Pharmaceutical Development and Technology

ISSN: 1083-7450 (Print) 1097-9867 (Online) Journal homepage: http://www.tandfonline.com/loi/iphd20

Transdermal Permeability of N-Acetyl-D-Glucosamine

Solomon T. Garner Jr., Bridg´ette J. Israel, Hishamaldin Achmed, Anthony C. Capomacchia, Trina Abney & Parastoo Azadi

To cite this article: Solomon T. Garner Jr., Bridg´ette J. Israel, Hishamaldin Achmed, Anthony C. Capomacchia, Trina Abney & Parastoo Azadi (2007) Transdermal Permeability of N-Acetyl-D-Glucosamine, Pharmaceutical Development and Technology, 12:2, 169-174, DOI: 10.1080/10837450701212560

To link to this article: http://dx.doi.org/10.1080/10837450701212560

Published online: 07 Oct 2008.

Pharmaceutical Development and Technology, 12:169–174, 2007

ISSN: 1083-7450 print / 1097-9867 online
DOI: 10.1080/10837450701212560

informa
healthcare

Transdermal Permeability of N-Acetyl-D-Glucosamine

Solomon T. Garner, Jr., Bridg´ette J. Israel, Hishamaldin Achmed, and Anthony C. Capomacchia
Department of Pharmaceutical and Biomedical Sciences, The University of Georgia, Atlanta, GA

Trina Abney and Parastoo Azadi
The University of Georgia, Complex Carbohydrate Center, Athens, GA

Transdermal permeation of N-acetyl-D-glucosamine (NAG), a metabolite of glucosamine was examined. Glucosamine salts are nutraceuticals used in the oral treatment of osteoarthritis. Sparse information is available regarding glucosamine and NAG transdermal or percutaneous transport and absorption. Permeability of NAG in various enhancer suspensions was evaluated by using shed snakeskin as a model membrane via Franz-type cell diffusion studies. Negligible permeability was observed for NAG in neat solutions of known membrane permeation enhancers ethanol, oleic acid, isopropyl myristate, and isopropyl palmitate, as well as from saturated solutions of NAG in water or phosphate buffer. Permeability measurements obtained from saturated solutions of NAG in DMSO and phosphate buffer solutions containing ethanol at 2%, 5%, 10%, 25%, and 50% demonstrated excellent permeation. Permeability coefficients of the phosphate buffer/ethanol solutions at 5%, 10%, and 25% were about threefold larger in value as those for saturated DMSO solution, whereas the 2% and 50% solution values were lower.

Keywords N-acetyl-D-glucosamine (NAG), glycosaminoglycan (GAG), osteoarthritis, transdermal permeation, skin permeation enhancers

BACKGROUND

The oral use of glucosamine supplements became popular after being featured in the book, *The Arthritis Cure* by Jason Theodasakis, MD.[1] Currently, glucosamine and its metabolites are not classified as drugs but as nutraceutical/dietary supplements under United States Food and Drug Administration's Dietary Supplement Health and Education Act of 1994 (DSHEA). Oral dosage formulations of N-Acetyl-D-glucosamine (NAG) and its parent compound glucosamine in salt form (sulfate, hydrochloride, etc.) are commercially available nutraceuticals and are commonly administered in conjunction with chondroitin sulfate, also a readily available nutraceutical. Glucosamine and chondroitin have been reported effective in the oral treatment of osteoarthritis but have not undergone the rigorous studies needed for FDA approval as pharmaceuticals.[1,2] The National Institutes of Health (NCCAM) (Bethesda, MD, USA) has an ongoing multicenter study—GAIT (Glucosamine/Chondroitin Arthritis Intervention Trial), which has evaluated the efficacy of orally administered glucosamine and chondroitin oral supplements. Published study results indicate that a combination of glucosamine and chondroitin might be most effective in patients with osteoarthritis with moderate to severe pain and in many cases obviates the need for NSAID use.[3]

Glucosamine and chondroitin salts are charged, highly polar, aqueous soluble, and apparently poor candidates for transdermal absorption. Currently, there are topical products containing these salts, and other ingredients, marketed as nutraceuticals for the treatment of osteoarthritis. NAG, an acetylated glucosamine metabolite, is less polar, uncharged, and appears to be a more likely candidate for transdermal or percutaneous absorption.

