Effects of Designed Ultrasonic Field in Different Frequency Sonophoresis Using the Carrier of Liposomes

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Abstract: The objective of the study was to investigate the skin permeability of designed diffuse ultrasonic field and the application of different frequency exposed on liposome transdermal delivery. The specimens were exposed to ultrasound by frequencies of 20, 60 kHz and 1 MHz with the intensities 0.43 W/cm^2. In the exposed experiments, the diffuse ultrasonic field was performed using an inclined incident transducer and a designed wedge in the 20 and 60 kHz. The frequency of 1 MHz transducer was operated directly in the skin sample. These exposure methods have been compared to the unexposed samples by recording the permeated depth of the rhodamine in the skin. Experimentally, the results revealed that the ultrasonic frequency of 60 kHz has a better permeated depth about 168 nm under the skin surface. In general, applied higher intensity of ultrasound gave greater permeated depth than lower intensity. However, safe application of higher intensity ultrasound should be practiced by careful selection of exposure parameters. It is the principle reason for the lower intensity applied in the study.
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1. Introduction

Transdermal delivery system is a simple and convenient drug delivery system. It offers several advantages than traditional drug delivery such as oral delivery and injections including elimination of the first-pass metabolism, lower the pain, and possible maintained release of drugs. However, human skin is an efficient barrier. The outer layer of this barrier called the stratum corneum, is the main structure to cause the low skin permeability of transdermal delivery of drugs. Therefore, it is difficult to deliver the higher molecular weight drugs across the skin. Several physical and chemical methods have been reported in the literature, which have successfully increased the level of drugs delivery across and into the skin. These methods, for example, include the ultrasound, chemical enhancers and electric fields [1-3].

The use of ultrasonic technology in the biological or medical application has been a convenient clinical tools and development for many years [4-5]. Ultrasound under suitable conditions has been shown to enhance the transdermal transport. This phenomenon is referred to as sonophoresis. Sonophoresis is a physical technique to enhance the transdermal delivery of drugs using ultrasound energy [6]. In the recent researches, low frequency ultrasound has been shown to be more enhancement in the transdermal delivery than the high frequency ultrasound [7]. This high efficiency of low frequency ultrasound creates from cavitation effects, which is important reason for skin permeabilization [8]. Cavitation is the gaseous nuclei growing in liquid under the ultrasound exposure. It involves either the rapid growth and collapse of a bubble (called the transient cavitation), or vibration motion of a bubble (called the stable cavitation) in the ultrasound field. Cavitation is affected by numerous parameters including the presence of cavitation nuclei. The cavitation nuclei may exist in many forms including microbubbles that are already existence in the liquid or made by artificial way. Many methods to enhance the cavitation have been reported in the literature. For example, researchers have used microspheres, silica particles, and ultrasonic contrast agents to enhance cavitation [9]. Ultrasonic contrast agents are typically gas-encapsulated microbubbles with diameters of the order of 1-10 μm. Contrast agents are filled with a gas that may be air or substance of higher molecular weight, such as perfluoropropane. The shell can be stiff or more flexible, and the shell thickness can vary from 10-200 nm. Liposome has a similar structure as the contrast agents. It has composite structures made of phospholipids and may contain small amounts of other molecules. The size of the liposomes can vary from low micrometer range to tens of micrometers. Liposomes are artificially prepared vesicles made of lipid bilayer. It can be filled with drugs, and used to deliver drugs for cancer and other diseases. Physical methods such as iontophoresis, ultrasound, and tape-stripping can further assist the delivery of drugs encapsulated in liposomes. Dahlan *et al.* have considered the effects of the low frequency ultrasound and liposomes on skin [10]. It has to notice that the liposomes can repair skin damage, which could limit the drug permeation. They find that the influence of liposome was evident within 5 min of its application, and the smaller liposomes were more effective at repairing skin disruption caused by sonication. In addition, they think the skin repair by liposomes seems to depend on the extent of the disruption caused by ultrasound application. Though the ultrasound can assist the transdermal delivery of drugs in liposomes, it still exist some questions. Such as the exposure of high intensity ultrasound will increase the temperature in the liquid. The thermal effect induced by high temperature will damage the liposome and render the drug inside ineffective. Thus, liposomes solution in and not in an ultrasonic field will be discussed in this study, and the permeation depth of the entrapped material within liposomes (rhodamine B) was compared. The diffuse ultrasonic field was performed using the combination of an inclined incident transducer and a designed wedge. To prevent the thermal effects appeared in the exposure experiment, the lower ultrasonic intensity was applied to drive the transducer. Three driving frequencies of the ultrasonic field are selected and the distribution conditions of skin permeation depth examined.

