Noninvasive Glucose Monitoring by Back Diffusion *via* Skin: Chemical and Physical Enhancements

Yaw-Bin HUANG,[a] Jia-You FANG,[b] Pao-Chu WU,[a] Tsung-Hsien CHEN,[a] Ming-Jun TSAI,[c] and Yi-Hung TSAI*,[a]

[a] *School of Pharmacy, Kaohsiung Medical University; 100 Shih-Chuan 1st Road, Kaohsiung, Taiwan:* [b] *Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University; Tao-Yuan, Taiwan: and* [c] *Department of Neurology, Yuan's General Hospital; Kaohsiung, Taiwan.* Received December 26, 2002; accepted March 31, 2003

Blood glucose levels are routinely obtained by invasive and painful methods using glucose meters and test strips. The development of less invasive or non invasive techniques would be beneficial for diabetes patients. In this study, a noninvasive method was evaluated using the back diffusion of glucose across skin with or without permeation enhancement methods. An *in vitro* model was utilized. The stratum corneum (SC) was the predominant barrier for both back and forward diffusion of glucose across skin. Surfactants with various charges and essential oils (cyclic monoterpenes) were used as chemical enhancers to promote the back diffusion of glucose. A cationic surfactant (benzalkonium chloride) showed the highest enhancement, followed by anionic and nonionic surfactants. *d*-Limonene and 1,8-cineole dispersed in appropriate proportions of ethanol could enhance the glucose diffusion after pretreatment of the skin surface. Electroporation, defined as a physical method, significantly increased the amount of glucose that diffused back. The percentages of diffused glucose by 300 V (volts) and 500 V high voltage pulses on skin for 10 min were found to be 45 and 75 times greater than the control group, respectively.

Key words glucose; back diffusion; skin; chemical enhancer; electroporation

Diabetes patients routinely check their glucose levels using the invasive method of pricking a finger. Most patients consider this the most onerous part of their diabetes therapy.[1)] Patients are anxious for less invasive or non invasive methods for glucose monitoring. Noninvasive methods for measuring glucose have been attempted by a number of workers with variable success rates. Currently, all glucose analysis is performed on fluids extracted from the body. Urine, saliva, and sweat are the sources of extracted fluids studied. However, glucose secretion into these extracted fluids appears to be significantly delayed and there is no linear correlation as there is for blood glucose.[1,2)]

Many studies have reported on glucose values in subcutaneous tissue and the relationship to blood glucose.[1,3)] Virtually all studies agree that glucose measured in subcutaneous tissue lags behind blood glucose only by 5—15 min and that the values are slightly lower than those of blood glucose. Although glucose is present in the body at reasonable levels, the very low passive permeability of this highly polar and water-soluble species across the lipophilic skin barrier precludes surface collection as a viable means.[4)] Some efforts have been made to enhance glucose transport across the skin, including: reverse iontophoresis, modulation of osmotic pressure, and tape-stripping.[4—6)]

A previous study by Pellett *et al.* (1999) suggests that significant amounts of glucose back diffused only after removal of the stratum corneum (SC).[6)] It is likely that any attempts to monitor blood glucose levels should require a certain degree of modification to the SC. The aim of the present study was to evaluate the influence of both chemical and physical methods, which have been shown to modulate the SC morphology, on the back diffusion of glucose across skin *in vitro*. Chemical enhancers such as surfactants and natural essential oils were used to reduce the barrier properties of skin and thus facilitate the back diffusion of glucose. The physical enhancement method used here was electroporation. Electroporation involves the creation of a transient elevation in the permeability of lipid bilayers of skin by applying short, high-voltage pulses. It has been demonstrated as a powerful method to overcome the SC barrier.[7,8)] Glucose metabolism within the skin was also determined in this study.