Glucosamine and its orally delivered salt forms are metabolized to NAG via the hexosamine pathway; glucosamine or galactosamine, plus a uronic acid, is incorporated as a disaccharide unit into all macromolecules requiring amino sugars, such as keratan sulfates, dermatan sulfates, chondroitin 4- and 6-sulfates, hyluronates, and heparin and heparan sulfates, to produce glycosaminoglycans (GAGs). GAGs are highly negatively charged molecules, with an extended conformation, that demonstrate high viscosity and

Received 9 October 2005, Accepted 1 October 2006.
Address correspondence to Anthony C. Capomacchia, Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, The University of Georgia, Athens, Georgia, 30602, USA; E-mail: tcapomac@rx.uga.edu

low compressibility—ideal as a lubricating fluid for anatomical joints. The majority of GAGs in the body are linked to core proteins to form proteoglycans or mucopolysaccarides, which are basic components of skin, tissue, and cartilage.[4,5]

INTRODUCTION

The study objectives were to evaluate stratum corneum permeability of NAG with use of shed snakeskin and to assess the feasibility of pursuing a percutaneous formulation for local therapy to osteoarthritic joints. In this connection, a provisional patent application, followed by patent application 235.00560201 was filed with the U.S. Patent and Trademark Office.[6] Permeability was evaluated by using NAG solutionss of various known membrane transport-enhancing reagents, ethanol, oleic acid, isopropyl myristate, and isopropyl palmitate; NAG solutions of water, phosphate buffer, phosphate buffer, and aqueous ethanol; and NAG saturated DMSO solution.

Oral administration of glucosamine, its salts, and NAG are affected by the liver's first-pass metabolism.[7] However, a more recent report indicates that these agents may be metabolized mostly in the gut rather than solely by the liver.[8] Few pharmacokinetic literature reports exist on the disposition of these agents in articular cartilage. Setnikar et al. reported on the pharmacokinetic properties of glucosamine in dogs and man.[9,10] It is estimated that approximately 87% of the original glucosamine oral dose is absorbed and excreted; <13% is widely distributed in the body; and <<1% reaches osteoarthritic joints. Chondroitin is known to degrade into its basic dissaccaride components within the gut prior to further metabolism.[11] Although only a small fraction of glucosamine reaches the articular cartilage target site, it is reported to exhibit a high potency; and together glucosamine and chondroitin therapy demonstrate therapeutic efficacies over time.[2] NAG was selected because it is an active metabolite and prodrug of glucosamine and, owing to its commercial availability, relatively low cost and stability. It possesses the following physical and chemical characteristics, making it a reasonable candidate for transdermal delivery and percutaneous absorption: 1) high potency, 2) reasonably lipid soluble, 3) low molecular weight, 4) unique biochemical pathway with active transport from blood into articular cartilage.[5]

MATERIALS AND METHODS

Chemicals

NAG, 99.9% purity was purchased from MP Biomedical (Aurora, OH). All enhancer reagents purchased for this study were 99.9+% pure. All other reagents were of analytical grade and used without further purification.