2. Theory

In an ultrasonic field, the force act on the particle is determined by a balance among the diffusion force, the gravitational force and the acoustic radiation force. When the acoustic standing wave field is produced in a dilute suspension of particles, the acting force is known as the primary acoustic radiation force. For a spherical particle with a radius R dispersed in an inviscid fluid, and an acoustic force due to a one-dimensional standing plane wave field this is described by

$$F_{ac} = 4\pi R^3 \kappa E_{ac} F \sin(2\kappa x) \qquad (1)$$

where x is the position of the particle relative to the nearest pressure antinode of the wave field; κ is the acoustic wave number; E_{ac} is the acoustic energy density, and F is the constant acoustic factor. The constant F is given by

$$F = \frac{1}{3}\left[\frac{5\Lambda - 2}{1 + 2\Lambda} - \frac{\gamma_p}{\gamma_f}\right] \qquad (2)$$

In Eq. (2), Λ is the ratio of particle density to fluid density and γ_p and γ_f are the compressibility of the particle and the fluid, respectively. Eq. (1) yields the acoustic radiation force and is reasonable for any particle that is much smaller than the acoustic wavelength. If the above condition is satisfied, then the acoustic constant factor F is independent of the size and shape of the particle [11]. Eq. (1) indicates that the primary acoustic radiation force can drive the particles to the pressure nodes or the antinodes of the acoustic field. When the constant acoustic factor F is positive, then the particles move toward the pressure nodes, if F is negative, then the particles are driven to the pressure antinodes.

3. Materials and methods

3.1 Diffuse ultrasonic field

To produce a wide and uniform exposure surface, the suitable design was needed. The acoustic field is about using the diffuse field theory of Sabine to create a uniform sound field for the radiation experiment [12-13]. With this theory, the ultrasonic beam had to be oblique incident to the finite boundary. After repeatedly reflection of the sound wave, a uniform sound field would be obtained in the surfaces of the space. The cuboid acrylic wedge, shown schematically in Fig. 1(a), with the bottom area of 62×65 mm and the height of 120 mm was used to create the uniform irradiation field. The top corner of the exposure wedge was made an oblique and triangle plane with the length of 75 mm to mount the ultrasonic transducer. Ultrasonic beam of the transducer was incident with the angle 45° from the oblique plane toward the boundary of the wedge at the far end. The transducers with the frequencies of 20 and 60 kHz were used to fix on the wedge. In Fig. 1(b), the acrylic case was applied as a boundary to fix the transducer of 1 MHz. The exposure area was determined as the boundary of the case. Furthermore, the exposure area indicated in the figure was used to contact the skin samples. All sampling positions of the exposure area were shown in the Fig. 2. Each permeated depth of six randomly selected regions of each sampling position was taken. The permeated depth was measured by Nikon C1 plus confocal microscopy. The mean values of permeated depth in the six regions was indicated the depth of one sampling position in the exposure area. An ultrasonic transducer was positioned above the sampling position A1 of the exposure area. Two custom built transducers with operating frequencies of 20 and 60 kHz (Broadsound Corporation, Taiwan) were used for application ultrasound. The exposure experiment of 1 MHz was operated by ultrasonic diathermy system (ZMI, ULS-1000). The exposure and measurement system with a diffusion field comprised an ultrasonic transducer that could produce a diffuse sound field was devised, and is presented in Fig. 3. The transducer was driven by a continuous sine wave from a function generator (GW instek, SFG-830). The intensity of the sound field was measured using a miniature PVDF ultrasonic hydrophone probe (Force Institute, MH28-10). In this experiment, the output intensities were set as 0.19 and 0.45 W/cm2. The signal obtained from the hydrophone was analyzed using a LeCroy WaveSurfer 422 digitizing oscilloscope. Ultrasound was exposed to the skin samples for 5 minutes to prevent the increasing temperature on the skin. All experiments were performed at room temperature. When the skin samples were exposed or sham-exposed to ultrasonic irradiation, the permeated depth distribution of liposomes, affected or unaffected by the ultrasonic waves, was visible.

