Amphiphilic Star-Like Macromolecules as Novel Carriers for Topical Delivery of Nonsteroidal Anti-Inflammatory Drugs

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ABSTRACT

The objective of this study was to evaluate amphiphilic star-like macromolecules (ASMs) as a topical drug delivery system. Indomethacin, piroxicam, and ketoprofen were individually encapsulated into the ASMs using coprecipitation. The effects of the ASMs on percutaneous permeation of nonsteroidal anti-inflammatory drugs (NSAIDs) across full thickness, hairless mouse skin were evaluated in vitro using modified Franz diffusion cells. In addition, solubility and in vitro release experiments were performed to characterize ASMs behavior in aqueous media. Poly(ethylene glycol) (PEG) and Pluronic P-85 were used as polymer controls to compare the role of PEG and amphiphilic behavior in the ASMs. In vitro release experiments indicated that ASMs can delay drug release ($P < .05$), whereas solubility measurements showed that ASMs can increase NSAIDs aqueous solubility ($P < .05$). Percutaneous permeation studies revealed that ASMs decreased both flux and Q_{24} of drugs compared with the control ($P < .10$). Skin pretreatment studies with ASM-containing solution before drug application demonstrated that pretreatment similarly influenced NSAID percutaneous permeation. In conclusion, ASMs likely slow drug permeation through 2 mechanisms, delayed drug diffusion from its core and skin dehydration by its shell. Thus, ASMs may be useful for delayed dermal delivery or prevention of compound permeation through the skin (eg, sunscreens, N,N-diethyl-m-toluamide [DEET]) from aqueous formulations.

KEYWORDS: topical drug delivery, NSAIDs, polymeric micelle, permeation, drug release

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of osteo- and rheumatoid arthritis as well as local inflammation.^{1,2} Oral therapy of NSAIDs is very effective, but the clinical use is often limited because of the potential to cause adverse effects such as irritation and ulceration of the gastrointestinal (GI) mucosa.^{3,4} The well-known side effects observed after oral administration of NSAIDs have accelerated the development of alternative pharmaceutical forms such as topical cream, gel, and foam formulations that allow local absorption at the inflammation site without adverse systemic reactions.^{5,6}

With topical dosage forms, great attention has been devoted to new formulations that can ensure adequate localization of the drug within the skin to enhance the local effect or increase the penetration through the stratum corneum and viable epidermis for a systemic effect.^{7,8} Polymeric micelles represent a novel type of drug delivery system that has generated much interest in many areas (eg, parenteral, oral, topical, ophthalmic, rectal delivery) because of their unique structure and properties.⁹ In contrast to conventional polymeric micelles, which consist of thermodynamic aggregates of amphiphilic molecules (eg, Pluronic P-85), amphiphilic star-like macromolecules (ASMs) have hydrophilic and hydrophobic segments that are covalently connected. Liu et al have demonstrated that one (individual) ASM behaves as a single micellar entity.¹⁰ Thus, the micelle structure is static rather than dynamic and maintained at all concentrations and in a variety of solvents.^{10,11} ASMs are degradable, highly branched, amphiphilic polymers with a hydrophobic core of alkyl chains surrounded by a hydrophilic surface layer of Poly(ethylene glycol) (PEG) (**Figure 1**). The hydrophobic core serves

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Figure 1. Structure of amphiphilic star-like macro-molecules.

as a reservoir for an insoluble drug, whereas the hydrophilic shell composed of PEG is responsible for micelle stabilization, aqueous solubility, and interactions with plasmatic proteins and cell membranes.^{8,9} In addition, ASMs possess a number of features that are advantageous for the topical route of drug administration, such as small particle size (30-50 nm), which enables close contact to the stratum corneum, and the ability to encapsulate and solubilize drug molecules hence controlling drug permeation into the skin.^{10,12}

The main objective of this study was to evaluate the ASMs as novel carriers for topical drug delivery of 3 NSAIDs: indomethacin (Ind), piroxicam (Pir), and ketoprofen (Ket). Furthermore, the ASMs were compared with PEG and a block copolymer micelle (Pluronic P-85) for percutaneous permeation.

