

1 RESEARCH ARTICLE

2 The Effect of Bubble Surface Charge on
 3 Phonophoresis: Implication in Transdermal
 4 Piroxicam Delivery

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10 ABSTRACT

11 It is over several decades that ultrasound is used to enhance the transdermal drug delivery (phonophore-
 12 sis). The mechanism of the enhancement is not fully understood and the ability of ultrasound on the en-
 13 hancement for some drugs is unclear. The effect of continuous wave 870 KHz ultrasound at intensity of 1
 14 W/cm² for 15 minutes on transdermal absorption of piroxicam from solution and gel formulations in hair-
 15 less rat skin was studied. Exposure to ultrasound increased the rate of diffusion from gel and solution of
 16 piroxicam to 10 and 3 times higher than that in skins not exposed to ultrasound. We strongly believe that
 17 the lower diffusion of piroxicam from the solution is caused by extra-bubbles generated by ultrasound. It
 18 can be suggested that cavitation activity and its negative surface charges play a dominant rule in phono-
 19 phoresis.

20 **Keywords:** Piroxicam, Phonophoresis, Cavitation, Micro-streaming

21 Transdermal drug delivery is designed to transfer
 22 drugs from the surface of the skin and through its vari-
 23 ous layers into the systemic circulation [1]. In normal
 24 condition, the diffusion of drug into the skin and beyond
 25 that is limited. This limitation is attributed to stratum
 26 corneum of the skin. In order to increase diffusion of
 27 drugs through the skin, physical & chemical approaches
 28 have been adopted to change stratum corneum proper-
 29 ties. Phonophoresis is a physical method to disturb the
 30 stratum corneum and is defined as the enhancement and
 31 movement of drugs through intact skin and into soft
 32 tissue under influence of ultrasound perturbation [2].
 33 Although significant attention has been devoted to the
 34 investigation of phonophoresis, its mechanism of action
 35 has not been clearly understood.

36 There are several parameters, which may affect the
 37 skin upon exposure of ultrasound. These parameters as
 38 summarised by Mitragotri are cavitation, thermal effect,
 39 induction/convection processes and mechanical effects.
 40 The role of cavitation in diffusion processes is well
 41 documented. Mitragotri stated that enhancement of drug
 42 by phonophoresis is due to disordering of lipid bilayer
 43 of stratum corneum which is resulted from cavitation
 44 effects [3]. In another report, Mitragotri concluded that

45 ultrasound has no effect on increasing diffusion of some
 46 drugs [4]. Some other researchers believed that the en-
 47 hancement effect of phonophoresis was more pro-
 48 nounced for polar compounds compared to non-polar
 49 compounds [5]. Although the importance of cavitation
 50 has been realised by other investigators, its effect on
 51 diffusion of drugs has not been fully described.

52 Bubbles are usually produced in the sound field
 53 from small inactive bubble nuclei which are normally
 54 present in a medium activated by the pressure fluctua-
 55 tion of the sound field. This causes volume pulsations of
 56 the nuclei, growth by rectified diffusion to resonant size,
 57 and concentration of acoustic energy in their vicinity
 58 [6,7]. The nuclei will grow by a process of rectified dif-
 59 fusion [7-9]. Research by Watmough on cavitations
 60 revealed that the mapping of ultrasound field on paper is
 61 strongly dependent on the charge of the ions of the dyes
 62 and presence of micro-bubbles [10,11]. Shiran has
 63 shown that there would be no pattern of ultrasound field
 64 in the absence of micro-bubble [12].

65 In this study, the effects of micro-bubbles and their
 66 electrical surface charges on the rate of drug diffusion in
 67 phonophoresis have been assessed.

