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Comparison of the effect of ultrasound and of chemical enhancers on transdermal permeation of caffeine and morphine through hairless mouse skin in vitro

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Abstract

The effect of ultrasound (US) on permeation of two model drugs, caffeine (CAF) and morphine (MOR), through hairless mouse skin in vitro was compared with that of three chemical enhancers. Low-frequency (40 KHz), low-power (< 0.5 W/cm^2) US was used; the effect of high-frequency US (1.5–3.0 MHz) was also evaluated in the case of CAF. The chemical enhancers, tested in combination with propylene glycol (PG), were benzalkonium chloride (BAC) oleyl alcohol (OA) and α-terpineol (TER). The high-frequency US enhancement of CAF transdermal flux was not statistically significant, while low frequency produced a small but significant increase of the enhancement factor. The effect of US on CAF permeation, however, was lower than that produced by chemical enhancers, in particular OA. The effect of low-frequency US on permeation of MOR was significantly greater (about 10-fold) when compared, on the same frequency and intensity basis, with the effect on CAF. The most active chemical enhancer for MOR, OA, had practically the same effect as low-frequency US. Sonicated skin, although showing slight histological changes, recovered its original low permeability characteristics after turning off sonication. Within the tested system, chemical enhancement appears to offer some advantages over low-frequency US.

Keywords: Transdermal drug delivery; Ultrasound; Phonophoresis; Chemical enhancers; Benzalkonium chloride; Oleyl alcohol; α-Terpineol; Hairless mouse skin; Caffeine; Morphine

1. Introduction

The constantly increasing commercial interest of transdermal products, and the recognition of the poor permeability characteristics of the skin have stimulated a great deal of research, aimed at enhancing transdermal drug delivery in a reproducible and reliable manner. The methods that have been explored to enhance transdermal penetration can be divided into two major categories: physical and chemical (Shah, 1994).

Among the physical methods, sonophoresis (or phonophoresis), that can be defined as ultrasonically-facilitated transport of drugs through the skin, has attracted in recent years the attention of several investigators (Skauen and Zentner, 1986;

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Brucks et al., 1989; Tyle and Agrawala, 1989; Bommannan et al., 1992a,b; McElnay et al., 1993; Meidan et al., 1995; Mitragotri et al., 1995, 1996). The most commonly used conditions for sonophoresis correspond to therapeutic/diagnostic US (frequency range, 1–10 MHz, intensity range, 0.2–3 W/cm^2): the typical enhancement of transdermal drug transport observed in this frequency/intensity range is less than 10-fold, although in many circumstances no or scarce enhancement has been detected. Low-frequency (20 KHz, 0.125 W/cm^2) pulsed US has been reported to be more effective, particularly in the case of proteins and hydrophilic drugs (Mitragotri et al., 1995, 1996) and of clobetasol 17-propionate (Fang et al., 1999).

A wide body of literature, on the other hand, deals with chemical enhancers (or absorption promoters): these act mainly by transiently altering the permeability characteristics of the stratum corneum, which forms the rate-limiting lipophilic skin barrier (Hsieh, 1994; Finnin and Morgan, 1999).

Aim of the present investigation was to compare the effect of US and of chemical enhancers on permeation of two model drugs, caffeine (CAF) and morphine (MOR), through hairless mouse skin in vitro. Low-frequency (40 KHz), low-power (< 0.5 W/cm^2) US was used; the effect of high-frequency US (1.5–3.0 MHz) was also evaluated in the case of CAF. The chemical enhancers, tested in combination with propylene glycol (PG), were representative of three main categories: surfactants (benzalkonium chloride, BAC), fatty alcohols (oleyl alcohol, OA) and terpenes (α-terpineol, TER). The investigation included a histological study aimed at verifying possible damages produced by low-frequency US to the treated skin.

2. Materials and methods

2.1. Materials

The following chemicals were used as received: caffeine (CAF, m.p. = 238 °C); benzalkonium chloride (BAC); propylene glycol (PG) (Carlo Erba, Milano, Italy), morphine hydrochloride trihydrate (MOR, m.p. ≈ 200 °C, SALARS, Como, Italy); oleyl alcohol (OA, Novol, Croda Italia srl, Pavia, Italy); α-terpineol (TER, Sigma Chemical Co, St. Louis, MO, USA). All other chemicals and reagents were of analytical grade.

