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*In-vitro*에서 전기이온영동법을 이용한 알렌드로네이트의 경피약물전달

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Iontophoretic Transdermal Delivery of Alendronate in Hairless Mouse Skin

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초록: 알렌드로네이트의 생체이용률을 높이고 경구복용시 발생하는 부작용을 해소하고자 경피약물전달시스템에 전기이온영동법, microneedle 등을 적용하여 *in-vitro* 시험 후 약물전달량을 HPLC-Flu를 이용하여 조사하였다. 전기이온영동시험을 위해 사용된 약물 패취는 UV중합법으로 합성하였으며, 이때 패취에 함유된 알렌드로네이트의 량은 5.0 mg/cm^{3}이었다. 0.25, 0.50 mA/cm^{2}의 전류를 인가한 경우, 약물전달량은 각각 0.80±0.03와 2.00±0.02 μg이었다. Microneedle로 전처리 후의 전달량은 각각 70.65±0.37와 162.23±0.40 μg으로 증가했다. 경피약물전달용 알렌드로네이트 패취의 생체적합성 평가는 ISO 10993에 따라 시험하였다.

Abstract: This study examined the transdermal delivery of alendronate across hairless mouse skin. The effects of iontophoresis, perforation with a microneedle, and a combination of a microneedle pretreatment and iontophoresis were evaluated *in vitro* test. Hydrogel patches were polymerized by UV polymerization to supply a hydrogel patch to the iontophoretic transdermal drug delivery system. The alendronate content in the iontophoretic delivery patch was 5.0 mg/cm^3. The amounts of alendronate that permeated across the hairless mouse skin when current densities of 0.25 and 0.50 mA/cm^2 were supplied to the iontophoretic alendronate patch were 0.80±0.03 and 2.00±0.02 μg, respectively. After pretreatment with a microneedle, the amounts of alendronate that permeated across the hairless mouse skin increased to 70.65±0.37 and 162.23±0.40 μg, respectively. The biocompatibility of the iontophoretic alendronate patch was examined according to the international standardization organization 10993.

Keywords: microneedle, iontophoresis, alendronate, transdermal drug delivery.

Introduction

Osteoporosis is the most common type of metabolic bone diseases worldwide with the aging population, particularly middle-aged women, being more susceptible. The World Health Organization (WHO) has defined osteoporosis as a bone mineral density (BMD) that is more than 2.5 standard deviations below the young adult peak.[1] According to WHO, osteoporosis is the second leading health care problem. In addition, the lifetime risk of osteoporosis-related fractures in men and women is approximately 13% and 35%, respectively. The field of osteoporosis, which was once dominated by hormone replacement therapy, witnessed the evolution of new therapeutic classes during the mid-1990s. Bisphosphates, such as alendronate and risedronate, are useful for preventing and treating all types of osteoporosis. Bisphosphonates have been shown to increase the bone mass in the spine and hips and reduce the incidence of fractures. Merck developed alendronate for the prevention treatment of osteoporosis. Alendronate is a nitrogen-based bisphosphate that is highly effective in the treatment of osteoporosis. It is an aminobisphosphate that inhibits osteoclast-related bone resorption.[2] Alendronate is 200 to 1000 times more active in inhibiting bone resorption than etidronate, and appears to increase the bone mass density by 4 to 7%. In postmenopausal osteoporosis, alendronate enhances the bone density in the hips and spine, decreases the level of bone loss, and reduces the risk of fractures. A weekly dose of alendronate

