometer and this depth becomes 700 micrometers in the skin with cups. Using US assisted transdermal Nano cups delivery system is better than using US assisted chemical enhancers **[13]**. Rangsimawong *et al* investigated the enhancement of transdermal delivery of galantamine HBr (GLT) by using low frequency sonophoresis (SN) and Limonene-containing PEGylated liposomes (PL). The results show that the application of SN with PEGylated liposomes (PL) increased the GLT penetration into and through the skin than GLT solution alone. limonene containing liposomes enhance fluidity of intracellular lipids in SC hence delivering GLT through intracellular pathway. in addition, SN might enhance drug permeation via intracellular pathway **[59]**. Zorec *et al*. studied the ability of US in combination with nanocarriers like liposomes and ethosomes to enhance transdermal drug delivery. Although a sonication for five minutes leads to better permeation across the skin compared to passive diffusion, the liposomal formulation enhanced insignificantly the permeation through skin compared to passive diffusion. This happened because the liposomal phospholipids may repair the defects in the skin occurred by Ultrasound. In addition, the alcoholic content present in ethosomes may interact with lipids in SC and inhibit the permeation of the skin. The combination between US and nanovesicles did not give synergistic effect **[60]**.

Table 1: studies on ultrasound in TDD

Drug/ molecules	study model	study method	results	reference
Brucine	porcine skin	Effects of ultrasound on the transdermal absorption of Brucine under the influence of various acoustic parameters, including frequency, amplitude and irradiation time. The transdermal conditions of yellow-green fluorescent nanoparticles and Brucine in skin samples were observed by laser confocal microscopy and ultraviolet spectrophotometry.	The permeability of the skin to the fluorescent label and Brucine (e.g., the depth and concentration of penetration) is increased compared to its passive diffusion permeability.to depth more than 110 micron	[61]
Diclofenac Sodium	3 D skin EpiDerm™ (Franz cell diffusion) (In vitro)	Ultrasound parameters: 20 KHz and 1MHz with different Us modes, amplitudes, sonication time and conditions	The most effective enhancing parameters were power sonication with 20 kHz frequency, 20% amplitude at continuous mode for 5 min.	[2]
Diclofenac sodium Ibuprofen, piroxicam and	cellulose and rabbit skin membranes (in vitro) Franz diffusion cell	High frequency US with different intensities ranging from 0.5 to 3 W/cm^2, continuous and pulsed mode.	Continuous ultrasound mode was more effective in enhancing drug transport than the pulsed mode. Diclofenac Sodium has the least flux. There is a therapeutic potential of ultrasound in transdermal delivery of NSAIDs and the synergistic effect of temperature and ultrasound operational parameters on drug transport.	[32]
D-mannitol physostigmine	rats and guinea pigs (in vivo)	Therapeutic range ultrasound (1 MHz, 1.5 W/cm2 continuous wave or 3 W/cm2 pulsed	The study demonstrated that US eliminates Lag time associated with TDD. In addition, permeability of rat skin to Physostigmine solution was relatively high.	[8]
Estradiol, Salicylic Acid, Corticosterone, sucrose, aldesrone, water and butanol	Human cadaver epidermis (in vitro) Hairless rat skin (in vivo)	20 kHz, 125mW/cm2, 100 msec, pulses applied every second	Low frequency US enhances transdermal transport of the drugs 1000 times higher than that enhanced by therapeutic US	[4]
Fentanyl Caffeine	human and hairless rat skin (in vitro)	20 kHz, continuous or discontinuous mode, intensity 2.5 W/cm2.	Low-frequency ultrasound enhanced the transdermal transport of both fentanyl and caffeine across human and hairless rat skin. Discontinuous mode is more effective for increasing transdermal penetration of fentanyl while Transdermal transport of caffeine was enhanced by both continuous and pulsed mode.	[5]
fluorescently labeled Oligonucleotides	Porcine skin (in vitro)	Low frequency ultrasound (20 kHz, 2.4 W/cm2)	Successful delivery of anti-sense oligonucleotides using low-frequency ultrasound.	[62]
Glucose Mannitol	porcine skin in vitro	High frequency US: pulsed mode50% duty cycle, intensities of 0.2 and 2W/cm^2	Only 25% of this enhancement was attributable to the increased temperature induced by US. It follows another mechanism, most probably cavitation, is principally responsible for the lowered skin barrier function observed.	[41]

Table 1 Continued….