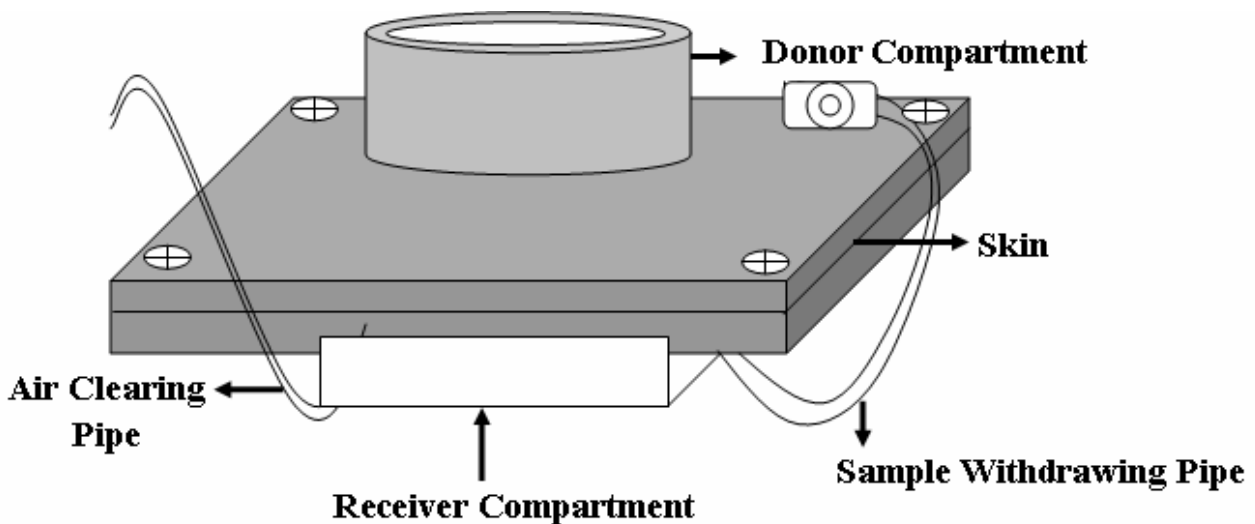


Fig. 1. Two compartments chamber used for mounting the isolated skin

MATERIALS AND METHOD

A costume-made chamber with two compartments was constructed as shown in Fig 1. The receiving compartment was covered with rubber (1 mm thickness) as an absorber to prevent standing wave formation and multiple reflections during sonification. The temperature of the skin inside receiving compartment was measured by a digital thermometer (LT Luton TM 905). Two plastic tubes (1 mm diameter) were fitted into the receiver compartment to take sample from, and to clear the air under the skin. A magnetic stirrer with Perspex cover and speed of 100 rpm placed in a ban to keep temperature constant at 33°C throughout the experiments. Male Wistar rats (200-300 g) were sacrificed by cervical dislocation and the abdominal hair was carefully removed with electrical clips.

The stratum corneum side of the prepared abdominal skin was mounted on ring-shaped donor compartment with 2.5 cm diameter; large enough to allow the ultrasound to pass through without any disturbances. The donor and receiver compartments were screwed together and 55 ml of normal saline (9 g/L) was injected into the receiver compartment and left for 15 hours in refrigerator. Prior to the experiment, the skin was washed with fresh normal saline. Pre-experimental solution was then replaced by fresh normal saline at 33°C.

A plane circular 870 KHz transducer, 4 cm in diameter powered by sonostat 633 generator, was placed vertically in the donor compartment. The fixed skin in the receiving chamber was covered either with 10 g of piroxicam gel (0.5%) or piroxicam solution (5×10 ml; 20 mg/ml) and exposed to ultrasound in continuous wave mode at intensity of 1W/cm² for 15 minutes. The equipment was calibrated by radiation force and dye/paper method before use. Each experiment was repeated four times and the mean of the values were obtained. Aliquots of 5 ml were withdrawn from the receiver chamber immediately after the end of sonification and then at 2-hour intervals up to 6 hours. After withdrawal of each sample, the same volume of normal saline was added to keep the receiver chamber volume constant during experiment. The drug concentration was determined using HPTLC system.

RESULTS

Fig. 2 shows the results of continuous wave ultrasound applied to the skin covered with the piroxicam gel. The rate of piroxicam diffusion through the skin, using gel formulation, was over 10 fold higher compared to its control for 6 hours of sampling, with sharp increase in piroxicam concentration two hours after the termination of sonification. The rate of diffusion of piroxicam from solution in

Table 1. The mean value of the amount of piroxicam from gel formulation in the receiver chamber with and without ultrasound (US) radiation

Time (minute)	Amount of Piroxicam (µg) (Mean ± SE)		t-test	Ratio of the amount of Piroxicam (with US/without US)
	Without (US)	With (US)		
15	0	0	-	00.0
120	3.71±0.5	40.3±4.5	S*	10.9
240	5.98±1.2	58.64±2.3	S	10.0
360	6.55±1.2	76.41±3.9	S	11.6
480	8.74±2.3	91.83±6.9	S	10.5

*S=significant (p<0.05)

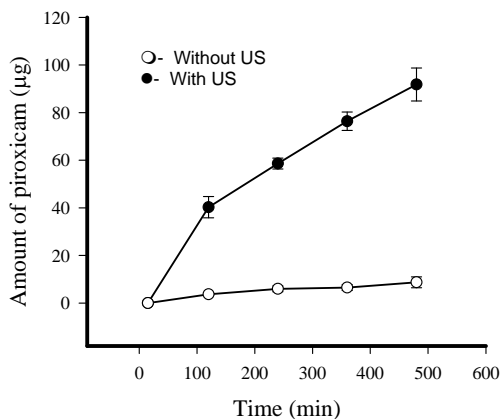


Fig. 2. The time-concentration curve of piroxicam gel upon 15 minutes ultrasound treatment (data are means ± standard error)