2.2. Determination of octanol-buffer partition coefficients of CAF and MOR

The apparent partition coefficients of CAF and MOR were determined at 37 °C by equilibrating solutions of the compounds in a n-octanol–66.7 mM phosphate buffer (pH 7.4) system. The calculated values were 0.95 for CAF and 0.66 for MOR (log P, -0.022 and -0.18, respectively).

2.3. Ultrasound apparatus

Low-frequency continuous-wave US (40 KHz, 0.13–0.44 W/cm^2) was produced using a custom-made wave generator/amplifier and a Murata 400ET180, 40 KHz transducer (Farnell Electronic Components Ltd., Leeds, UK). The driving frequency and voltage were monitored using an oscilloscope (Matsushita Comm. Ind., Co, Ltd., Japan). High-frequency continuous-wave US (1–3 MHz, 0.01–2 W/cm^2) was produced using a wave generator (Wavetek 50 MHz pulse/Function Generator Model 166; Promeda SpA, Milano, Italy), a power amplifier (R.F. EIN Model 240L 20 kHz–10 MHz; 0.1–200 W/50 W; Vianello SpA, Milano, Italy) and a set of commercially available diagnostic US transducers (Gilardoni, Milano, Italy). In all tests, the transducers were driven at their fundamental resonant frequency, after confirming this parameter using a network analyzer. The intensity of US emission was measured with an Ultrasonic/Megasonic Energy Meter II fitted with a pb-308/bx-308 all quartz probe (ppb Inc., Palo Alto, CA, USA).

2.4. In vitro skin permeation experiments

Male hairless mice (Strain MF1-hr/hr/Ola, Nossan S.r.l., Correzzana, Milano, Italy) aged 7–10 weeks were used throughout. The permeation tests were carried out using vertical cells

(Gummer et al., 1987) with an effective diffusional area of 1.2 cm^2, whose lower (receiving) section was thermostated at 37 °C. The overall procedure and the preparation of skin samples have been described in a previous paper (Monti et al., 1995). As donor phase, the following solutions (1.0 ml) in isotonic, 66.7 mM pH 7.4 phosphate buffer were used: CAF, 1.0% w/v, MOR, 0.2% w/v. US energy was applied by immersing the tip of the transducer into the solution, at a distance of about 3 mm from the skin. During sonication, the temperature of the donor solution was monitored using a thermocouple digital thermometer (Minitherm HI 8053, Hanna Instruments Ltd., Leighton Buzzard, Bedfordshire, UK). Each permeation test was performed at least in triplicate.

The US conditions (frequency and power) used in the study are indicated in Table 1.

The effect of skin pretreatment with different enhancers (BAC, OA and TER) on penetration of CAF and MOR from aqueous solutions was investigated as follows, using the vertical cells mentioned in the previous paragraph. Propylene glycol (PG) solutions (1.0 ml) containing 1.0% v/v of each enhancer were introduced into the upper section of the assembled cells. After 30 min the solutions were removed, and the treated skin was gently swabbed with filter paper to remove any residue. CAF or MOR (1.0 ml, same solutions used in the US tests) were then introduced into the permeation cells.

The receiving phase consisted in all cases of isotonic, 66.7 mM, pH 7.4 phosphate buffer containing 0.003% w/v sodium azide to prevent bacterial growth. In all cases, at predetermined time intervals samples of the receiving phase were withdrawn for analysis, and replaced with an equal volume of fresh solution. Also in this case, each permeation test was performed at least in triplicate.

2.5. Analytical methods

The concentration of drugs in the samples was determined by HPLC (liquid chromatograph with LC 6A pump and 20 μl Rheodyne injector, SPDM6A photo-diode array detector and computer integrating system; Shimadzu Corp., Kyoto, Japan). The column (30 cm × 3.9 mm) was packed with μ-Bondapack C18 (pore size 10 μm, Waters, USA-Milford, MA). CAF analysis was carried out using 30:70 v/v methanol–water as mobile phase (flow rate 1.5 ml/min): the retention time and the detection wavelength were 9.0 min and 273 nm, respectively. In the case of MOR the mobile phase (flow rate = 1.0 ml/min) was 50:50 v/v methanol–pH 6.98 buffer. The buffer consisted of 0.1% w/v $(NH_4)_2CO_3$ and 0.01 M

