International Journal of Pharmaceutics 385 (2010) 37–41

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Efficiency of low-frequency ultrasound sonophoresis in skin penetration of histamine: A randomized study in humans

Annabel Maruani[a,b,c,*], Emilie Vierron[a,d,e], Laurent Machet[a,b,c], Bruno Giraudeau[a,d,e], Alain Boucaud[f]

[a] Université François Rabelais Tours, France
[b] INSERM, U930 Tours, France
[c] CHRU de Tours, Service de Dermatologie, France
[d] INSERM, CIC 202, France
[e] CHRU de Tours, France
[f] Transderma Systems, Tours, France

ARTICLE INFO

Article history:
Received 24 May 2009
Received in revised form 6 October 2009
Accepted 8 October 2009
Available online 27 October 2009

Keywords:
Low-frequency ultrasound
Sonophoresis
Clinical study
Histamine
Allergy

ABSTRACT

Low-frequency ultrasound (US) applied to skin (sonophoresis) has been investigated to enhance the transdermal transport of various drugs. Histamine is usually used in allergy investigations. We aimed to investigate, in a randomized study, the transdermal penetration of histamine with sonophoresis. Ten subjects were included. Their right forearm was divided into three zones, which were randomly assigned a treatment: no US, US_1 ($I_1 = 2.72\ W/cm^2$), US_2 ($I_2 = 3.50\ W/cm^2$). The primary outcome was area of induced papule, which revealed histamine penetration. Secondary outcomes were echographic measurement of papule (skin thickness) and pruritus. Measurements were taken immediately after US application and after 30 min, 2 h and 24 h. Arm zones without US application showed no papules induced by histamine; 9/10 subjects receiving US showed papules. Their mean size increased with increased intensity of US but not significantly. The skin thickness increased with US. Pruritus occurred in 7/10 cases after US and histamine. The adverse events were skin erythema, pain and tinnitus. Though this study included a few number of patients, it confirms that sonophoresis enhances skin penetration of histamine. This technology could be used at therapeutic levels: histamine could be used with sonophoresis as a positive control in allergy testing instead of prick tests, which involve skin disruption with a lancet.

1. Introduction

Histamine is a low-molecular-weight molecule (almost 110 Da) largely synthesized by basophile granulocytes and involved in several immune/inflammatory functions in addition to its dominant role in type I hypersensitivity reactions (Akdis et al., 2008). Its chemical structure is $C_5H_9N_3$ (2-(4-imidazolyl)ethylamine). It is synthesized by histidine decarboxylase, starting from the amino acid histidine. In medical practice, histamine is used in allergy testing to investigate drug hypersensitivity: histamine prick tests are used as positive controls (Liccardi et al., 2008; Malling, 1984). Prick tests involve skin disruption with a lancet before deposition of the drug. Test results are considered positive if an itchy papule appears a few minutes after the deposition of the molecule.

Low-frequency sonophoresis has been investigated to enhance the transdermal transport of various drugs of low and even high molecular weight (such as insulin, 6 kDa) (Boucaud et al., 2002; Smith et al., 2003). Most investigations have been performed *in vitro* in excised skin and *in vivo* in animals (Byl, 1995; Liu et al., 2006; Mitragotri et al., 1995; Mitragotri and Kost, 2001). In humans, sonophoresis has been shown to be safe under defined parameters of ultrasound (US) delivery (Maruani et al., submitted for publication). As compared with injections, sonophoresis is noninvasive, because it does not disrupt the skin.

We aimed to investigate, in a randomized study of healthy human subjects, the transdermal penetration of histamine with sonophoresis.

2. Materials and methods

2.1. Participants and setting

The study was carried out from July to December 2006, in the Clinical Investigation Center of the University Hospital of Tours, France. The subjects were healthy volunteers who were 18 years or older, were not pregnant or breastfeeding, did not use topical therapy, and had neither dermatological nor neurological disease.