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(70 mg) or a daily dose of alendronate (10 mg) has been proven to have a positive effect on the BMD.[3-7] Although alendronate is an effective drug for osteoporosis, its bio-availability is quite low when taken orally. In women, the oral bioavailability of alendronate was independent of dose (5 to 80 mg) and averaged (90% confidence interval) 0.76% when taken with water in the fasting states followed by a meal 2 hours later. The bioavailability was similar in men.[8] The bio-availability was decreased to an estimated 0.46% and 0.39% when aldendronic acid was administered 60 or 30 min before breakfast.[9] Therefore, alendronate must be taken with plain water only at least 30 min before the first meal, beverage or medicinal product of the day. Other beverages, food and some medicinal products are likely to reduce the absorption of alendronate. In addition, it is not recommended that the patient lie down for at least 30 min after taking the me-dication. Certain people, including those who have difficulty in swallowing, cannot take the bisphosphonates orally. In these people, another bisphosphate, pamidronate, can be administered intravenously. The following people also should not take bisphosphates: people with certain disorders of the esophagus or stomach, women who are pregnant or nursing, people with low levels of calcium in the blood, and people with severe kidney disease.

As mentioned above, the oral bioavailability of alendronate was < 1.0% and the stratum corneum is a barrier to drug permeation. Because of the low permeability of the skin, only a few drugs such as fantanyl, nicotine, nitroglycerin, sco-polamine, etc, have been used for transdermal drug delivery. Several skin penetration enhancing technologies have been developed, such as iontophoresis,[10-13] electroporation,[14] sono-phoresis,[15] microneedle,[16] combinated microneedle and iontophoresis.[17]

Iontophoresis involves the use of a low-density electric current to drive charged molecules across the skin. The idea of applying an electric current to enhance the penetration of a charged compound into the tissue was first proposed by Pivati Veratti in 1747.[18]

This paper reports the transdermal delivery of alendronate. Iontophoresis and a combined microneedle pretreatment and iontophoresis were carried out using hairless mouse skin in an attempt to increase the permeation of alendronte. The effects of current density, and micro-needle pretreatment were investigated.

Experimental

Materials. Sodium 2-acrylamido-2-methylpropanesulpho-nate (NaAMPS) was purchased from Sigma-Aldrich Inc. (St. Louis, MO., USA) as a 50% aqueous solution, and acrylic acid (3-sulphopropyl) ester (SPA) is available commercially from Rasching (Deutsch) in the sodium form of a solid. Poly (ethylene glycol) diacrylate (PEGDA) cross-linker was obtained from Sigma-Aldrich Inc. (St. Louis, MO., USA). The initiator, 1-hydroxycyclohexyl phenyl ketone, which is marketed under the trade name of Irgacure 184, was acquired from Ciba Inc. (Basel, Switzerland). Alendronate was supplied by Hwail Pharm Co., Ltd. (Seoul, Korea). Citric acid, 9-fluorenyl methylchlorifomate (FMOC), and sodium diphosphate deca-hydrate, which were used to determine the amount of alendro-nate using HPLC, were purchased from Sigma-Aldrich Inc. (St. Louis, MO., USA), and Junsei Chemical Co., Ltd. (Tokyo, Japan), respectively. Methanol and acetonitrile were supplied by Burdick & Jackson (Ulsan, Korea). All solutions were prepared in deionized water (18 MΩ) obtained from an AquaMax™-Ultra (Younglin Instrument, Seoul, Korea) water system.

Preparation of Skin. Seven week old hairless mice (Orient Bio Inc., Seongnam, Korea) were euthanized by a cervical dislocation. The full-thickness skin was excised and the excess subcutaneous fat was carefully trimmed off. The excised skin was kept in a deep-freezer at −80 ℃ until used. Immediately before the experiment, the skins were taken out and left to thaw at room temperature.

Polymerization of Iontophoretic Alendronate Hydrogel Patches. A UV polymerization technique was used to polymerize the iontophoretic alendronate hydrogel patch. The polymerization process is as follows. A monomer solution was produced by mixing a 50% NaAMPS aqueous solution equivalent with a 50% SPA aqueous solution. Glycerol was then added as a chemical enhancer. Alendronate and potassium chloride were dissolved in a 0.05 M phosphate buffer adjusted to pH 7.0 to prepare the drug solution. Finally, the monomer and drug solutions were mixed and the initiator and crosslink reagent were then added. The mixture was transferred to a petri dish, and exposed to UV radiation using the RC-747 UV system purchased from Xenon (Woburn, MA, USA).