3.2 Material and skin preparation

Skin exposure experiments were carried out in vitro with full thickness pig skin of the ear (Yorkshire). Superfluous tissues such as fat and muscle were removed. Skin was cut into square pieces (10×10 cm), and was stored in a freezer until the experiments were performed. Egg yolk phosphatidylcoline (EPC) and cholesterol (Sigma Chemical Co., St. Louis, MO) in the molar ratio of 4:1 were mixed in a round-bottomed flask. The fluorescence materials (rhodamine) were dissolved in the suspension. Then the suspension was prepared by dissolving in chloroform. Subsequently, the organic solvent was evaporated under the vacuum, and the lipid film formed was then left under a stream of nitrogen to remove traces of the organic solvents. The resulting dried lipid film was dispersed with a buffer solution (Hepes: 0.1 M, pH 5). The solution was vortex mixed above the phase-transition temperature (room temperature) and yielded the lipid suspensions. Lipid suspensions were operated with the mechanical shaking for 30min. After that, the ultrasonic processor was used to crushing the lipid membrane and obtained liposomes with the diameter of 200 nm.

4. Results and discussions

Table 1 presents the permeated depth of liposomes at each sampling position for exposed or sham-exposed to ultrasonic irradiation with three different frequencies. In this table, the permeated depth of liposomes, are presented in a unit of micrometer. Sham irradiation experiments are used to compare the influence of the ultrasonic irradiation in the liposomes. In addition, the sham irradiation experiments were measured the permeated depth after maintained the liposome solution about 30 min in the skin. Figure 4 shows the permeated depth distribution of the exposure area of the skin samples without exposure to ultrasound, based on the color plot. The sampling position A1 to A9 indicate the relative position in the exposure area. The color scale is given by MATLAB package, and expanded from 130 to 200. The average value of permeated depth of liposomes in the skin sample is about 138 μm, as indicated in Table 1. Based on the value of permeated depth, the distribution of the liposomes was about 130 to 145μm in the Z-axis. In this condition, the attraction of molecule and the absorption of the skin afford the liposomes to permeate the skin sample.

Figures 5(a)-(c) plot the distribution of permeated depth with ultrasound exposure obtained from the data in Table 1. In the 20 and 60 kHz exposure experiment, the sound beam is incident into the cuboid acrylic wedge to produce a diffuse ultrasonic field and expose the skin sample. Figures 5(a) plot the results of exposure to the ultrasonic frequency of 20 kHz in the intensity of 0.45 W/cm^2. In this image, the distribution of permeated depth is from 148.3 to 173.3 μm, and the average permeated depth of liposomes is 159 μm, as shown in Table 1. It must be notice that the thermal effects induced by ultrasound will be avoided in this research. Thus the ultrasonic transducer does not contact the skin sample directly in the exposure experiments and the shorter exposure time can reduce the temperature rise. Comparing to the sham irradiation results, the average permeated depth under the ultrasonic exposure is increased about 20 μm. The greatest depth was 173.3 μm and appeared in the sampling position A5. Figures 5 (b) plots the distribution of permeated depth exposed to the ultrasonic frequency of 60 kHz with the intensity of 0.45 W/cm^2. In this image, the distribution of permeated depth is from 151.7 to 185 μm. The average permeated depth of liposomes is 168 μm, as shown in Table 1. The average permeated depth is exceeded about 30 μm to the sham-exposed result. It is also better than the result of 20 kHz about 10 μm. As can be seen in the Table 1, all sampling positions appeared more than 165 μm permeated depth except A7 and A9. The greatest depth was 185 μm and appeared in the sampling position A2. Figures 5 (c) plots the distribution of permeated depth exposed to the ultrasonic frequency of 1 MHz. Comparing to the wedge exposure, this experiment is used the traditional sonophoresis apparatus. The ultrasonic transducer will contact the skin sample directly. It must be notice that the exposure area is about 30×30 mm. The average permeated depth of liposomes is 159.5 μm and the greatest depth is 167.7 μm appeared in the sampling position A2. The average permeated depth is exceeded about 20 μm to the sham-exposed result.