* Corresponding author at: Service de Dermatologie, CHRU de Tours, Avenue de la République, 37044 Tours Cedex 9, France. Tel.: +33 2 47 36 62 56; fax: +33 2 47 36 62 56.
E-mail address: annabel-maruani@wanadoo.fr (A. Maruani).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.10.024

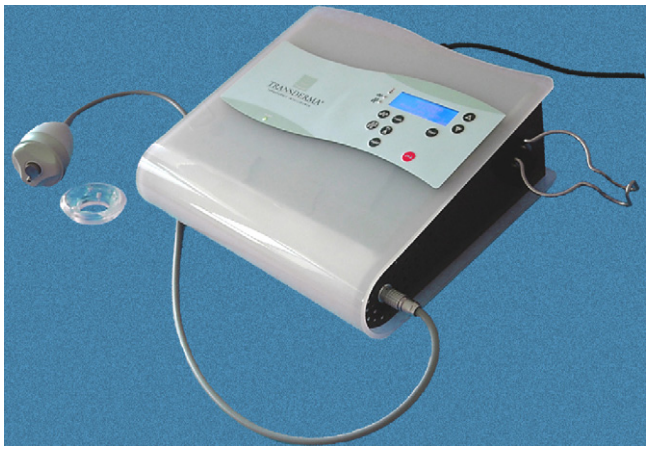

Fig. 1. Photo of the low-frequency ultrasound device.

Each volunteer gave their signed informed consent to be in the study. Before inclusion, each subject received a prick test with histamine on the left forearm as a positive control and a prick test with glycero-saline phenol as a negative control. To be included in further steps of the study, the subjects had to be correctly reactive to prick tests: a positive reaction for the histamine prick test was the development of a papule ≥2 mm in both diameters (if no papule with negative control) or a papule at least twice as large in both diameters than the papule induced by the negative control prick test.

The protocol was approved by the Ethics Committee of the University Hospital of Tours, France (registration 2006-15).

2.2. Ultrasound device

The US equipment (Fig. 1) was developed by Transderma Systems (Tours, France). The working frequency of the device was 36 kHz. The tip of the device included a cylindrical ultrasonic horn (titanium) 1.8 cm in diameter housed in a tube made of Delrin. The device also included a "cavitation chamber" consisting of an O-ring with a 7-cm^2 inner surface placed between the skin surface and the ultrasonic horn and containing 10 ml of saline ([NaCl] = 0.9%) as a coupling medium. During the experiments, the tip of the ultrasonic horn was always positioned at 5 mm from the skin. The power unit of the device allowed for choosing various parameters, including intensity, continuous or pulsed mode, and application time. Before each application, the equipment was tuned to ensure that the electrical signal matched the resonance frequency of the US probe. Low-frequency US was applied in pulsed mode to minimize thermal effects for 5 min. US intensity was determined by a calorimetric method commonly used for measurement of low-frequency US intensity (Weimann and Wu, 2002). Two schemes of US were investigated: 2 s on/5 s off, intensity (I) I_1 = 2.72 W/cm^2 (US_1); and 3 s on/5 s off, I_2 = 3.50 W/cm^2 (US_2).

2.3. Protocol and blinding

The subject sat on a medical chair. The research engineer "(AB)" defined three zones on the anterior side of the right forearm (Fig. 2) and randomized the zones for treatment according to a computer-generated randomization sequence: one zone received no US, one received US_1 and the last zone received US_2. If toxic effects appeared (pain > 40 on a 0–100-mm visual analogue scale [VAS], or necrosis), the engineer discontinued US delivery. The dermatologist "(AM)" blinded to the application of US, received the subject in another room and applied one drop of histamine on each zone of the forearm immediately after US. After 30 min, 2 h and 24 h, arms were measured for induced papules, and secondary outcomes were observed.

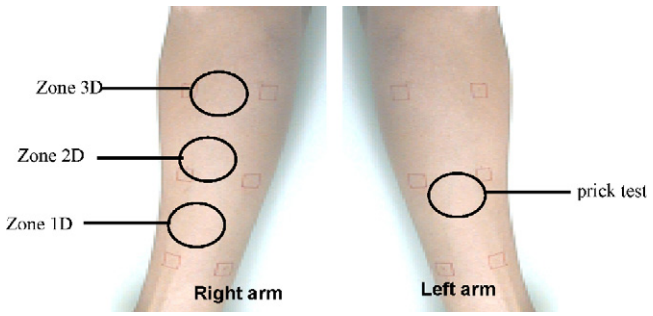

Fig. 2. Definition of three zones on the anterior side of the right forearm.

2.4. Outcome measurement

The primary outcome was penetration of histamine, as assessed by the size of the induced papule; the area was calculated according to the two diameters measured by the dermatologist.