Table 1
Effect of sonication on in vitro transdermal permeation of caffeine (CAF) and morphine (MOR)

Drug	Ultrasound conditions KHz (mW/cm^2)	Flux, J ± S.E. ($\mu g/cm^2$ h)	Lag time ± S.E. (h)	Enhancement factor (*E*)
CAF	No ultrasound	2.6 ± 0.4	0.56 ± 0.20	1.0
	1500 (14)	3.8 ± 0.3	0.79 ± 0.15	1.5 ± 0.30
	1500 (42)	9.2 ± 1.3	0.62 ± 0.14	3.2 ± 0.45
	3000 (14)	2.8 ± 0.5	1.39 ± 0.30	1.1 ± 0.21
	3000 (42)	4.1 ± 0.2	0.78 ± 0.10	1.6 ± 0.38
	40 (130)	2.8 ± 1.1	0.57 ± 0.20	1.0 ± 0.38
	40 (325)	4.3 ± 1.1	0.73 ± 0.06	1.5 ± 0.28
	40 (440)	11.1 ± 0.7[a]	0.69 ± 0.09	3.8 ± 0.18[a]
MOR	No ultrasound	0.3 ± 0.06	0.08 ± 0.01	1.0
	40 (130)	1.2 ± 0.13	0.25 ± 0.09	4.1 ± 0.44
	40 (325)	1.4 ± 0.48	0.20 ± 0.08	4.5 ± 1.61
	40 (440)	11.9 ± 1.62[a]	0.30 ± 0.10	39.8 ± 5.34[a]

Each permeation test was performed at least in triplicate.
[a] significantly different from control.

$(NH_4)_2HPO_4$. The retention time and the detection wavelength were 7.0 min and 212 nm, respectively.

2.6. Statistical evaluation of data

Statistical differences between means were assessed by ANOVA (StatView software, Abacus Concepts inc., Berkeley, CA). The evaluation included calculation of means and standard errors, and group comparisons using the Fisher PLSD test. Differences were considered statistically significant at $P < 0.05$.

2.7. Histological studies

The effect of low-frequency US on skin structure was assessed by histological studies performed by light microscopy. The following skin samples were examined: (1) freshly excised skin, after removal of fat and subcutaneous tissues; (2) skin as above, mounted in the diffusion cell and kept in contact with donor (no drug) and receiving phase for 5 h; (3) skin as in 2, with drug (CAF or MOR) in the donor phase; (4) skin as in 3, after 5 h exposure to US (frequency, 40 KHz; intensity, 440 mW/cm^2). All samples were fixed in 10% formaldehyde solution, then were dehydrated by sequential immersion in ethanol at increasing concentrations, and embedded in paraffin. Slices (3–4 μm) obtained with a knife microtome were mounted on slides, stained with hematoxylin/eosin, and observed at 175–250-fold magnification to assess structural changes.

3. Results

3.1. Effect of ultrasound

The steady-state flux, J (given by $Q/A \times t$, where Q is the amount of permeant diffusing across the area A in time t) was calculated by linear regression analysis of permeation data. The enhancing effect of sonication on drug permeation was expressed as US enhancement factor (E), calculated from the ratio Jb/Ja, where Ja and Jb are the average fluxes in the absence and in presence of US, respectively. The skin permeation parameters (flux, lag time, enhancement factor) calculated for CAF and MOR in the absence and in the presence of US of different frequencies and intensities are reported in Table 1.

The transdermal flux of CAF under passive diffusion conditions (no sonication) was 2.6 ± 0.4 μg/cm^2 h. The drug flux under sonication exhibited a different behavior, depending on the US characteristics. When continuous, high-frequency (1.5 or 3.0 MHz) US was applied, no significant enhancement was observed when the intensity was 14 mW/cm^2. At 42 mW/cm^2, the enhancement factors were 3.2 and 1.6 for 1.5 and 3.0 MHz, respectively. The difference between the two factors was not statistically significant.

Further permeation tests on CAF were carried out using low-frequency (40 KHz) US, whose intensity was varied in the range 130–440 mW/cm^2. As shown in Table 1, sonication at 130 mW/cm^2 did not apparently modify the CAF permeation rate. When the intensity was raised to 325 and 438 mW/cm^2 the enhancement factors were 1.51 and 3.81, respectively. The latter value was significantly different from the control.