MATERIALS AND METHODS

Materials ^{3}H-glucose (1 mCi/ml) and ^{3}H-H_2O (tritiated water; 1 mCi/ml) were purchased from NEN Life Science Products, Inc. (U.S.A.). Dextrose was obtained from Shiyakyu Co. (Japan). *N*-(2-Hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) and sodium laurylsulfate (SLS) were supplied by Sigma Co. (U.S.A.). *d*-Limonene and Span85 were purchased from Tokyo Kasei Industrial Co. (Japan). Benzalkonium chloride (BC) was supplied by Nacalai Co. (Japan). 1,8-Cineole was purchased from Hsin Jung Perfumery Co. (Taiwan). All other chemicals and solvents were analytical grade and used as received.

Bioconversion of Glucose in Skin Skin from 250 mg male Wistar rats (6—8 weeks old; 200—250 g) was homogenized in 1.5 ml HEPES buffer with 133 mM NaCl for 10 min. The homogenized suspension was diluted with 1 ml HEPES and 133 mM NaCl. Then 2.5 ml of glucose solution (800 mg/dl) was added to the suspension and the suspension shaken in a water bath (37±1 °C). At appropriate intervals (1, 3, 6 h), 300 μl samples were withdrawn. After centrifugation at 12000 rpm for 10 min, 50 μl of clear supernatant was analyzed using a blood glucose monitor (Advantage Co., U.S.A.) to determine the glucose concentration.

***In Vitro* Back Diffusion Experiments** Side-by-side diffusion cells were used in the *in vitro* experiments. Male Wistar rats (6—8 weeks old) were sacrificed by ether and shaved, full-thickness skin was excised from the abdominal region.

* To whom correspondence should be addressed. e-mail: fajy@mail.cgu.edu.tw

To obtain SC-stripped skin, adhesive tape (Four Pillars®, Taiwan) was applied to the rat skin under uniform pressure and then removed. This procedure was repeated 20 times. The subdermal phase of side-by-side cells consisted of 8 ml of HEPES containing 133 mM NaCl; the ^{3}H-glucose and glucose concentrations were 4 μCi/ml and 400 mg/dl respectively. ^{3}H-water (4 μCi/ml) was also used in the experiments. The SC phase consisted of 8 ml of HEPES containing 133 mM NaCl. The available back diffusion area between the cells was 0.770 cm^2. The stirring rate and temperature were kept at 700 rpm and 37 °C, respectively. After an appropriate period of time, the solution adjacent to the SC side was removed by successive washing with HEPES, and the remaining samples were wiped with a cotton wool swab. All washes were added together to analyze ^{3}H by scintillation.

Pretreatment of Chemical Enhancers Five hundred μl of enhancer solution such as Span85, Tween80, SLS, and BC, at concentrations of 0.5, 1.0, and 1.5% was deposited onto the SC surface. The vehicle of these enhancers was redistilled water. Four percent cyclic monoterpenes in 30%, 50%, or 90% EtOH/water were also used to pretreat the skin. The excised skin samples were pretreated with the test enhancers for 30 min. After pretreatment, the enhancer solution was removed, the skin surface wiped with a cotton wool swab, and the *in vitro* back diffusion experiment was conducted.

Treatment of Physical Electroporation Electroporation was applied using an exponential decay pulse generator (ECM 630 Electro Cell Manipulator®, BTX Co., U.S.A.). Platinum electrodes (1×2 cm^2, 99.99%) were used and each was located 3 cm from the skin. The anode was positioned in the SC side and the cathode was placed in the subdermal side. Unless otherwise noted in Table 2, the electroporation protocol was 1 pulse per 30 s, applied for 10 min; the pulse voltage was 300 V and pulse duration was 200 ms (*e.g.* 300 V, 200 ms/30 s for 10 min). The voltages were recorded as applied values but not transdermal values.

Scintillation Assay Quantification of ^{3}H-glucose and tritiated water was conducted using a liquid scintillation counter (Tri-Carb® Liquid Scintillation Analyzer Model 2100TR, U.S.A.). The samples were mixed with an appropriate volume of scintillation cocktail and the radioactivity was determined.