Secondary outcomes consisted of the following:

- echographic measurement of the induced papule by the dermatologist; skin thickness was measured by high-resolution, B-mode, real-time US (DermCup 2020®, GIP Ultrasons, Tours, France) with use of a 20-MHz center-frequency transducer (Machet et al., 2006);
- pruritus induced by skin application of histamine, self-reported by the subject to the dermatologist and self-evaluated according to a scale of 1 = low intensity of itching; 2 = moderate intensity; 3 = high intensity; and 4 = very high intensity;
- adverse effects, which were systematically recorded by the research engineer and by the dermatologist: subjects were especially asked about pain, self-reported on a VAS ranging from 0 (no pain) to 100 (maximal pain).

All these measurements were taken immediately after US application and after 30 min, 2 h and 24 h.

2.5. Statistical methods

We planned to include 10 subjects in this pilot study. Demographic data and measurement of papule area are presented as mean ± standard deviation or frequencies, or median [interquartile range]. Data for secondary outcomes are presented as mean ± standard deviation for baseline data and mean variation from baseline for further measures. A Wilcoxon signed rank test for paired data was used to compare the two US intensities for papule area. Statistical analyses involved the use of SAS (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant.

3. Results

Ten healthy subjects were included (8 females; age range 19–51 years); an itchy papule developed after the histamine prick test in all subjects, and no subject showed a reaction to the negative prick control.

3.1. Primary outcome

Arm zones without US showed no papules induced by histamine. In 9 of 10 patients, arm zones with US, whether US_1 or US_2, showed

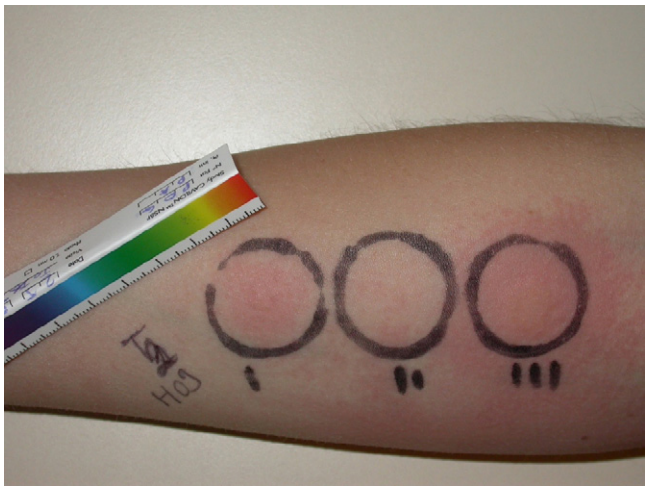

Fig. 3. Papules observed 30 min after the deposition of histamine on skin: one zone showed a large papule (zone III, after US_2), one a moderate papule (zone I, after US_1) and one no papule (zone II, no US previously applied).

Table 1
Area of papule induced by the deposition of histamine: measurements obtained 30 min after the application or not ultrasound in 10 patients.

	Number of subjects showing a papule	Area of the papule (mm^2), median [interquartile range]
No US	0	–
US_1	9	39.3 [12.6–121.0]
US_2	9	62.8 [16.5–141.4]

US: ultrasound; US_1: ultrasound with intensity = 2.72 W/cm^2; US_2: ultrasound with intensity = 3.50 W/cm^2.

a papule (Fig. 3). After 30 min, the area of papule, the primary outcome of the study, was significantly greater with than that without US (Table 1). The mean size of papules was greater with higher intensity of US ($US_2 > US_1$) but not significantly ($P = 0.789$). The papules had partially disappeared after 2 h (4 papules persisted with US_2 and 3 with US_1), and only 3 patients showed a small papule after 24 h.

3.2. Secondary outcomes

Measures of skin thickness of papules induced by histamine was measured by echography (Fig. 4) and reflected dermal edema subsequent to histamine absorption, are in Table 2. The results confirm clinical measures: dermal edema increased with US application, and no edema occurred after deposition of histamine without US. The size of the edema was high after 30 min and decreased at 2 h, and no edema persisted at 24 h. With high doses of US (US_2), the median values of edema were higher than those with US_1.