The transdermal permeation parameters of MOR under low frequency (40 KHz) sonication are also shown in Table 1. By increasing the US intensity from 130 to 325 mW/cm^2 the enhancement factors rose to 4 and 4.5, respectively (difference from control not statistically significant). A further increase to 440 mW/cm^2 produced a significant permeability increase: the enhancement factor was 39.8, about 10-fold higher than those observed at the lower intensities.

As shown in Fig. 1, after turning off US the transdermal fluxes of CAF and MOR decreased to lower values (3.1 ± 0.11 and 1.5 ± 0.16 μg/cm^2 h, respectively) which, although slightly higher, were not significantly different from those measured in the absence of sonication.

In no case significant effects of US on permeation lag times were detected; the temperature of the donor phase (26.5–27 °C) was not influenced by sonication.

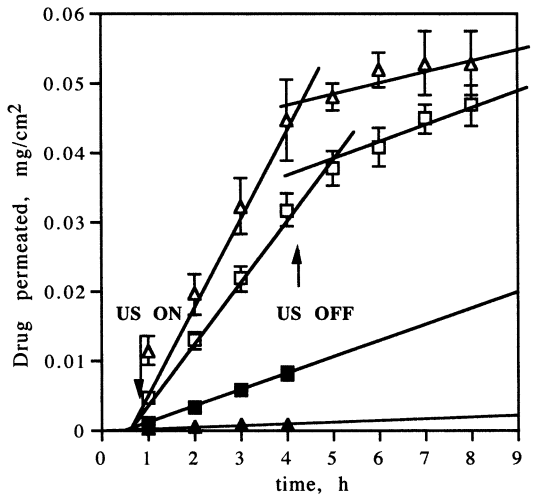

Fig. 1. Effect of US on CAF and MOR permeability through hairless mice skin. (□) CAF, US (40 KHz, 440 mW/cm^2) applied for 4 h; (■) CAF no US; (△) MOR, US (40 KHz, 440 mW/cm^2) applied for 4 h; (▲) MOR no US. For CAFF no US (■) and MOR no US (▲) lines, error bars were smaller than symbols.

3.2. Effect of chemical enhancers

The transdermal penetration parameters of CAF and MOR observed after pretreatment of the skin with the chemical enhancers dissolved in PG are listed in Table 2. BAC, OA and TER produced a 10.7, 19.6 and 6.6-fold enhancement of CAF flux, respectively. No remarkable variation of lag time with respect to the control was observed with BAC and TER, while a significant decrease of this parameter was produced by OA. The permeation of MOR was also significantly increased after skin pretreatment with OA ($E = 32.7$); the effect of BAC was smaller ($E = 5.6$) while TER was completely ineffective.

3.3. Histological studies

In the control skin sample (1) the normal epidermal layers, even if very thin, were clearly apparent. Sample 2, kept in contact for 5 h on both sides with the buffer solution and not sonicated, did not show any significant change with respect to 1, except for the presence of a few, very small intercellular vacuoles. Samples 3 (same as 2, but with CAF or MOR in the donor phase) were identical with 2. Samples 4 (same as 3, after 5 h sonication at 40 KHz, 440 mW/cm^2) showed, as possible effects of cavitation, some intercellular vacuoles, sometimes confluent, intercalated between the epidermal layers. The layers themselves did not show any perceptible alteration.

4. Discussion

CAF, considered a relatively hydrophilic, poorly skin-permeable molecule, is a widely used model drug for transdermal permeation studies. The influence of high- and low-frequency US on its transdermal transport has been investigated (Machluf and Kost, 1993; Mitragotri et al., 1995).