RESULTS AND DISCUSSION

Back Diffusion of Tritiated Water and Glucose across Intact and SC-Stripped Skin It is possible that glucose is metabolized during its outward transport across the human skin.[4] The same phenomenon is observed in rat skin.[9] Glucose metabolizing enzymes are present in the epidermis and the formation of lactate over water and carbon dioxide is favored. Therefore, the possibility of glucose metabolism within skin was investigated by an *in vitro* method in this study. There was no reduction in the amount of intact glucose during the first 6 h of incubation in rat skin homogenates (Fig. 1), indicating no metabolism occurred within this period. The glucose amount greatly decreased after 6 h incubation. The glucose concentration was even below the detectable limit of the blood glucose monitor after 15 h of incubation. Glucose already present in the epidermis and dermis may be extracted and measured to interfere with the results of back diffusion experiments.[4] Hence, the homogenized skin was incubated alone for 15 h to determine whether the glucose existed in the skin or not. The result also shows that no detectable amount of glucose was observed in the homogenized skin solution (Fig. 1). In order to exclude the influence of glucose metabolism in the skin, the sampling period of the following back diffusion was set at 6 h.

The changes in water permeability due to skin structure alterations can be used as a predictive tool for the flux of other moderate sized polar substances.[10] Table 1 shows that the percentage of tritiated water that back diffused through the SC-stripped skin is 37.08-fold higher than that through intact rat skin, indicating SC was the principal barrier in the back diffusion of it and the other polar substances. The percentage of glucose by forward diffusion across the SC-stripped skin was significantly higher (*t*-test, $p<0.05$) than that by back diffusion (Table 1). Glucose should first be partitioned into the epidermis, following which it permeats across the dermis in forward diffusion across SC-stripped skin, whereas back diffusion is in the opposite direction. The results in Table 1 seem to indicate that glucose partitioning into viable epidermis was easier than partitioning into dermis.

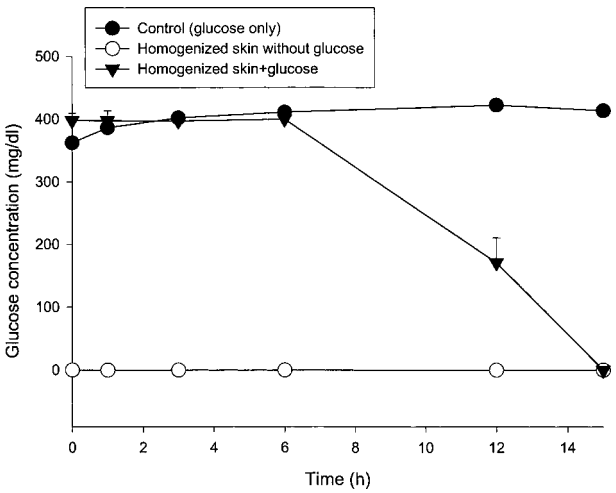

Fig. 1. Glucose Concentration Determined after 15 h Incubation in Rat Skin Homogenates

Each value represents the mean±S.D. (n=3).

Table 1. Percentages of Water and Glucose That Forward or Back Diffused across Intact and SC-Stripped Skin after 6 h Application

Mode	Percentage diffused across intact skin at 6 h (%)	Percentage diffused across SC-stripped skin at 6 h (%)	ER
Back diffusion of H_2O	0.995±0.408	36.876±11.516	37.08
Back diffusion of glucose	0.032±0.013	0.403±0.327	12.79
Forward diffusion of glucose	0.044±0.003	64.518±11.426	1466.32

ER=enhancement ratio=percentage across SC-stripped skin/percentage across intact skin. Each value represents the mean±S.D. (n=4).