Skin Permeation Experiments. Franz diffusion cells and modified iontophoresis Franz cells,[19] which were designed for iontophoretically driven lateral transport within the skin, were purchased from Lab Fine (Anyang, Korea) and Laboratory Glass Apparatus Inc. (CA, USA), respectively. The skins were mounted on top of the diffusion cells or modified diffusion cells. The available surface area of the diffusion cells and modified diffusion cells were 0.64 cm^2 and 0.98 cm^2, res-pectively. The volume of the acceptor compartment in the diffusion cell and modified diffusion cell were 5.0 and 6.0 mL, respectively. The acceptor compartments of the cells

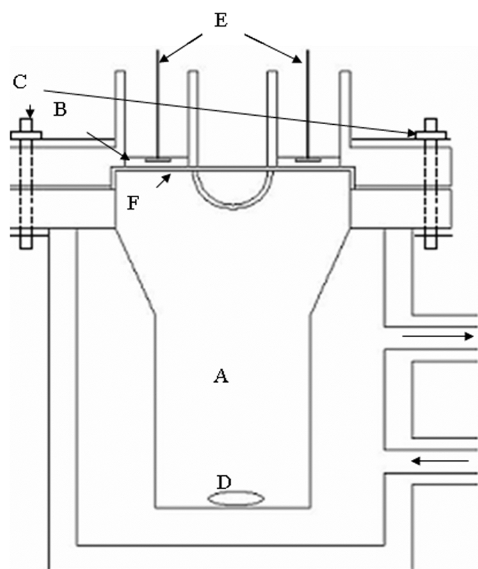

Figure 1. Schematic diagram of the iontophoretic alendronate delivery system used modified iontophoresis Franz Cell: (A) acceptor chamber with capacity for 6.00 mL buffer; (B) alendronate iontophoretic hydrogel; (C) clamp; (D) magnetic stirrer; (E) electrode; (F) hairless mouse skin.

were filled with 0.05 M phosphate buffered saline(pH 7.4) and stirred at 600 rpm in a pyrostat at 35.0±0.5 ℃.

Constant Current Density. Two current densities were used, 0.25 and 0.50 mA/cm^2. A direct current was applied to the iontophoretic patch through platinum wires, 0.5 mm and 3.5 cm in diameter and length, respectively. The Pt wires were immersed in the iontophoretic alendronate hydrogel patch. Amount of alendronate that permeated across the hairless mouse skin was determined according to the current density and the number of times the skin had been pretreated with the micro-needle roller. Figure 1 shows the iontophoresis alendronate delivery system using the modified iontophoresis Franz cell.

Skin Pretreatment with Micro-needle Roller. Passive diffusion, iontophoresis and iontophoresis after micro-needle pretreatment through the hairless mouse skin were carried out to determine the amount of alendronate delivered. The length and density of the micro-needles were 500 μm and 34 ea/cm^2, respectively. The micro-needle roller was supplied by Honam Petrochemical Corp.(Daejeon, Korea).

Determination of Alendronate by HPLC. A few methods for the determination of alendronate have been reported. These include spectrophotometric[20,21] and liquid chromatographic[22] methods. In this study, alendronate sodium was determined by HPLC after derivation with 9-fluorenyl methylchlorifomate(FMOC).[23] The HPLC system consisted of a pump (616, Waters, NY, USA), and a fluorescence detector(470, Waters, NY, USA) set at 260 and 310 nm for excitation and emission, respectively. Separation was carried out on a 150×4.6 mm I.D. column(Thermo, MA, U.S.A.) filled with a Hypersil C_{18} station phase with a particle size of 5 μm. An isocratic elution system was used with the mobile phase consisting of a mixed organic solution (acetonitrile:methanol, 1:1) and buffer(25 mM citric acid and 25 mM sodium di-phosphate decahydrate without pH adjustment).[24] The flow-rate was 1 mL/min at 35℃.