Analysis

NAG analysis was carried out by using high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD); Dionex, Sunnyvale, CA, USA): Dionex DX-500 HPLC system consisting of a GP40 gradient pump, ED40 Electrochemical detector, AS3500 autosampler and PeakNet Chromatography Workstation.[1215] The HPAE-PAD was equipped with CarboPac™ PA20 (3 × 150 mm), analytical anion-exchange column for the rapid, high-resolution separation of monosaccharides and disaccharides, using pulsed amperometric detection,[12–15] a CarboPac PA20 analytical guard column (3 × 30 mm), and a carbonate trap column (25 × 15 mm). Mobile phase (A) was degassed and deionized water. Mobile phase (B) consisted of 0.02 N NaOH prepared with deionized water and filtered with 0.45-µm filters in a solvent filtration apparatus (Waters-Millipore, Milford, MA, USA) that was degassed under vacuum. The mobile phase system was run at a gradient concentration of 16 mM NaOH at a flow rate of 0.5 mL/min. A standard calibration curve of NAG (Figure 1) was obtained with linear regression and value of $R^2 = 0.9934$ in 0.1 M phosphate buffer at pH 7.4. Each sample set was run with external standards. The sample concentration values were obtained via the Peak Net software. These values were compared with to those obtained by calculations of the peak area and peak height observed as functions of the standard curve linear regression equation. The instrument LOD (limit of detection) was 0.05 ng/mL.

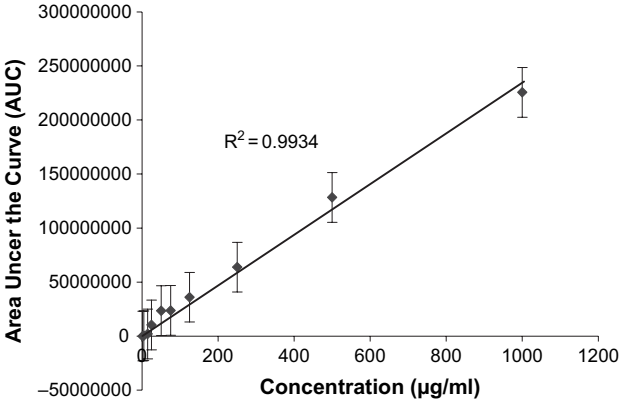

Figure 1. Standard calibration curve of NAG ($R^2 = 0.9934$) obtained in 0.1M phosphate buffer at pH 7.4.

In Vitro Membrane Permeation

Shed snakeskins were used as a model membrane for all permeation studies using the NAG solutions in known membrane permeation enhancers; ethanol, oleic acid, isopropyl myristate, and isopropyl palmitate; saturated solutions of NAG in water and in phosphate buffer; as well as in DMSO solution (Table 1; Figure 2) and phosphate buffer containing ethanol at 2%, 5%, 10%, 25%, and 50% solutions (Table 2; Figure 3). The skins were stored at 20°C before use; a piece of the dorsal section was trimmed to fit the Franz cell and hydrated at 37°C for 30 min.[16] Franz-cell diffusion experiments were carried out. For experiments using ethanol, oleic acid, isopropyl myristate, isopropyl palmitate, DMSO, and phosphate buffer containing ethanol at 2%, 5%, 10%, 25%, and 50% solutions in the donor phase, receptor cells were filled with 7.4 pH 0.1 M phosphate buffer, and the donor cell was filled with the corresponding NAG solution. Receptor solutions were maintained at 37°C and stirred with a magnetic stirrer. The snakeskins were mounted between the receptor and donor cells. The surface exposed to diffusion was 2.54 cm^2 (diameter 1.8 cm) and the receptor cell volume was 6 cm^3. The donor cell was covered with plastic film. The system was allowed to equilibrate at 37°C for 2 hr before each experiment. To the donor cells, 5 mL of the NAG-enhancer solution (100 mg/mL) maintained at 37°C were added. Samples were taken at intervals over a 24-hr period, 200-µL samples of receptor solutions were taken and replaced with fresh buffer; experiments were conducted in triplicate. The amounts of NAG that permeated through the snakeskin were determined by HPAE-PAD.