Back Diffusion of Glucose Enhanced by Surfactant Pretreatments Since the SC layer was a barrier to obstruct the back diffusion of glucose, enhancement methods to modify the SC structure are needed for glucose back diffusion to achieve successful noninvasive monitoring. Surfactants with different head group chemistries, including nonionic, anionic, and cationic, were studied to increase back diffusion of glucose. After pretreatment with Span85 and Tween80 on the SC side of the skin for 30 min, neither of the nonionic surfactants at low concentrations (0.5%, 1.0%) enhanced the percentage of glucose diffused (Fig. 2). When the concentration of nonionic surfactants increased to 1.5%, there was a significant increase (t-test, $p<0.05$) in the back diffusion of glucose. The glucose diffusion enhancing ability of Tween80 was greater than that of Span85.

Ionic surfactants are known to induce a higher degree of SC disruption than nonionic surfactants.[11] As shown in Fig. 3, both 1.0% and 1.5% SLS, an anionic surfactant, can enhance the percentage of diffused glucose. There was no significant difference (t-test, $p>0.05$) between the enhancement ratios (ER) of 1.0% and 1.5% SLS. The enhancement of skin conductivity is strongly correlated with the enhancement of skin permeability for hydrophilic solutes.[12] A previous study has shown that 1.0% SLS can greatly raise the conductivity of skin,[11] and thus may increase the permeability of hydrophilic solutes across skin such as glucose. Although there was no significant increase in the diffused glucose percentages when pretreating with 0.5% and 1.0% BC, a cationic surfactant, the highest glucose diffusion activity was seen with 1.5% BC (Fig. 2). Treatment of the SC with cationic surfactants can markedly increase the transport of hydrophilic substances.[13,14] Application of even diluted solutions of cationic surfactants to the skin changes the structure of the lipid bilayers in the skin.

Back Diffusion of Glucose Enhanced by Essential Oil Pretreatment *d*-Limonene and 1,8-cineole were utilized in the present study to examine their ability to enhance the back diffusion of glucose across skin. Ethanol at different proportions was used as the solvent for these cyclic monoterpenes. There were no linear relationships between the ethanol proportions and ER values of glucose diffusion after pretreatment with *d*-limonene or 1,8-cineole (Fig. 3). Pretreatment with 4% terpenes in 95% ethanol produced an even greater retardation of glucose diffusion. As a function of the solubility, partition behavior between vehicle and skin, and skin structural modification, many mechanisms are found in which ethanol affects the skin permeation of drugs (Ho *et al.*, 1994; Zhao and Singh, 2000).[15,16] Although an appropriate portion of ethanol may increase the solubility and thus the skin permeability of drugs, changes in skin structure induced by ethanol at high proportions can reduce the permeation of some drugs.[17,18] Protein denaturation in SC layers may be involved in this reduction of permeation. Another explanation of the lack of linear relationship is that the partitioning of terpenes to SC was less from vehicles with a higher ethanol proportion than a lower proportion because of the lipophilic characteristics of *d*-limonene and 1,8-cineole.

d-Limonene generally appears to be more potent at increasing glucose diffusion than 1,8-cineole (Fig. 3). The lipophilicity of *d*-limonene is higher than that of 1,8-cineole. It has been suggested that the increase in lipophilicity of the

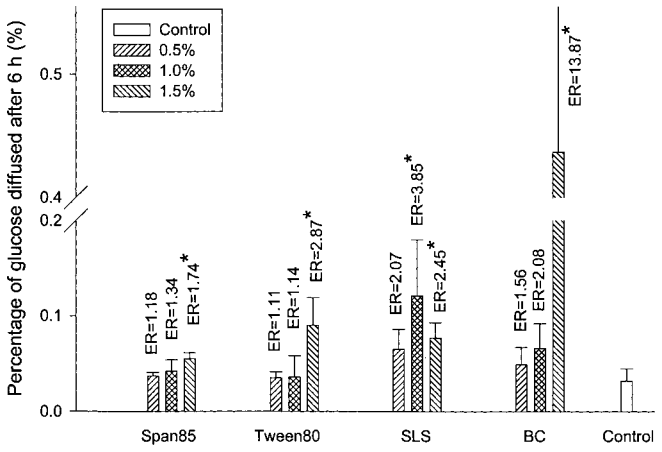

Fig. 2. Percentages of Glucose That Back Diffused after 6 h across Rat Skin Pretreated by Various Surfactants with Different Concentrations for 30 min

Each value represents the mean±S.D. ($n=4$).