Stock solutions of alendronate sodium trihydrate were prepared by dissolving approximately 10 mg in 10 mL of distilled water. Standard solutions at concentrations of 0.5 ~40.0 μg/mL were then prepared by dilution using distilled water. The fluorescence derivative agent, FMOC, was made by dissolving approximately 5 mg in 20 mL of acetonitrile. The derivation procedure involved the addition of 200 μL of 1.0 M sodium carbonate buffer(pH 11.9) to 270 μL of the sample followed by the addition of 100 μL of the FMOC solution. After 3 min, 200 μL of a 1.0 M citric acid solution was added to adjust the pH, and 20 μL of the sample was injected into the HPLC.

Biocompatibility of Alendronate Patch. The alendronate patch must be biocompatible in order for it to be used as a medical device. Therefore, the *in vitro* cytoxicity, skin irritation, intra-cutaneous reactivity and sensitization of the alendronate patch were examined. All biocompatible tests were carried out by the Korean Testing and Research Institute for the Chemical Industry in accordance with the international standardization organization 10993. ISO 10993 is a family of standards which provides means to the medical device manufacturer to identify and perform the different types of biological evaluations. This series of standards is the result of efforts to combine and harmonize numerous International and national Standards and guidelines concerning the biological evaluation of medical devices. As such the Standard is increasingly gaining acceptance in an otherwise complex global environment of diverse national regulations and requirements.

Results and Discussion

Iontophoretic Alendronate Patch. The alendronate iontophoretic patch, which had been polymerized by UV radiation, was transparent, adhesive and conducting. The content of water and water activity were 43.48±0.84%, and 0.66±0.01%, respectively. Generally, fungi and bacteria cannot propagate at water activities < 0.7.

The pH of the alendronate patch was 7.0, which is very important for iontophoretic drug delivery. When the pH decreases, the concentration of hydrogen ions increases and

a vascular reaction is initiated due to C−fibre activation. At pH 5.5 and below, there is an increased risk of vascular reactions due to the high concentration of hydrogen ions rather than the compound used.

Effect of Current Density. The hairless mouse skin under physiological conditions was subjected to a direct current to determine if iontophoresis of the stratum corneum is possible. A constant current caused skin irritation, and burns due to changes in pH. Therefore, the current density was limited to less than 0.50 mA/cm^2.

Figure 2 shows the amounts of alendronate that permeated across the hairless mouse skin at constant direct current densities of 0.25 and 0.50 mA/cm^2. When a current was not applied to the alendronate patch, alendronate did not permeate across the hairless mouse skin. The amounts of alendronate that permeated across the hairless mouse skin were proportional to the current density and time. As shown in Figure 2, increasing the current density from 0.25 to 0.50 mA/cm^2 resulted in an increase in the amount of alendronate that permeated across the hairless mouse skin of applied current. When the current densities applied to the iontophoretic alendronate patch were 0.25 mA/cm^2 and 0.50 mA/cm^2 for 120 min, amount of alendronate that permeated across the hairless mouse skin and the steady state flux were 0.80± 0.03 μg and 2.00±0.02 μg, and 0.41 μg/cm^2 · hr and 1.02 μg/cm^2 · hr, respectively.