Table 1

Physiochemical data obtained for the permeation of N-acetylglucosamine (NAG) through shed snake skin using dimethyl sulfoxide (DMSO) solution in the donor phase and pH 7.4 phosphate buffer in receptor phase (from Figure 2)

Parameter	
J_{ss} (µg/cm^2/hr)	8.28
Cumulative 24-hr steady-state permeation (µg)	510.5
Permeation coefficient, k_p (cm/hr) × 10^3	0.425
% NAG in receptor phase after 24 hr	2.57

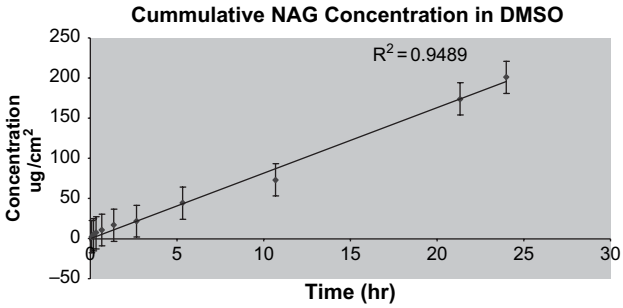

Figure 2. N-acetylglucosamine (NAG) permeation through shed snakeskin at 37.5°C (cumulative concentration vs. time) in DMSO solution (100 mg/mL). Each point represents the mean ± D, n = 3.

Data Treatment

Permeation was determined from the increasing amount of NAG in the receptor medium, and cumulative steady-state permeation from plots of cumulative amount of NAG, per unit area (µg/cm^2) that permeated through the snakeskin versus time (Figures 2 and 3); where the slope of the linear portion of the plot was used to calculate the steady-state flux, J_{ss}; µg/cm^2/h.[16–18] NAG permeability coefficients (kp) were calculated from the expression kp = $J_{ss} \times \Delta C$, where J_{ss}, and ΔC are the flux and concentration change, respectively (Tables 1 and 2). Permeation was rapid with almost no lag time observed for NAG in DMSO (Figure 2; Table1)[20]; and also for permeation of the ethanol-laced phosphate buffer solutions of NAG after examination of the intersection of the linear portion of the plots with the x-axis (Figure 3; Table 2).

Table 2

Physicochemical data obtained for the permeation of N-acetylglucosamine (NAG) through shed snake skin via phosphate buffer containing ethanol at 2%, 5%, 10%, 25%, and 50% solutions in the donor phase and pH 7.4 phosphate buffer in the receptor phase

% Ethanol, enhancing agent	2	5	10	25	50
J_{ss} (µg/cm^2/hr); from linear portion of curves in Figure 3	6.88	26.8	29.5	25.5	5.14
Cumulative 24-hr permeation (µg)	196	572.8	572.1	482.6	54.0
Permeation coefficient k_p (cm/hr) × 10^3	0.347	1.38	1.52	1.31	0.264
% NAG in receptor phase after 24 hr	0.98	2.89	2.88	2.44	0.273

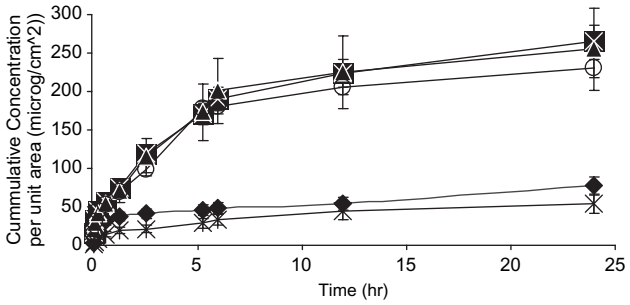

Figure 3. The effect of ethanol concentration on N-acetylglucosamine (NAG; 100 mg/mL) permeation at 37.5°C through shed snakeskin (cumulative concentration, μg/cm^2, vs. time). Each point represents the mean ± D, n = 3. 2% ethanol: ◆; 5% ethanol: ■, 10% ethanol: ▲, 25% ethanol: ❍, 50% ethanol: ×.