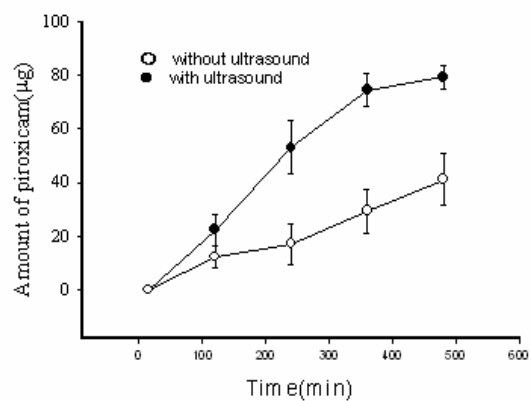


Fig 3. The time-concentration curve of piroxicam solution upon 15 minutes ultrasound treatment (data are means ± standard error)

Table 2. The mean value of the amount of piroxicam in solution form in the receiver chamber with and without ultrasound (US) radiation

Time (minute)	Amount of Piroxicam (µg) (Mean ± SE)		t-test	Ratio of the amount of Piroxicam (with US/without US)
	Without (US)	With (US)		
15	0	0	-	0.0
120	12.3±4.3	22.43±5.9	NS*	1.8
240	17.16±7.6	53.16±10	S**	3.1
360	29.52±8.2	74.47±6	S	2.5
480	41.24±9.8	79.45±4.5	S	2.0

*NS= no significant, **S=significant (p<0.05)

the presence and absence of ultrasound application is illustrated in Fig. 3. The graph shows an increase in piroxicam concentration in receiver chamber. The absolute increase in concentration is about 3 times higher than its control with very low diffusion rate two hours after termination of sonication.

The mean of the piroxicam concentrations found in receiver chamber with or without ultrasound radiation are shown in Tables 1 and 2 for gel and solution respectively. The solution of piroxicam in the absence of ultrasound also shows a more linear penetration of the drug compared to the gel formulation. A maximum 7°C temperature increase was recorded for both sets of experiments.

DISCUSSION

The results indicate that ultrasound has an enhancing effect on transdermal drug delivery. This enhancement is believed to be attributed to several factors such as thermal, cavitation and convective effects [3]. Ueda and co-workers found different diffusion rate for polar and non-polar drug, but they related this increase to thermal effect and diffusivity of the drug across the stratum corneum [5]. The temperature rise in this study was about 7°C, which according to previous studies can not have a great effect on the enhancement of piroxicam diffusion through the skin [2,3].

The amount of piroxicam measured in receiver chamber from the gel formulation was almost 10 times higher than the control value, which was statistically significant. The concentration of piroxicam measured for the solution of piroxicam was almost three times higher compared to its control, even with higher concentration of drug applied to the skin. The increase of piroxicam permeation through the skin in both gel and solution forms upon ultrasound exposure signifies the involvement of other mechanisms rather than the change in the temperature alone. Since the increased permeation from gel is more pronounced than that from solution and also the possibility of bubble formation in solution is more than that in the gel, the bubble activation should be reviewed.

It seems that cavitation was an important factor which caused the rate of diffusion of piroxicam from solution to be less than that from the gel despite the higher concentration of drug in the solution. Usually, there are some bubble nucleus existing in the liquid medium and these nuclei will grow under rectified diffusion.

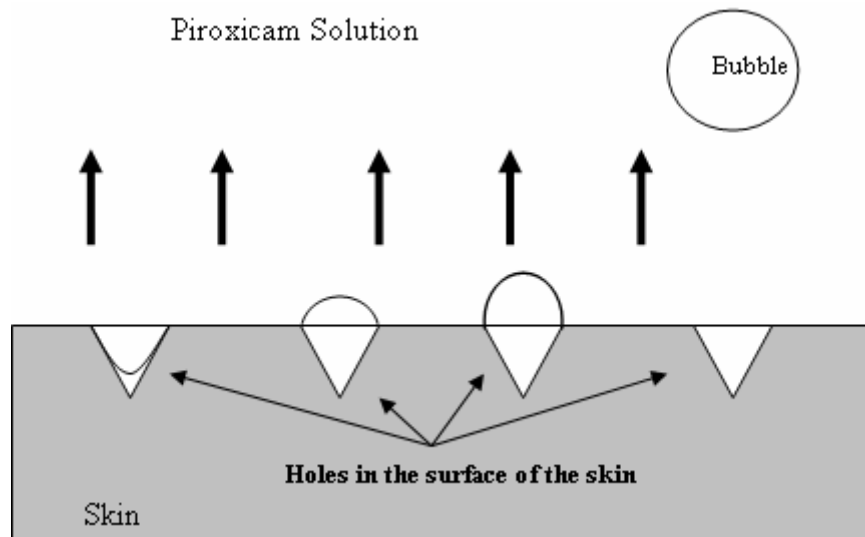


Fig. 4. Formation of bubble on the surface of the skin during sonication in solution