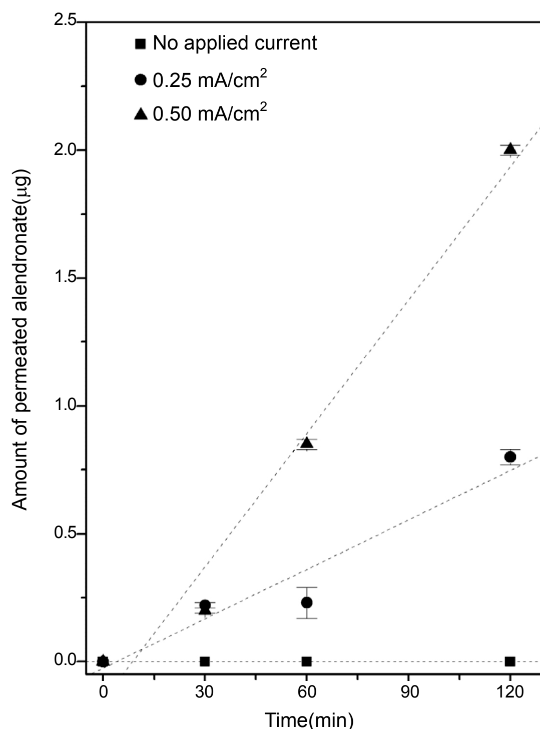

Figure 2. Effect of current density on permeated amount from iontophoretic alendronate patch. Data represents mean±S.D. on n=3 samples.

Effect of the Skin Pretreatment. A micro−needle roller was used to improve the level of alendronate permeation across the hairless mouse skin. The height and density of the micro−needle was 500 μm, 32 ea/cm^2, respectively. The micro−needle was rolled up in a perpendicular, horizontal trajectory on the hairless mouse skin. The alendronate patch was placed on the skin treated with the micro−needle roller for 300 min. Table 1 shows the amounts of alendronate that permeated across the treated hairless mouse skin.

As shown in Table 1, alendronate is permeated after 180 min. There was an increase in the amounts of alendronate that permeated across the hairless mouse skin after 8 and 12 cycles of the micro−needle treatment. That means that micro−needle rolling affects the permeation of alendronate from iontophoretic alendronate patch through the skin. After more than 8 cycles of rolling, the amount of alendrate that permeated across the hairless mouse skin was not influenced by the number of rolling cycles. The alendronate patch was not swelled. That means that the micro−needle roller can puncture the skin but migration does not occur through the punctures.

In order to improve the permeation alendronate, across the hairless mouse skin a combination of a pretreatment with the micro−needle roller and iontophoresis was performed on the iontophoretic alendronate patch. After treating the skin with the micro−needle roller, the iontophoretic alendronate patch was placed on the treated hairless mouse skin. A constant current was applied to the iontophoretic alendronate patch through the Pt wire. After 30, 60, and120 min, the amount of alendronate that permeated across the hairless mouse skin to the acceptor buffer solutions was determined by HPLC. As shown as Figure 3, the amount of alendronate that permeated across the hairless mouse skin was pro−portional to the applied current density and micro−needle rolling cycles. When 0.50 mA/cm^2 applied to the iontophoretic patch, amounts of alendronate that permeated across the hairless mouse skin was higher than that observed at 0.25 mA/cm^2.

Biocompatibility of the Alendronate Patch. The alendronate

Table 1. Effect of Micro-Needle Rolling Cycle on Permeated Amount by Passive Diffusion from Alendronate Patch

(unit : μg)

	Passive Diffusion Time (min)		
	60	180	300
Non treatment	ND	ND	ND
4 cycles	ND	ND	5.35±0.02
8 cycles	ND	48.80±0.13	52.40±0.81
12 cycles	Tr	44.90±2.51	57.66±1.39

ND: non detection, Tr: Trace.