Determination of Partition Coefficients

The oil/water partition coefficient for NAG was determined by using n-octanol/phosphate buffer (pH 5.5, 6.5, and 7.4 at 0.1 M) and n-octanol/water (Table 3).[19] In each case 5 mL of n-octanol was mixed with 5 mL of aqueous solutions containing NAG and shaken at 37°C for 24 hr. The mixture was centrifuged, and the organic and aqueous phases were separated. NAG concentration in the filtrates was determined by HPAE-PAD after appropriate dilution.

RESULTS AND DISCUSSION

Initial permeability investigations were carried out by using shed snakeskin as a model membrane to human skin, a widely recognized model for preliminary studies due to its similarity in composition to the human stratum corneum.[20,21] Negligible NAG transport was observed from neat saturated solutions of the membrane permeability enhancers; pure ethanol, oleic acid, isopropyl myristate, and isopropyl palmitate. No permeation was observed from the aqueous saturated solutions of NAG in water or phosphate buffer solutions (pH 5.5, 6.5, and 7.4; 0.1 M). This indicates that permeation of NAG probably does not involve interactions with intercellular lipids as presented in Barry's lipid-protein partitioning theory.[18,22] Partition coefficients are contained in Table 3; the NAG oil-water partition coefficient is shown to increase concomitantly with the increase in buffer solution pH, no doubt owing to the greater concentration of unionized species at pH values greater than the pKa of 6.73 (Table 3). The theoretically calculated permeation coefficient (k_p) of 1.9×10^{-4} is similar in magnitude to those we report in Tables 1 and 2.

DMSO was chosen for evaluation as a benchmark permeation enhancer due to its physical properties and well-documented enhancement properties.[22] Enhancers in the category of DMSO (also ethanol) are reported to disrupt intercellular lipids of the stratum corneum by increasing a drug's partitioning into the stratum corneum with a concomitant increase in drug permeation through the intercellular junctions.[23–25] From the plot containing cumulative NAG concentration per unit area (μg/cm^2) versus time, NAG's in vitro flux was 8.28 μg/cm^2 (Figure 2; Table 1). The assumption is that NAG's high polarity and low partition coefficient (Table 3) contributes to its permeation in DMSO and poor permeability in purely aqueous solution owing to solubility considerations. The study shows that DMSO allows NAG to be transported immediately and continuously as seen in Figure 2 with a linear concentration increase over time.

NAG was also incorporated in ethanol/buffer solution at various ethanol concentrations. Ethanol is known to promote transdermal penetration and percutaneous absorption of many drugs.[26] NAG transdermal transport was not observed from phosphate buffer or pure ethanol where it is highly soluble and insignificantly soluble, respectively. NAG permeation was observed in sink conditions from phosphate buffer containing ethanol at 2%, 5%, 10%, 25%, and 50% (Figure 2). The cumulative concentration of the solutions containing 5% and 10% ethanol are very similar after 24 hr; the 25% solution slightly lower; and the 2% and 50% ethanol in buffer solutions deliver comparatively less NAG. Beyond 50% ethanol concentration in buffer, the NAG precipitated. The flux values for 5%, 10%, and 25% ethanol concentrations are also close in value, whereas the 50% and 2% values are significantly lower.

Table 3
Experimentally determined partition coefficients for N-acetyl-D-glucosamine (NAG) [pKa 6.73] in pH solutions 5.5, 6.0, 6.5, 7.4, and water; and permeation coefficient theoretically calculated according to Potts-Guy equation using our experimentally derived value for NAG Octanol/water ratio of 0.116.[15]

Octanol/pH 5.5buffer	Octanol/pH 6.5 buffer	Octanol/pH 7.4 buffer	Octanol/water	k_p(cm/hr)[19]
0.085	0.089	0.110	0.116	1.9×10^{-4}

The results indicate that thermodynamic and solubility effects may control NAG permeation in DMSO at 100%, and in the different concentrations of ethanol buffer solutions.[27] NAG in vitro flux and cumulative permeations from 5%, 10%, and 25% ethanol solution were larger in magnitude than that observed in DMSO and are also larger than those recently reported for glucosamine sulfate in a transdermal permeation study.[28] The results indicate that a solution concentration of 5–25% ethanol as an enhancer in delivery vehicles appears to be an excellent starting point toward a percutaneous formulation of NAG.