Figs. 6(a)-(c) show the effects of the ultrasound exposure and thus clarify the change in the permeated depth of the sampling position between the exposed or sham-exposed to ultrasonic irradiation. These figures plot the average values of permeated depth as a function of sampling position at frequency of 20, 60 kHz and 1 MHz, respectively. One sampling position represents an arithmetic mean over six sampling points. As can be seen in these figures, the permeated depth of treated samples are greater than the sham-exposed skin. In the sampling position A1, the permeated depth of the exposed samples are over 170 μm than the control samples in the frequencies of 20 kHz and 60 kHz in Figs. 6(a)-(b). Based on the corresponding dimensions of the wedge presented in Fig. 2, the sound beam is incident with the angle 45° . When ultrasound is applied, the sound wave is initially reflected from the boundary of the wedge and the reflected beam points to the sampling position A1, A2, A4 and A5. The first reflected sound wave penetrate through the wedge and produce greater acoustic radiation force. Thus, the acoustic radiation force affects the liposomes and pushes them down to the skin. It can be seen that the two figures has greater permeated depth in the sampling positions in the A2 and A5. Fig. 6(c) is the average values of permeated depth as a function of sampling position for the skin sample with exposure frequencies 1 MHz. In Figs. 6(c), the distribution of the permeated depth is from 148.7 to 167.7 μm. Notably, the exposure area in the 1 MHz irradiation experiments is smaller than the wedge experiments. Thus, it can be seen that under the 60 kHz irradiation, the average depth result and the distribution of the permeated depth is greater than the other frequencies.

Table 1. The permeated depth of the different sampling positions are exposed to ultrasound at two output intensities by using the 20, 60 kHz and 1 MHz frequencies. The unit of the recorded values are micrometer. In this table, the (AVG) is the average permeated depth in the series of sampling positions.

Frequency / sampling position	Sham exposed	20 kHz	60 kHz	1 MHz
A1	130	171.7	173.3	160
A2	130	158.3	185	167.7
A3	133.3	150	165	157.3
A4	136.7	161.7	165	164.7
A5	145	173.3	176.7	163
A6	141.7	158.3	168.3	154.7
A7	145	153.3	158.3	148.7
A8	145	153.3	171.7	157.3
A9	138.3	148.3	151.7	162
AVG	138	159	168	159.5

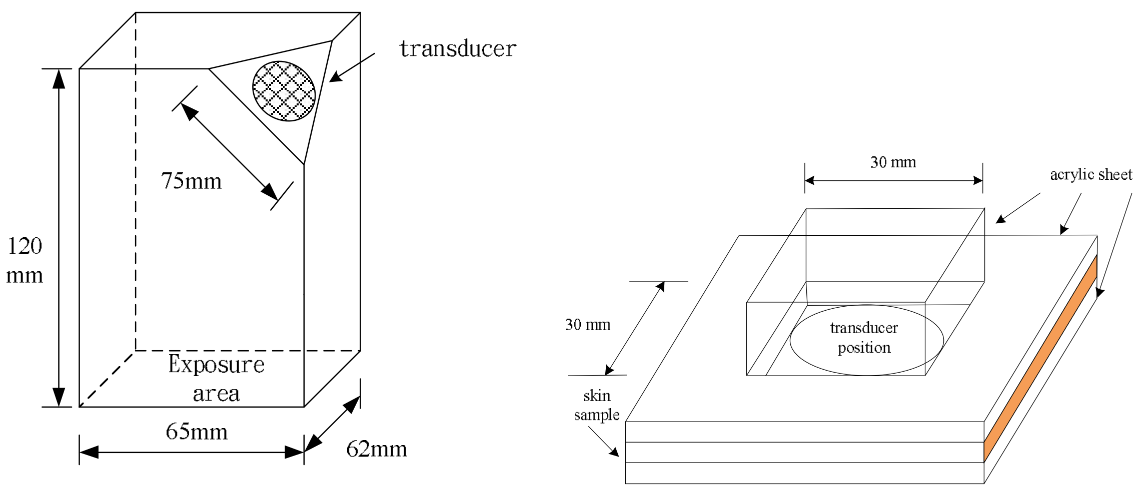

Figure 1. (a)The dimensions of the exposure wedge. The orientation of the transducer is fixed in the corner of the wedge. (b)The exposure chamber used in the 1 MHz experiments.

5. Conclusions

This study examined various subjects. First, the design wedge with inclined incidence of sound wave were applied to investigate the permeated effects of ultrasound. Second, three ultrasonic frequencies of 20, 60 kHz and 1 MHz were applied. Third, the average permeated depth of liposomes in each experiment were described and the permeated depth distribution of the sampling position in the skin samples were compared. An ultrasonic intensity of 0.45 W/cm^2 and the frequency of 60 kHz permeated the liposomes more effectively than other setup. An appropriate ultrasonic frequency inclined incident into the designed wedge could induce the permeability of liposomes and increased the permeated depth of particles in skin samples.