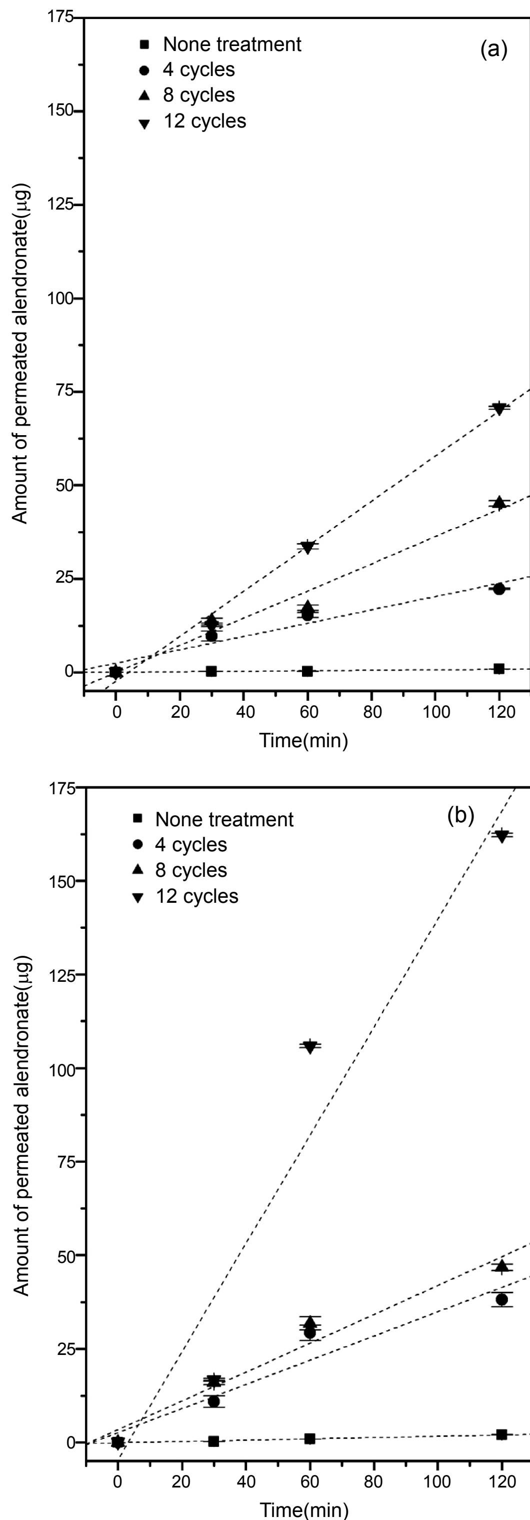

Figure 3. The effects of current density and micro-needle rolling cycle on permeation of alendronate from iontophoretic alendronate patch across hairless mouse skin. Results are expressed as mean±S. D. of two experiments: (a) 0.25 mA/cm^2; (b) 0.50 mA/cm^2.

patch must be biocompatible in order for it to be used as a medical device. The *in vitro* cytoxicity, skin irritation, intracutaneous reactivity, and sensitization tests were carried out according to the international standardization organization 10993. The *in vitro* cytotoxicity test is a method for determine if the material induces cellular toxicity. The level of cytotoxicity of the alendronate patch was scale 1, which means the alendronate patch is mildly cytotoxic but is still suitable as a medical device. There was no mortality, clinical signs, body weight changes, skin irritation or skin responses observed after the rest, skin irritation, intracutaneous reactivity and sensitization examinations. According as these evaluation, the alendronate patch is considered to be biocompatible and suitable for use as a medical device.

Conclusions

This study examined the feasibility of administering an alendronate dose by transdermal delivery of alendronate across hairless mouse skin. Iontophoresis and combined iontophoresis and micro-needle methods were introduced in an attempt to improve the level of alendronate permeation. Alendronate was contained in the iontophoretic patch, which was polymerized with NaAMPS and SPA by UV polymerization. In the passive diffusion experiments, alendronate did not permeate from the iontophoretic alendronate patch across the hairless mouse skin. In the combined iontophoresis and skin treatment with the micro-needle roller for 120 min, increasing the current density from 0.25 to 0.50 mA/cm^2 resulted in a doubling of the amount of alendronate that permeated across the hairless mouse skin. Biocompatibility tests, such as the *in vitro* cytoxicity, skin irritation, intracutaneous reactivity and sensitization, were also carried out on the iontophoretic alendronate patch. The patch was found to be biocompatible and deemed suitable for further testing as a medical device.

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References

1. J. A. Kanis, WHO Study Group, *Osteoporos Int.*, pp 368-381 (1994).
2. K. Skorey, H. D. Ly, J. Kelly, M. Hammond, C. Ramacjamdran, Z. Huang, M. J. Gresser, and Q. Wang, *J. Biolo. Chem.*, **272**, 22472 (1997).
3. U. A. Liberman, S. R. Weiss, J. Bröll, H. W. Minne, H. Quan, N. H. Bell, J. Rodriguez-Portales, R. W. Dowms, Jr., J.