Table 2
Effect of chemical enhancers on in vitro transdermal permeation of caffeine (CAF) and morphine (MOR)

Drug	Enhancers	Flux, J ± S.E. (μg/cm^2 h)	Lag time ± S.E. (h)	Enhancement factor (E)
CAF	None	2.6 ± 0.4	0.56 ± 0.20	1.0
	BAC	27.9 ± 8.5	0.74 ± 0.03	10.7 ± 3.27
	OA	50.8 ± 3.1[a]	0.06 ± 0.01	19.6 ± 1.21[a]
	TER	18.7 ± 8.1	0.42 ± 0.01	6.6 ± 2.55
MOR	None	0.3 ± 0.06	0.08 ± 0.01	1.0
	BAC	1.7 ± 0.03	0.83 ± 0.17	5.6 ± 1.10
	OA	9.8 ± 1.80[a]	0.16 ± 0.12	32.7 ± 6.23[a]
	TER	0.3 ± 0.03	0.48 ± 0.10	0.9 ± 0.11

Each permeation test was performed at least in triplicate.
[a] significantly different from control.

Some investigations have also dealt with transdermal administration of MOR. Attempts at improving the poor skin permeation properties of this drug, attributed to its high hydrophilicity (Roy and Flynn, 1988, 1989) have been made using different approaches: chemical enhancers (Mahjour et al., 1989; Sugibayashi et al., 1989; Morimoto et al., 1993), prodrug derivatization (Drustrup et al., 1991) or iontophoresis (Ashburn et al., 1992).

The permeation data obtained in the present study in the absence of chemical or US enhancement indicated an approximately 10-fold greater transdermal flux for CAF with respect to MOR, in agreement with the slightly greater lipophilicity of the former drug (experimentally determined log P, -0.022 and -0.18 for CAF and MOR, respectively). The high-frequency US enhancement of CAF permeation was not statistically significant, in agreement with the findings of Mitragotri et al. (1995), who used frequencies in the range 1–3 MHz and intensities of up to 2.0 W/cm^2. Conversely, low frequency, relatively high-intensity US (0.44 W/cm^2) produced a small but significant increase of the enhancement factor of CAF ($E = 3.8$). This also is in agreement with the findings of Mitragotri et al. (1996), who reported on the enhanced transdermal transport of CAF induced by low-frequency US, and attributed it to greater cavitation effects with respect to therapeutic US. The effect of US on CAF permeation, however, was moderate when compared with that of chemical enhancers, in particular, OA, whose enhancement factor was 19.6.

The effect of low-frequency US on permeation of MOR was significantly greater (about 10-fold) when compared, on the same frequency and intensity basis, with the effect on CAF. The most active chemical enhancer for MOR, oleyl alcohol (OA), had practically the same effect as US on permeation of this drug. This finding confirms the greater enhancement activity of US on hydrophilic molecules with respect to less hydrophilic ones, noticed by Meidan et al. (1995) who hypothesized that transfollicular pathways are more susceptible to ultrasonic enhancement than are transcellular processes. Cavitation-induced lipid bilayer disordering has been indicated as the most important cause for ultrasonic enhancement of transdermal transport (Mitragotri et al., 1995). The slight histological changes observed in the sonicated skin might be attributed to this effect, but the alterations seems to be reversible since the permeation rate practically returned to the basal values after turning off sonication (Fig. 1).

Of the tested chemicals, OA proved the most active enhancer for both CAF and MOR. OA in combination with PG (as in the present case) was found an effective enhancer e.g. for naloxone (Aungst et al., 1986) and for metronidazole (Hoelgaard et al., 1988).

In conclusion, the present results with the model drug CAF confirm the greater effect of low-frequency US on skin permeation in vitro. The comparison of US and chemical enhancement indicates a slight superiority of the combination OA/PG over low-frequency US. Concerning MOR, significantly increased transdermal fluxes were produced by both low-frequency US and by OA in combination with PG. Whether therapeutically effective amounts of MOR might be delivered transdermally by any of these methods remains open to question.

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References

Ashburn, M.A., Stephen, R.L., Ackerman, E., Petelenz, T.J., Hare, B., Pace, N.L., Hofman, A.A., 1992. Iontophoretic delivery of morphine for postoperative analgesia. J. Pain Symptom Management 7, 27–33.

Aungst, B.J., Rogers, N.J., Shefter, E., 1986. Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–234.

Bommannan, D., Okuyama, H., Stauffer, H., Guy, H.R., 1992a. Sonophoresis I. The use of high-frequency ultrasound to enhance transdermal drug delivery. J. Pharm. Res. 9, 559–564.

Bommannan, D., Menon, G.K., Okuyama, H., Elias, P., Guy, H.R., 1992b. Sonophoresis II. Examination of the mecha-

nism(s) of ultrasound-enhanced transdermal drug delivery. J. Pharm. Res. 9, 1043–1047.