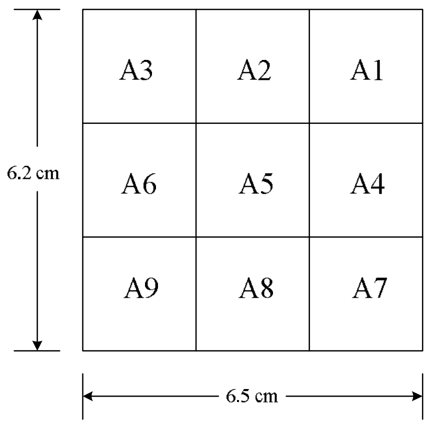

Figure 2. The sampling positions of the exposure area applied in the experiments. The diameter is about 62×65 mm in the wedge bottom. The sampling positions of ultrasonic diathermy system is the same as this figure, only the exposure area of ultrasonic diathermy system is about 30×30 mm.

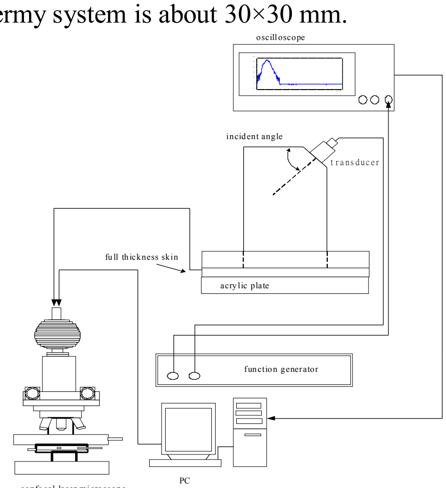

Figure 3. Schematic diagram of the isonation and measurement apparatus used in the exposure experiments.

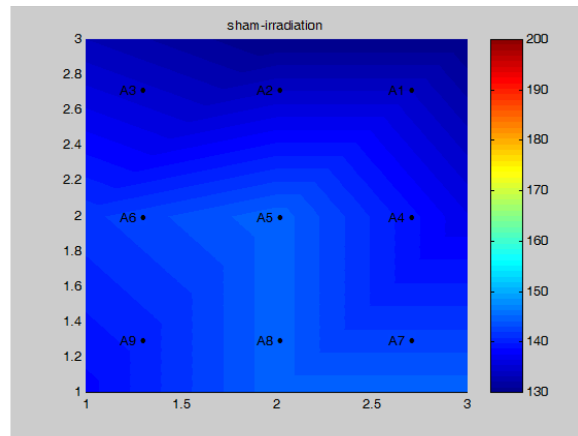

Figure 4. Color mapping of the permeated depth distribution for the skin sample with no sound field applied. Color plot corresponds to the magnitude of depth value. A1 to A9 is the sampling position with respect to the skin sample.

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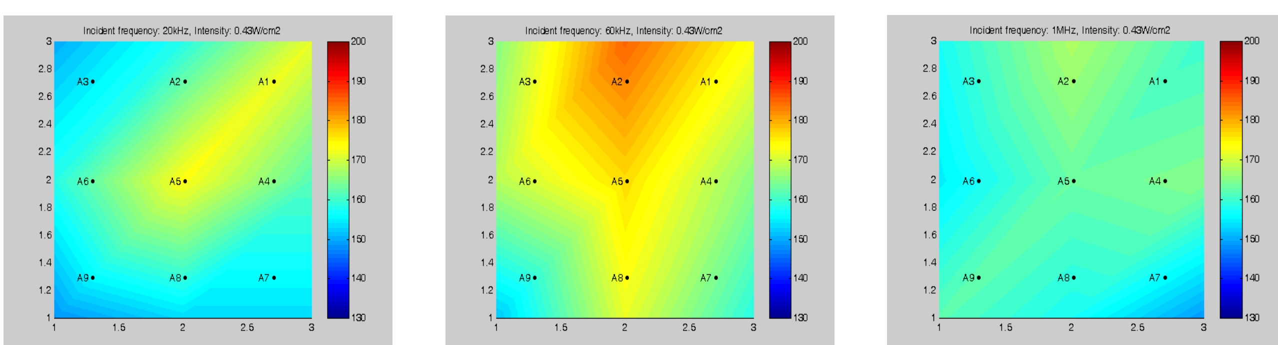

(a) (b) (c)

Figure 5. Color mapping of the permeated depth distribution for the skin sample with exposure frequencies of 20, 60 kHz and 1MHz: (a) demonstrate the frequency of 20 kHz with intensities 0.45 W/cm^2, (b)(c) demonstrate the frequency of 60 kHz and 1 MHz.