No pruritus was reported after histamine application without US. After US_1, pruritus was reported for 7 of 10 cases (score = 1 in 5 cases, score = 2 in 2 cases); after US_2, pruritus was reported in 7 cases (score = 1 in 3 cases, score = 2 in 2 cases, score = 3 in 2 cases). No pruritus persisted at 2 h or 24 h.

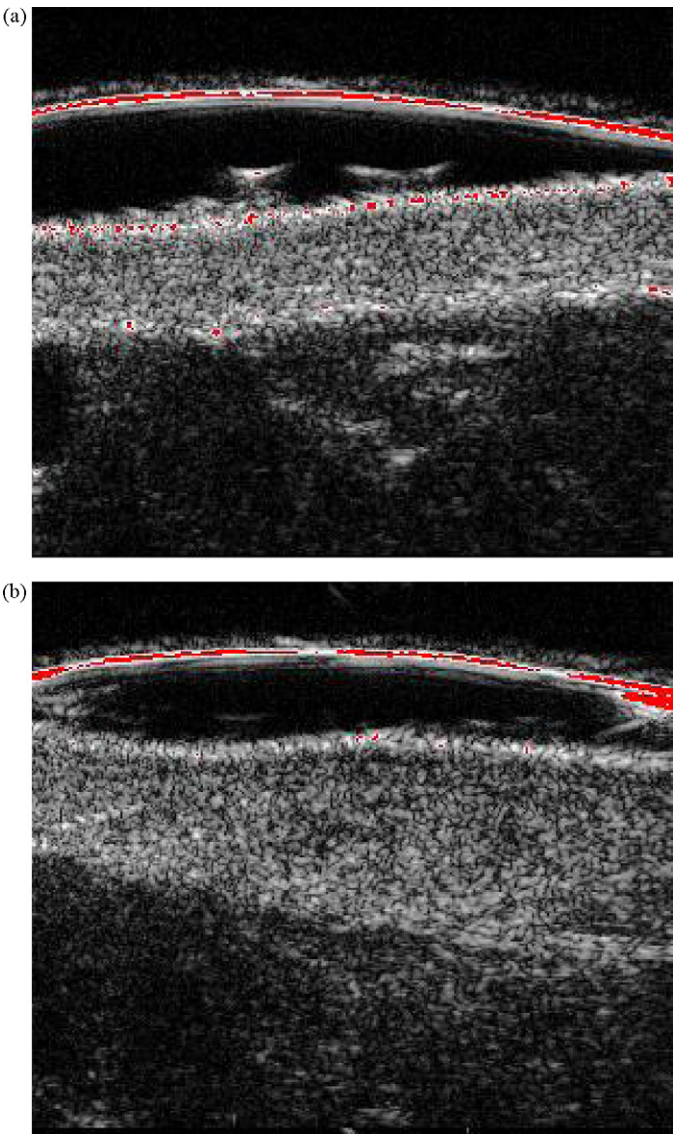

Fig. 4. High-resolution US imaging of skin thickness showing an increase of dermal edema (papule) after 30-min histamine application on skin that previously received low-frequency US. (a) Before US and histamine; (b) 30 min after histamine application.

No toxic effects were observed. The adverse events reported during or after US application were skin erythema on the site of US application in 9 cases with US_1 and US_2 and were persistent at 24 h in 4 cases; purpura on the site of US application (1 case with US_2, which disappeared at 24 h), punctiform excoriation (1 case, regressive after 48 h); tinnitus during US application, without

Table 2
Measurement of dermal edema with ultrasound imaging.

	Dermal edema (mm), median [IQR]			
	T0	T1/2	T2	T24
No US ($n = 10$)	1.00 [0.90–1.00]	1.00 [0.90–1.00]	1.00 [0.90–1.10]	0.95 [0.90–1.00]
US_1 ($n = 10$)	1.05 [0.90–1.10]	1.30 [1.10–1.60]	1.15 [0.90–1.50]	1.05 [1.00–1.40]
US_2 ($n = 10$)	0.95 [0.90–1.10]	1.60 [1.00–1.90]	1.40 [1.00–1.50]	1.05 [0.90–1.30]

US: ultrasound; US_1 = 2.72 W/cm^2; US_2 = 3.50 W/cm^2. IQR: interquartile range. T0: before application of US and histamine; T1/2: 30 min after the application of histamine (with or without previous US); T2: after 2 h; T24: after 24 h.