In conclusion, DMSO is an excellent skin penetration enhancer for NAG. DMSO is generally used in veterinary drug delivery, and the use of DMSO in NAG formulations may be useful for localized osteoarthritis treatment in animals because DMSO is not an FDA-approved excipient for human use in topical or transdermal pharmaceutical products. Results also show that ethanol enhances the permeation of NAG in the concentration range of 5–25%, in shed snakeskin. This finiding indicates that formulations containing 5–25% ethanol may enhance NAG permeation in humans.

ACKNOWLEDGMENTS

This work was supported in part by NIH grant GM066321–03; the Alfred P. Sloan Foundation Minority Ph.D. Program; the Department of Pharmaceutical and Biomedical Sciences; and a private grant from AC Capomacchia.

REFERENCES

1. Theodasakis, Jason; Fox, Barry; Adderly, Beverly. *The Arthritis Cure* 1st ed. St. Martin's Press: New York, 1997.
2. McAlindon, T.E.; LaValley, M.P.; Gulin, J.P.; Felson, D.T. Glucosamine and chondroitin for treatment of osteoarthritis: A systematic quality assessment and meta-analysis. JAMA. **2000**, *283*, 1469–1475.
3. Glucosamine/Chondroitin Arthritis Intervention Trial (GAIT). National Center for Complementary and Alternative Medicine (NCCAM), and National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS). September 1999. (http://nccam.nih.gov/research/results/gait/qa.htm)
4. Merrick, J.M.; Roseman, S. Glucosamine metabolism. J. Biop. Chem. **1960**, *5*, 235–242.
5. Milewski, S. Glucosamine-6-phophate synthase. Biochim. Biophys. Acta **2002**, *1597*, 173–192.
6. Capomacchia, A.C.; Garner, S.T.; Beach, W.J. NSAID/ Glucosamine Mutual Prodrug and Uses Thereof. US Provisional Patent Application No. 60/560,128. 2004; Patent Application 235.00560201, Glucosamine and glucosamine/ anti-inflammatory mutual prodrugs, compositions and methods, Capomacchia, AC; Garner, ST; Beach, WJ., 'Glucosamine; filed April 7, 2005.
7. Setnikar, I.; Giacchetti, C.; Zanolo, G. Pharmacokinetics of glucosamine in the dog and in man. Arzneimittel-Forschung **1986**, *36* (4), 729–735.
8. Aghazadeh-Habashi, A.; Sattari, S.; Pasutto, F.; Jamali, F. Single dose pharmacokinetics and bioavailability of glucosamine in the rat. J. Pharm. Pharm. Sci. **2002**, *5* (2), 181–184.
9. Setnikar, I.; Palumbo, R.; Canali, S.; Zanolo, G. Pharmacokinetics of glucosamine in man. Arzneimittel-Forschung **1993**, *43* (10), 1109–1113.
10. Setnikar I; Rovati L C Absorption, distribution, metabolism and excretion of glucosamine sulfate: A review. Arzneimittel-Forschung **2001**, *51* (9), 699–725.
11. Lamari, F.N.; Militsopoulou, M.; Mitropoulou, T.N.; Hjerpe, A.; Karamanos, N.K. Analysis of glycosaminoglycan-derived disaccharides in biological samples by capillary electrophoresis and protocol for sequencing glycosaminoglycans. Biomed. Chromatogr. **2002**, *16*, 95–102.
12. Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector. Technical Note 21, Dionex Corp., Sunnyvale, CA, USA.
13. Clarke, A.P.; Jandik, P.; Rocklin, R.D.; Liu, Y.; Avdalovic, N. An integrated amperometry waveform for the direct, sensitive detection of amino acids and amino sugars following anion exchange chromatography. Anal. Chem. **1999**, *71*, 2774–2781.
14. Campo, G.M.; Campo, S.; Ferlazzo, A.M.; Vinci, R.; Calatroni, A. Improved high-performance liquid chromatographic method to estimate amino sugars and its application to glycosaminoglycan determination in plasma and serum, J. Chromatogr. B **2001**, *765*, 151–160.
15. LaCourse, W.R. *Pulsed Electrochemical Detection in High-Performance Liquid Chromatography*. John Wiley & Sons Inc.: New York, 1997.
16. Itoh, T.; Xia, J.; Magavi R.; Nishihata, T.; Rytting, J.H. Use of shed snake skin as a model membrane for in vitro percutaneous penetration studies: Comparison with human skin. Pharm. Res. **1990**, *7*, 1042–1047
17. Bach, M.; Lipppold, B.C. Percutaneous penetration enhancement and its quantification. Eur. J. Pharm. Biopharm. **1998**, *46*, 1–13. Guidance for Indusry:
18. Hadgraft, J.; Guy, R.H.; Feasibility assessment in topical and transdermal delivery: Mathematical models and in vitro studies. *Transdermal Drug Delivery*, 2nd ed. Marcel Dekker, Inc.: New York, 2003, 1–23.
19. Bernacki, R.J.; Sharma, M.; Porter, N.K.; Rustum, Y.; Paul, B.; Korytnyk, W. Biochemical characteristics, metabolism and antitumor activity of several acetylated hexosamines. J. Supramol. Struct. **1977**, *7*, 235–250.
20. Xudong Yuan and A.C. Capomacchia. The binary eutectic of NSAIDs and two-phase liquid system for enhanced membrane permeation. Pharm. Dev. Technol. **2005**, *1*, 1–10.
21. Franz, T.J.; Lehman, P.A.; Kagy, M.K. Dimethyl sulfoxide. *Percutaneous Enhancers*; Smith, E.W.; Maibach, H.I. Eds.; CRC Press, Inc.: Boca Raton, FL, **1995**, 112–127.
22. Barry, B.W. Lipid-protein-patitioning theory of skin penetration enhancement. J. Control. Release **1991**, *15*, 237–248.