169sion and form bubble in a sound field. There are three 207piroxicam concentration is maintained at its lowest
 170possible areas in which bubbles may form: in the liquid 208level, less piroxicam enters the skin. There are greater
 171medium, on the surface of the skin and inside the epi-209numbers of induced gas bubble in piroxicam solution
 172dermis. Close examination of the surface of the skin 210compared to gel formulation, which can repel the nega-
 173shows that the surface of the skin contains a large num-211tively-charged piroxicam ions. This explains why the
 174ber of holes about 0.4mm in diameter [13]. These holes 212amount of piroxicam measured from sonicated solution
 175accommodate gas nuclei and when sonicated, the air 213of piroxicam with five times higher concentration was
 176will expand forming gas bubbles and because of the 214lower than that from the gel. Such large difference has
 177temperature rise in the skin, rectified diffusion may also 215not been observed in the control groups. In fact, the
 178contribute to their growth, as shown in Fig. 4. Mitragotri 216penetration of drugs through the skin in control experi-
 179et al. and Wu et al. strongly believed that the growth of 217ments with piroxicam solution was slightly higher.
 180bubble by rectified diffusion does take place in the 218Bommannan et al. reported that penetration rate of drug
 181keratinocytes and lipid bilayer [3,14]. It was also be- 219through the skin for 2 MHz ultrasound was less than
 182lieved that the presence of cavitation is the possible 220that for 16 MHz ultrasound [2]. They also stated that the
 183mechanism of the passage of high molecular weight 221treatment with ultrasound at 10 and 16 MHz signifi-
 184drugs through the skin [14]. They realized that the cavi- 222cantly increased penetration of drug (almost 5 fold).
 185tation influenced the penetration of drug through the 223They could not observe significant difference between
 186skin, but the mechanism of action was not completely 224the ultrasound-treated and controls at these frequencies.
 187explained. It was reported by a number of researchers 225They concluded that “the enhancing effect of sonopho-
 188that the gas bubbles possessed a considerable negative 226resis is due to direct effect of ultrasound on, presuma-
 189electrical charges on the surface [10,15-20]. Margulis 227bly, stratum corneum”. According to Hueter and Bolt
 190and Shiran experimentally measured the charge on bub- 228“above 10 MHz frequency, a vapour type of cavitation
 191ble surface in distilled water to be 3×10^{-13} C and 10×10^{-229} is unlikely to occur” [22]. The presence of cavitation
 192¹³ C respectively [19,20]. Watmough et al. suggested 230and presumably the negative charges of the drug ions
 193that the charges on acoustically-excited bubbles are 231suppressed the rate of penetration at 2 MHz frequency,
 194greater than gas/water interface in the absence of ultra- 232while there were no bubbles at frequency of 10 and 16
 195sound [10]. They also stated that ultrasound is capable 233MHz. The evidence provided here also suggests that it
 196of maintaining the surface charges on the bubbles even 234took almost 2 hours before the bubble (on the surface
 197in the presence of ions which would otherwise neutral- 235and possibly inside the keratinocytes and lipid bilayer)
 198ize the charge.

236could dissolve and the passage for piroxicam diffusion
 199 The charged bubbles would thus be expected to at- 237be cleared to accelerate the drug passage.
 200tract the cationic drug ions and thus increase the local 238 In conclusion, this study has demonstrated that ultra-
 201concentration of drug. Anionic drug ions, on the other 239sound significantly enhanced transdermal absorption of
 202hand, would be repelled by the electric field set up by 240piroxicam from solution and gel formulations. It also
 203micro-bubbles. Since piroxicam ions are negatively 241suggests that the cavitation and its surface charge play a
 204charged, local stirring caused by micro-streaming in- 242major rule in this enhancement. We also believe that the
 205creases this depletion of piroxicam when resonant bub- 243size of bubble over the surface of the skin, keratinocytes
 206bles are excited by high local intensity [21]. Where the 244and lipid bilayer does affect the rate of permeation.

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