Brucks, R., Nanavaty, M., Jung, D., Siegel, F., 1989. The effect of ultrasound on the in vitro penetration of ibuprofen through human epidermis. Pharm. Res. 6, 697–701.

Drustrup, J., Fullerton, A., Christrup, L., Bundgaard, H., 1991. Utilization of prodrugs to enhance the transdermal absorption of morphine. Int. J. Pharm. 71, 105–116.

Fang, J.-Y., Fang, C.-L., Sung, K.C., Chen, H.-Y., 1999. Effect of low frequency ultrasound on the in vitro percutaneous absorption of clobetasol 17-propionate. Int. J. Pharm. 191, 33–42.

Finnin, B.C., Morgan, T.M, 1999. Transdermal penetration enhancers: applications, limitations and potential. J. Pharm. Sci. 88, 955–958.

Gummer, C.L., Hinz, R.S., Maibach, H.I., 1987. The skin penetration cell: a design update. Int. J. Pharm. 40, 101–104.

Hoelgaard, A., Møllgaard, B., Baker, E., 1988. Vehicle effect on topical drug delivery. IV. Effect of *N*-methylpyrrolidone and polar lipids on percutaneous drug transport. Int. J. Pharm. 43, 233–240.

Hsieh, D.S., 1994. Understanding permeation enhancement technologies. In: Hsieh, D.S. (Ed.), Drug Permeation Enhancement. Theory and Application. Marcel Dekker, New York, pp. 3–17.

Machluf, M., Kost, J., 1993. Ultrasonically enhanced transdermal delivery. Experimental approaches to elucidate the mechanism. J. Biomater. Sci. Polym. Ed. 5, 147–156.

Mahjour, M., Mauser, B.E., Fawzi, M.B., 1989. Skin permeation enhancement effects of linoleic acid and azone on narcotic analgesics. Int. J. Pharm. 56, 1–11.

McElnay, J.C., Benson, H.A.E., Hadgraft, J., Murphy, T.M., 1993. The use of ultrasound in skin penetration enhancement. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York, pp. 293–309.

Meidan, V.M., Walmsley, A.D., Irwin, W.J., 1995. Phonophoresis: is it a reality? Int. J. Pharm. 118, 129–149.

Mitragotri, S., Edwards, D.A., Blankschtein, D., Langer, R., 1995. A mechanistic study of ultrasonically-enhanced transdermal drug delivery. J. Pharm. Sci. 84, 697–706.

Mitragotri, S., Blankschtein, D., Langer, R., 1996. Transdermal drug delivery using low-frequency sonophoresis. Pharm. Res. 13, 411–420.

Monti, D., Saettone, M.F., Giannaccini, B., Galli-Angeli, D., 1995. Enhancement of transdermal penetration of dapiprazole through hairless mouse skin. J. Controlled Release 33, 71–77.

Morimoto, Y., Sugibayashi, K., Kobayashi, D., Shoji, H., Yamazaki, J., Kimura, M., 1993. A new enhancer-coenhancer system to increase skin permeation of morphine hydrochloride in vitro. Int. J. Pharm. 91, 9–14.

Roy, S.D., Flynn, G.I., 1988. Solubility and related physicochemical properties of narcotic analgesics. Pharm. Res. 5, 580–586.

Roy, S.D., Flynn, G.I., 1989. Solubility behavior of narcotic analgesics in aqueous media. Solubilities and dissociation constants of morphine, fentanyl and sufentanyl. Pharm. Res. 6, 147–151.

Shah, V.P., 1994. Skin penetration enhancers: Scientific perspectives. In: Hsieh, D.S. (Ed.), Drug Permeation Enhancement. Theory and Application. Marcel Dekker, New York, pp. 19–23.

Skauen, D.M., Zentner, G.M., 1986. Phonophoresis. Int. J. Pharm. 20, 235–245.

Sugibayashi, K., Sakanoue, C., Morimoto, Y., 1989. Utility of topical formulations of morphine hydrochloride containing azone and N-methyl-2-pyrrolidone. Selective Cancer Ther. 5, 725–744.

Tyle, P., Agrawala, P., 1989. Drug delivery by phonophoresis. Pharm. Res. 6, 355–361.