Dequeker, M. Favus, E. Seeman, R. R. Recker, T. Capizzi, A. C. Samtora II, A. Lombardi, R. V. Shan, L. J. Hirsch, and D. B. Karpf, *N. Engl. J. Med.*, **333**, 1437 (1995).
4. C. V. Odvina, J. E. Zerwekh, D. S. Rao, N. Maalouf, F. A. Gottschalk, and C. Y. C. Pak, *J. Clin. Endo. & Meta.,* **90**, 1294 (2005).
5. D. M. Black, D. E. Thompsom, D. C. Bauer, K. Ensrud, T. Musliner, M. C. Hochberg, M. C. Nevitt, S. Suryawanshi, and S. R. Cummings, *J. Clin. Endo. & Meta.,* **85,** 4118 (2000).
6. E. Orwoll, M. Ettinger, S. Weiss, A. Miller, D. Kendler, J. Graham, S. Adami, K. Weber, R. Loernc, P. Pietschmann, K. Vandormael, and A. Lombardi, *N. Engl. J. Med.,* **243**, 604 (2000).
7. H. G. Bone, D. Hosking, J.−P. Devogelaer, J. R. Tucci, R. D. Emkey, R. P. Tonino, J. A. Rodriguez−Porales, R. W. Downs, J. Gupta, A. C. Santora, and R. A. Liberman, *N. Engl. J. Med.,* **350,** 1189 (2004).
8. B. J. Gertz, S. D. Holland, W. F. Kline, B. K. Matuszewski, A. Freeman, Q. Hui, K. C. Lasseter, J. C. Mucklow, and A. G. Porras, *Clin. Pharm. Ther.*, **58,** 288 (1995).
9. A. B. Ragsdale, T. A. Barringer III, and G. D. Anastasio, *Arch. Fam. Med.*, **7,** 583 (1998).
10. M. B. Delgado−Charro and R. H. Guy, *Pharam. Res.,* **20**, 1508 (2003).
11. D. Marro, R. H. Guy, and M. B. Delgado−Charro, *J. Control. Release*, **70**, 213 (2001).
12. T. Tomohira, Y. Machida, H. Onishi, and T. Nagai, *Inter. J. Pharm.,* **155**, 231(1997).
13. C. T. Costello and A. H. Jeske, *Phys. Ther.,* **75** , 554 (1995).
14. M. R. Prausnitz, V. G. Bose, R. Langer, and J. C. Weaver, *Proc. Natl. Acad. Sci. U. S. A.*, **90**, 10504 (1993).
15. S. E. Cross and M. S. Roberts, *Current Drug Delivery*, **1**, 81 (2004).
16. J. Ji, R. E. H. Tay, J. Miao, and C. Iliescu, *J. Phys.: Conference Series,* **34**, 1127 (2006).
17. W.−M. Wu, H. Todo, and K. Sugibayashi, *J. Control. Release,* **118**, 189 (2007).
18. S. Rawat, S. Vengurlekar, B. Rakesh, S. Jain, and G. Spikarti, *Indian J. Pharm. Sci.,* **70**, 5 (2008).
19. P. Glikfeld, C. Cullander, R. S. Hinz, and R. H. Guy, *Pharm. Res.,* **5**, 443 (1988).
20. E. A. Taha and N. F. Youssef, *Chem. Pharm. Bull.*, **51**, 1444 (2003).
21. J. Kuljanin, L. Janković, J. Nedeljković, D. Prstojević, and V. Marinković, *J. Pharm. Biomed. Anal.*, **28**, 1215 (2002).
22. S. K. A. Deeb, I. I. Hamdan, and S. M. A. Najjar, *Talanta,* **64**, 695 (2004).
23. P. Ptáček, J. Klíma, and J. Macek, *J. Chrom. B,* **767**, 111 (2002).
24. M.−H. Yun, J.−S. Woo, and K.−I. Kwon, *Arch. Pharm. Res.*, **29**, 328 (2006).