Table 3
Pain induced by histamine prick tests and application of ultrasound.

	Pain on a 0–100-mm visual analog scale after US application			
	N	Mean ± SD	Median [IQR]	Min–Max
Prick test	4	22.5 ± 17.6	22.5 [7.5–37.5]	5.0–40.0
No US	5	0.0 ± 0.0	0.0 [0.0–0.0]	0.0–0.0
US_1	10	21.0 ± 32.1	10.0 [0.0–20.0]	0.0–90.0
US_2	10	28.7 ± 30.6	10.0 [7.0–50.0]	0.0–80.0

US: ultrasound; $US_1 = 2.72\ W/cm^2$; $US_2 = 3.50\ W/cm^2$. IQR: interquartile range; SD: standard deviation.

acoustic alteration (4 cases, which disappeared as soon as US was discontinued); and pain (Table 3).

4. Discussion

This simple, blinded, randomized clinical study demonstrates transdermal penetration of histamine by sonophoresis, although the number of subjects were small; the penetration was revealed clinically by itchy skin papules and echographically by increased skin thickness. Skin not receiving US showed no papules. Altogether, papules were increased with increased intensity of low-frequency US. Indeed, the higher the US intensity, the more efficient the transdermal penetration, but toxic effects increase in the same way (Boucaud et al., 2001; Machet and Boucaud, 2002). With very high US intensity, superficial necrosis (dermis) and deep necrosis (fascias and muscles) have been described in animals (Singer et al., 1998). In the present study, the US_1 intensity was sufficient to induce papules, which were well tolerated, because they provoked almost the same pain as prick tests, which are commonly used.

Increased transdermal permeability with US application is linked in part to the thermal effects of US and is mainly attributed to cavitation (Simonin, 1995; Tezel et al., 2002). Cavitation consists of the generation of gas bubbles, which oscillate and may implode at the skin surface, thus provoking disorganization and/or creation of an aqueous pathway through the stratum corneum.

Adverse events linked to US application were increased with increased intensity of US ($US_2 > US_1$), as we expected. Erythema and purpura are directly linked to thermal and cavitation effects on skin but were quickly regressive in all cases. Tinnitus has been described during application of high- and low-frequency US (Lenhardt et al., 2002). In a study of sonophoresis tolerance in humans, tinnitus was reported in 23.5% of cases (Maruani et al., submitted for publication); it was of moderate intensity, always resolved after withdrawal of US delivery, and showed no acoustic alteration. Tinnitus occurs with bone conduction of US waves in the ear (Cai et al., 2002).

In our study, pain, which may be induced by sonophoresis, was not constant and showed large variations among subjects. One possible explanation for these variations could be the varied densities of hair follicles among subjects. With application of low-frequency US, gaseous microbubbles entrapped at the root of hair follicles may induce cavitation and pain. Pain occurred with application of US only and disappeared with discontinuation of US. The assessment of pain induced by prick tests has never been investigated, although prick tests are commonly used in allergy investigations. In this study, the variation in pain was large (5–40 on a 0–100-mm VAS), with mean 22.5; pain seemed to be slightly increased during US_1 (21/100) but was slightly less than during US_2 (28.7/100).

The results of this study on 10 subjects confirm that sonophoresis enhances transdermal penetration of histamine as revealed by papule measurement. Only a few studies have reported on the *in vivo* penetration of drugs in humans, and in the future, this technology could be used at therapeutic levels (Becker et al., 2005; Katz et al., 2004; Santoianni et al., 2004). For instance, histamine could be used with sonophoresis as a positive control in allergy tests instead of histamine prick tests, which involve skin disruption with a lancet.

Conflict of interest

Dr. Alain Boucaud is a member of Transderma Systems, which supplied the ultrasound device.

Acknowledgements

The authors thank Jean-Marc Grégoire for technical assistance and Aurélie Avarguès and Catherine Roussel for their support. The study was funded by a “Projet Hôpital Promoteur” (HP) financing.