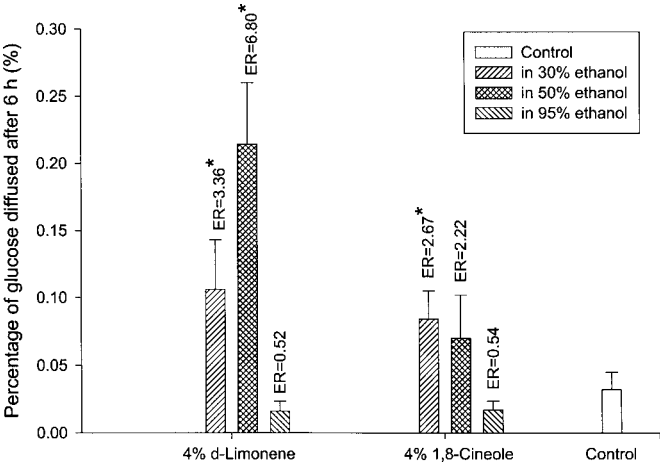

Fig. 3. Percentages of Glucose That Back Diffused after 6 h across Rat Skin Pretreated by 4% *d*-Limonene or 1,8-Cineole with Different Proportions of Ethanol as Vehicle for 30 min

Each value represents the mean±S.D. ($n=4$).

terpene enhancers is associated with an increase in the ability to disrupt the highly lipophilic lipid lamella of the SC.[19] *d*-Limonene distributed into or attacked the SC very quickly, making it readily permeable to glucose. The higher enhancement activity of *d*-limonene relative to 1,8-cineole can also be attributed to its lower solubility in an ethanol/water solution. *d*-Limonene could not be completely dissolved at a concentration of 4% in 30% or 50% ethanol. The thermodynamic activity of *d*-limonene is thought to have already attained a maximum level in this condition.[19,20]

Back Diffusion of Glucose Enhanced by Electroporation Solvent flow (electroosmosis) generated during iontophoresis (low voltage, continuous constant current) can be used to convect glucose, a neutral molecule, which back diffuses across the skin, thereby enhancing its flux.[4,5] Hence another electric enhancement method—electroporation (high voltage pulse for a very short duration)—may be useful to enhance the back diffusion of this non electrolyte. While iontophoresis acts primarily on the permeant, electroporation acts on the skin with some driving forces on the permeant during a pulse.[8] The cumulative amounts of glucose back

diffused across skin during 6 h by electroporation are shown in Fig. 4. Compared to passive diffusion, the application of high voltage pulses significantly increased the skin permeation of glucose. Application of 100 V, 300 V, and 500 V pulses produced approximately 10-, 45-, and 55-fold increases, respectively in glucose diffusion as compared to passive diffusion. A correlation exists between the voltages applied and the percentages of glucose diffused during a 6 h period (correlation coefficient $r=0.975$). The result is consistent with known mechanisms for single bilayer electroporation, which demonstrates that the pore characteristics and sizes can be influenced significantly by pulse voltage.[8,21] Another observation is that the ER value after application of 300 V and 500 V electroporation was significantly higher than that of passive diffusion across SC-stripped skin. This may again indicate that SC layers are not the only barrier for glucose back diffusion.