23. Williams, A.C.; Barry, B.W. Skin absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. **1992**, *9*, 305–353.
24. Sinha, V.R.; and Kaur, M.P. Permeation enhancers for transdermal drug delivery. Drug Dev. Ind. Pharm. **2000**, *26*, 1131–1140.
25. Berner, B.; Liu, P. Alcohols, percutaneous penetration enhancers. *Alcohols*. Smith, E.W.; Maibach, H.I. Eds.: CRC Press: Boca Raton, FL, 1995, 45–60.
26. Kurihara-Bergstrom, T.; Flynn, G.L.; Higuchi, W.I. Physicochemical study of percutaneous absorption enhancement by dimethyl sulfoxide: Dimethyl sulfoxide mediation of vidarabine (ara-A) permeation of hairless mouse skin. J. Inv. Derma., **1987**, *89*, 274–280.
27. Magnusson, B.M.; Walters, K.A.; Roberts, M.S. Veterinary drug delivery: Potential for skin penetration enhancement. Adv. Drug Del. Rev., **2001**, *50*, 205–227.
28. Kanwischer, M.; Kim, S.-Y.; Bian, S.; Kwon, A.E.; Kim, J.S.; and Kim, D.-D. Evaluation of the physicochemical stability and skin permeation of glucosamine salts. Drug Dev. Ind. Pharm. **2005**, *31* (1), 91–97.