References

Akdis, C.A., Jutel, M., Akdis, M., 2008. Regulatory effects of histamine and histamine receptor expression in human allergic immune responses. Chem. Immunol. Allergy 94, 67–82.
Becker, B.M., Helfrich, S., Baker, E., Lovgren, K., Minugh, A., Machan, J.T., 2005. Ultrasound with topical anesthetic rapidly decreases pain of intravenous cannulation. Acad. Emerg. Med. 12, 289–295.
Boucaud, A., Garrigue, M.A., Machet, L., Vaillant, L., Patat, F., 2002. Effect of sonication parameters on transdermal delivery of insulin to hairless rats. J. Control. Release 81, 113–119.
Boucaud, A., Montharu, J., Machet, L., Arbeille, B., Machet, M.C., Patat, F., Vaillant, L., 2001. Clinical, histologic, and electron microscopy study of skin exposed to low-frequency ultrasound. Anat. Rec. 264, 114–119.
Byl, N.N., 1995. The use of ultrasound as an enhancer for transcutaneous drug delivery: phonophoresis. Phys. Ther. 75, 539–553.
Cai, Z., Richards, D.G., Lenhardt, M.L., Madsen, A.G., 2002. Response of human skull to bone-conducted sound in the audiometric-ultrasonic range. Int. Tinnitus J. 8, 3–8.
Katz, N.P., Shapiro, D.E., Herrmann, T.E., Kost, J., Custer, L.M., 2004. Rapid onset of cutaneous anesthesia with EMLA cream after pretreatment with a new ultrasound-emitting device. Anesth. Analg. 98, 371–376.
Lenhardt, M.L., Richards, D.G., Madsen, A.G., Goldstein, B.A., Shulman, A., Guinta, R., 2002. Measurement of bone conduction levels for high frequencies. Int. Tinnitus J. 8, 9–12.
Liccardi, G., Passalacqua, G., Falagiani, P., Russo, M., D'Amato, G., 2008. The effect of multiple allergens on histamine release in vivo assessed by skin prick test. Allergy 63, 1559–1560.
Liu, H., Li, S., Pan, W., Wang, Y., Han, F., Yao, H., 2006. Investigation into the potential of low-frequency ultrasound facilitated topical delivery of cyclosporin A. Int. J. Pharm. 326, 32–38.
Machet, L., Boucaud, A., 2002. Phonophoresis: efficiency, mechanisms and skin tolerance. Int. J. Pharm. 243, 1–15.
Machet, L., Ossant, F., Bleuzen, A., Grégoire, J.M., Machet, M.C., Vaillant, L., 2006. High-resolution ultrasonography: utility in diagnosis, treatment, and monitoring dermatologic diseases. J. Radiol. 87, 1946–1961.
Malling, H.J., 1984. Skin prick testing and the use of histamine references. Allergy 39, 596–601.
Maruani, A., Vierron, E., Machet, L., Giraudeau, B., Halimi, J.M., Boucaud, A., submitted for publication. Tolerance of low-frequency ultrasound sonophoresis, and efficacy on skin penetration of histamine: a double-blind randomised study on humans.
Mitragotri, S., Blankschtein, D., Langer, R., 1995. Ultrasound-mediated transdermal protein delivery. Science 269, 850–853.
Mitragotri, S., Kost, J., 2001. Transdermal delivery of heparin and low-molecular weight heparin using low-frequency ultrasound. Pharm. Res. 18, 1151–1156.
Santoianni, P., Nino, M., Calabro, G., 2004. Intradermal drug delivery by low frequency sonophoresis (25 kHz). Dermatol. Online J. 10, 24.
Simonin, J.P., 1995. On the mechanisms of in vitro and in vivo phonophoresis. J. Control. Release 33, 125–141.

Singer, A.J., Homan, C.S., Church, A.L., McClain, S.A., 1998. Low-frequency sonophoresis: pathologic and thermal effects in dogs. Acad. Emerg. Med. 5, 35–40.
Smith, N.B., Lee, S., Shung, K.K., 2003. Ultrasound-mediated transdermal in vivo transport of insulin with low-profile cymbal arrays. Ultrasound Med. Biol. 29, 1205–1210.
Tezel, A., Sens, A., Mitragotri, S., 2002. Investigations of the role of cavitation in low-frequency sonophoresis using acoustic spectroscopy. J. Pharm. Sci. 91, 444–453.
Weimann, L.J., Wu, J., 2002. Transdermal delivery of poly-l-lysine by sonomacroporation. Ultrasound Med. Biol. 28, 1173–1180.