Drug transport by electroporation can occur during pulsing by electrophoresis and diffusion through new aqueous pores and/or after pulsing by diffusion through the electropermeabilized skin.[22] The importance of electrophoresis in transdermal transport by electroporation has been demonstrated for charged molecules. Because glucose is neutral, an increased transport due to electrophoresis during pulsing can be ruled out.[23] Even though electrophoresis must take place during pulsing, its impact on transport could be low because of the short duration of current application. After pulsing for 10 min, the cumulative amount of glucose remained elevated until the end of the experiments (Fig. 4). This may suggest the creation of a drug reservoir within the skin and/or a persistent change in skin permeability due to altered skin structure.[7] Glucose is a very hydrophilic molecule. The high affinity of hydrophilic molecules to viable epidermis/dermis may lead to a large reservoir effect after electroporation.

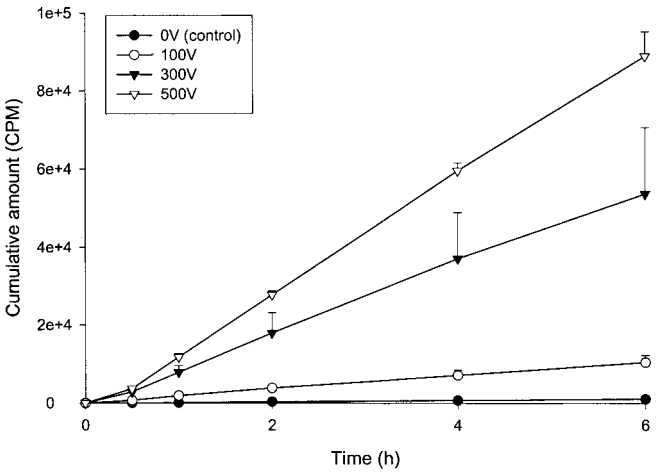

Fig. 4. Cumulative Amount–Time Profiles of Glucose That Back Diffused *via* Rat Skin after Electroporation with Different Voltage Pulses

Each value represents the mean±S.D. ($n=4$).

Electroporation with various pulsing numbers was investigated to examine the influence of pulses on the back diffusion of glucose. Glucose diffusion was studied after application of 5, 10, and 20 single pulses. As shown in Table 2, the ER value of diffused glucose after 5 pulses is similar to that after 10 pulses. Multiplying the number of pulses to 20 significantly enhanced the glucose diffusion compared to a fewer number of pulses. When increasing the pulsing frequency but keeping the total pulsing time constant, no significant difference (ANOVA test, $p>0.05$) was observed in the glucose diffused with various pulsing frequencies (Table 2). This suggests that the enhancement of glucose diffusion by electroporation is related to the total time pulsed no matter what pulsing frequency is applied.

CONCLUSIONS

The results of the present study illustrate the influence of chemical enhancers and physical electroporation on the back diffusion of glucose across rat skin. SC layers were proven to be the predominant barriers for the forward diffusion of glucose. On the other hand, other mechanisms or barriers contribute the retardation of glucose back diffusion. Pretreatment with surfactants on the skin enhanced the back diffusion of glucose at different levels. BC showed the greatest effect, followed by SLS, Tween80, and Span85. 4% *d*-Limonene or 1,8-cineole, two cyclic monoterpenes, dispersed in appropriate proportions of ethanol could increase the cumulative amount of glucose by back diffusion. Electroporation significantly enhanced the diffusion of glucose, which is a neutral molecule. The various electric factors tested in the study of electroporation were pulse voltage, pulse number, and pulse frequency. The greatest influence was found for the pulse voltage, followed by pulse number. The pulse frequency did not influence the diffusion of glucose across the skin. The results of this study may be useful for the development of noninvasive glucose monitoring devices in the future. Further study is needed to investigate the *in vivo* and clinical efficacy of these enhancement methods on the back diffusion of glucose to sufficiently monitor glucose levels.