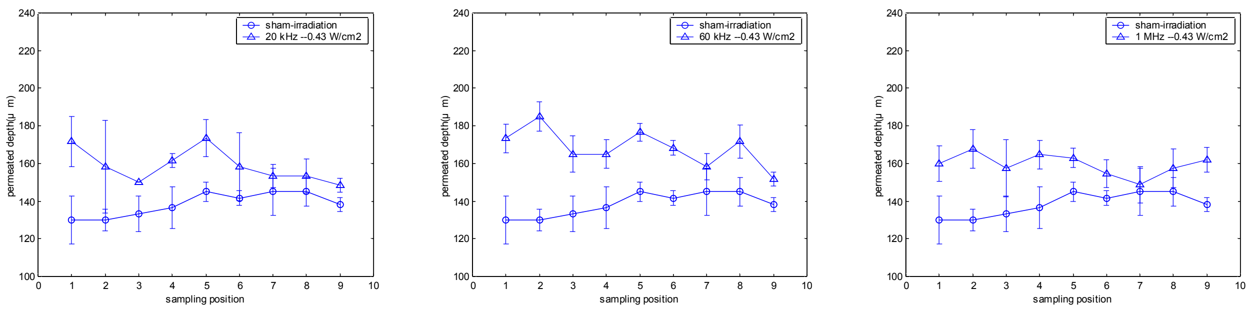

Figure 6. The average values of permeated depth as a function of sampling position for the skin sample with exposure frequencies of 20, 60 kHz and 1 MHz: (a) demonstrate the frequency of 20 kHz with intensities 0.45 W/cm^2, (b)(c) demonstrate the frequency of 60 kHz and 1 MHz.

References

1. El-Kamel AH, Al-Fagih IM, Alsarra IA. Effect of sonophoresis and chemical enhancers on testosterone transdermal delivery from solid lipid microparticles: an in vitro study. Current Drug Delivery, 2008,5:20-26.
2. Suhonen TM, Bouwstra JA, Urtti A. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. J Control Rel, 1999,59:149-161.
3. Prausnitz MR, Bose V, Langer R, Weaver JC. Electroporation of mammalian skin: A mechanism to enhance transdermal drug delivery. Proc Natl Acad Sci USA, 1993,90:10504-10508.
4. Song Y, Moshal KS, Krasna MJ. Using technique of Video-Assistant Thoracic Surgery (VATS) and small incision for diagnosis and treatment of pleurapulmonary diseases under local anesthesia. Life Sci J, 2009,6(2):83-87.
5. Aboserea M, Abdelgawad M, Wafik W. Early detection of breast cancer among females at fakous district, sharqia governorate, egypt. Life Sci J, 2011,8(1):196-203.
6. Mitragotri S. Low frequency sonophoresis. Adv Drug Deliv Rev, 2004,56:589-601.
7. Hideo Ueda, Mizue Mutoh, Toshinobu Seki, Daisuke Kobayashi, Yasunori Morimoto. Acoustic Cavitation as an Enhancing Mechanism of Low-Frequency Sonophoresis for Transdermal Drug Delivery. Biol Pharm Bull, 2009, 32(5):916-920.
8. Mitragorti S, Blankschtein D, Langer R. Transdermal Drug Delivery Using Low Frequency Sonophoresis. Pharm Res, 1996, 13:411-420.
9. Madanshetty SI, Apfel RE. Acoustic micro-cavitation: Enhancement and applications. J Acoust Soc Am, 1991, 90:1508-1514.
10. Dahlana A, Alpara HO, Murdan S. An investigation into the combination of low frequency ultrasound and liposomes on skin permeabilitys. Int J of Pharm, 2009, 379(1):139-142.
11. Gupta S, Feke DL. On the Use of Acoustic Contrast to Distinguish between Agglomerates of Finely Dispersed Polymeric Particles. J Acoust Soc Am, 1998, 104(4):2189-2191.
12. Sabine WC. *Collected Papers on Acoustics*, Dover, New York, 1964.
13. Huang YC. Effects of Ultrasonic Diffuse Field on Particle Dispersion in Liquid. J Chin Inst Eng, 2010, 33(1):111-119.

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