Insulin	**human skin-mimicking materials (in vitro) . 3% agar hydrogel . porcine skin**	**3% agar hydrogel: (0.25–1.40 MPa peak rarefaction focal pressure—PRFP) porcine skin was insonated at 1.00 and 1.40 MPa PRFP**	**In agar gel, both insulin penetration depth and concentration only increased significantly in the presence of inertial cavitation, with up to a 40% enhancement. In porcine skin the amount of fluorescent insulin was higher in the epidermis of those samples that were exposed to ultrasound compared to the control samples, but there was no significant increase in penetration distance.**	**[11]**
Insulin	**Japanese white rabbits**	**Ultrasound was delivered over relatively longer periods with aqueous drug forms instead of the cream or gel used in previous reports**	**US exposure with insulin to the skin induced sufficient effects of blood glucose lowering in diabetic rabbits**	**[63]**
Magnesium ascorbyl phosphate (MAP)	**agarose phantom and pig skin (in vitro) mice (in vivo)**	**US plus micro bubbles MBs in 0.1% agarose solution combined with MAP (UMB1), and by 48% and 206%, respectively, when treated with US plus MBs in 0.15% agarose solution and MAP (UMB2).**	**The survival of MBs with US is affected by the viscosity of the surrounding medium, and that in mice, treatment with US plus MBs in a suitable agarose gel can increase skin permeability and enhance transdermal MAP delivery.**	**[64]**
Methotrexate	**human cadaver skin (in vitro)**	**20 kHz frequency 2 min application, and 6.9 W/sqcm intensity**	**The skin became markedly more permeable to methotrexate after the treatment by sonophoresis than by iontophoresis. Drug distribution in the skin layers revealed a significantly higher level of methotrexate in the sonicated skin than that in iontophoresis and untreated groups**	**[12]**
Salicylic Acid	**Hairless guinea pigs**	**US frequencies: 2,10, 16 Total drugs absorbed was quantified by determining the amount of S.A present in tape strips and eliminated in urine**	**Sonophoresis for 20 min at 2MHz caused no significant increase in S.A delivery over passive diffusion Treatment with US at 10 and 16 MHz significantly elevated S.A transport by 4- fold and 2.4-fold**	**[30]**
Tetanus toxoid	**Mice (in vivo)**	**Using of low-frequency ultrasound as a potent physical adjuvant for successful transcutaneous immunization (TCI).**	**low-frequency ultrasound enhances the immune response induced by topical application of tetanus toxoid**	**[65]**

CONCLUSION:

In spite of the several advantages of transdermal drug delivery over oral drug delivery, studies on TDD are increasing. Ultrasound is one of a promising method used to enhance drug penetration across the skin. Based on previous studies, transdermal penetration of drug using ultrasound depends on different parameters such as frequency, intensity, application time, mode of ultrasound, distance between ultrasound horn and using coupling agent. Low frequency ultrasound has been verified as a method of enhancement drug penetration through the skin. Low frequency is responsible for enhancing cavitation activity which is considered the main mechanism in transport drugs across the skin using ultrasound. Studies on the promotion of cavitation activity were performed. One method to enhance cavitation activity is using dual frequency, whereas most of published studies approved that dual frequency has a positive effect on the skin permeability. Most recently, cavitation activity can be increased by using high frequency ultrasound with ultrasound coupling agent (UCA). Using engineered bubbles such as UCA with high frequencies may increase skin permeability without causing skin damage. In addition, current published results are encouraging using ultrasound for decreasing blood glucose level, since several authors investigated transdermal insulin delivery using ultrasound. On the other hand, several studies confirmed the success of ultrasound in transdermal NSAIDs transport such as ketoprofen and diclofenac. In order to make continuous development in drug delivery, a combination between ultrasound and chemical compounds like surfactants has been performed. The most currently novel studies in TDD concentrate on making a combination between lipids based nano carriers and ultrasound to deliver drugs efficiently. Since most investigations on TDD using ultrasound have been performed in vitro, further investigations are needed to prove the effectiveness of ultrasound in enhancement TDD by assessment in vivo.

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