Table 2. Percentages of Glucose That Back Diffused across Rat Skin after Electroporation with Different Pulsing Modes

Electroporation treatment		Percentage of glucose diffused at 6 h (%)	ER
Various voltage	0 V	0.032±0.013	—
	100 V, 200 ms/30 s for 10 min	0.312±0.054	9.57
	300 V, 200 ms/30 s for 10 min	1.412±0.448	44.94
	500 V, 200 ms/30 s for 10 min	2.349±0.168	74.55
Various pulses	300 V, 200 ms/30 s for 2.5 min	0.889±0.106	28.20
	300 V, 200 ms/30 s for 5 min	0.761±0.089	24.16
Various pulsing frequency	300 V, 200 ms/7.5 s for 2.5 min	1.516±0.344	48.11
	300 V, 200 ms/15 s for 5 min	1.269±0.154	40.28

ER=enhancement ratio=percentage with electroporation/percentage without electroporation. Each value represents the mean±S.D. ($n=4$).

REFERENCES

1) Ginsberg B. H., *Clin. Chem.*, **38**, 1596—1600 (1992).
2) Ben-Aryeh H., Cohen M., Kanter Y., Szargel R., Laufer D., *J. Diabet. Complications*, **2**, 96—99 (1988).
3) Klonoff D. C., *Diabetes Care*, **20**, 433—437 (1997).
4) Rao G., Glikfeld P., Guy R. H., *Pharm. Res.*, **10**, 1751—1755 (1993).
5) Rao G., Guy R. H., Glikfeld P., LaCourse W. R., Leung L., Tamada J., Potts R. O., Azimi N., *Pharm. Res.*, **12**, 1869—1873 (1995).
6) Pellett M. A., Hadgraft J., Roberts M. S., *Int. J. Pharmaceut.*, **193**, 27—35 (1999).
7) Jadoul A., Preat V., *Int. J. Pharmaceut.*, **154**, 229—234 (1997).
8) Banga A. K., Bose S., Ghosh T. K., *Int. J. Pharmaceut.*, **179**, 1—19 (1999).
9) Frienkel R. K., Traczyk T. N., *J. Invet. Dermatol.*, **67**, 557—581 (1976).
10) Magnusson B. M., Runn P., Koskinen L.-O. D., *Acta Derm-Venereol.*, **77**, 264—267 (1997).
11) Tezel A., Sens A., Tuchscherer J., Mitragotri S., *J. Pharm. Sci.*, **91**, 91—100 (2002).
12) Mitragotri S., Farrell J., Tang H., Terahara T., Kost J., Langer R., *J. Control. Release*, **63**, 41—52 (2000).
13) Rantuccio F., Scardigno A., Conti A., Sinise D., Coviello C., *Contact Dermatitis*, **5**, 392—397 (1979).
14) Fang J. Y., Tsai M. J., Huang Y. B., Wu P. C., Tsai Y. H., *Drug. Dev. Res.*, **40**, 56—67 (1997).
15) Ho H. O., Huang F. C., Sokoloski J. D., Sheu M. T., *J. Pharm. Pharmacol.*, **46**, 636—642 (1994).
16) Zhao K., Singh J., *J. Pharm. Sci.*, **89**, 771—780 (2000).
17) Inagi T., Muramatsu T., Nagai H., Terada H., *Chem. Pharm. Bull.*, **9**, 1707—1714 (1981).
18) Fang J. Y., Kuo C. T., Huang Y. B., Wu P. C., Tsai Y. H., *Int. J. Pharmaceut.*, **176**, 157—167 (1999).
19) El-Kattan A. F., Asbill C. S., Kim N., Michniak B. B., *Int. J. Pharmaceut.*, **215**, 229—240 (2001).
20) Obata Y., Takayama K., Maitani Y., Machida Y., Nagai T., *Biol. Pharm. Bull.*, **16**, 312—314 (1993).
21) Prausnitz M. R., *J. Control. Release*, **40**, 321—326 (1996).
22) Jadoul A., Lecouturier N., Mesens J., Caers W., Préat V., *J. Control. Release*, **54**, 265—272 (1998).
23) Vanbever R., Leroy M., Préat V., *J. Control. Release*, **54**, 243—250 (1998).