MATERIALS AND METHODS

Materials

Indomethacin, ketoprofen, piroxicam, trifluoroacetic acid (TFA), diethyl ether, and phosphate buffer tablets were purchased from Sigma-Aldrich (St Louis, MO). Pluronic P-85 was a gift from BASF Corporation (Mount Olive, NJ). Amino-terminated PEG (MW 5000) was obtained from Shearwater Polymers (Huntsville, AL). The ASM, Core(hex)PEG5 with molecular weight of 17 000 Da, was synthesized according to previously described methods.¹⁰ Purified water, metha-

nol, ethanol (70 vol%), and acetonitrile were high-performance liquid chromatography (HPLC) grade and purchased from Fisher Scientific (Atlanta, GA).

Drug Encapsulation into ASMs

Drug-loaded ASMs were prepared using a coprecipitation technique.¹³ Briefly, ASMs and the drug (indomethacin, ketoprofen, or piroxicam) were dissolved in a 1:1 mixture of methylene chloride and methanol and then precipitated into diethyl ether (1:60). The precipitate was washed twice with diethyl ether, collected, and dried overnight under vacuum. The drug content was assessed by HPLC methods as described below.¹⁴

Sample Preparation

The composition of investigated samples is listed in **Table 1**. The concentration of the polymers (ASM, PEG, and Pluronic P-85) was kept constant at 5 wt% for all formulations. The final drug concentration was determined by the maximum encapsulation efficiency of the ASM; all formulations containing indomethacin, piroxicam, and ketoprofen were maintained at 0.7, 0.4, and 1.2 wt%, respectively.

Ind-ASM, Pir-ASM, and Ket-ASM formulations were prepared by dissolving corresponding drug-loaded ASMs in water. Formulations containing PEG and Pluronic P-85 were prepared by dissolving the polymer in water, followed by addition of the appropriate amount of the drug. Control samples were aqueous suspensions (Ind-sus, Pir-sus, and Ket-sus) prepared by simply adding appropriate amounts of drug into the water. All formulations were prepared at room temperature (25°C ± 2°C) and stirred at 100 rpm for 10 minutes.

HPLC Analytical Methods

HPLC analysis was performed using a Hewlett-Packard 1100 with an auto sampler equipped with a quaternary pump and variable-wavelength detector. All samples were analyzed using a reverse phase C18 column (Microsorb-MV C18 15 cm, 5 µm). The mobile phase ratio was acetonitrile-water (0.1% TFA) 80:20, and flow rate was 1 mL/min. Indomethacin was detected at 320 nm, piroxicam at 340 nm, and ketoprofen at 264 nm.^{15,16}

Table 1. Composition of the Investigated Samples*

Ingredients (wt%)	Ind-sus	Ind-ASM	Ind-PEG	Ind-plur	Pir-sus	Pir-ASM	Pir-PEG	Pir-plur	Ket-sus	Ket-ASM	Ket-PEG	Ket-plur	ASM-pt	PEG-pt
Indomethacin	0.7	-	0.7	0.7	-	-	-	-	-	-	-	-	-	-
Piroxicam	-	-	-	-	0.4	-	0.4	0.4	-	-	-	-	-	-
Ketoprofen	-	-	-	-	-	-	-	-	1.2	-	1.2	1.2	-	-
Ind-loaded ASMs	-	5.0	-	-	-	-	-	-	-	-	-	-	-	-
Pir-loaded ASMs	-	-	-	-	-	5.0	-	-	-	-	-	-	-	-
Ket-loaded ASMs	-	-	-	-	-	-	-	-	-	5.0	-	-	-	-
Pluronic P-85	-	-	-	5.0	-	-	-	5.0	-	-	-	5.0	-	-
PEG	-	-	5.0	-	-	-	5.0	-	-	-	5.0	-	-	5.0
ASMs	-	-	-	-	-	-	-	-	-	-	-	-	5.0	-
Water to	100	100	100	100	100	100	100	100	100	100	100	100	100	100

*ASM indicates amphiphilic star-like macromolecule; Ind, indomethacin; Ket, ketoprofen; PEG, poly(ethylene glycol); Pir, piroxicam; plur, Pluronic P-85; pt, pretreatment; and sus, suspension.

Solubility Measurements

Drug solubility was measured using previously described methods of Fini et al.¹⁷ Briefly, samples (**Table 1**) were prepared as described above and agitated for 24 hours at 37°C. After this period, the samples were centrifuged (model EBA 12, Hettich, Tuttlingen, Germany) at 3500 rpm for 15 minutes. The supernatants were filtered using a 0.45 µm poly(tetrafluoroethylene) (PTFE) filter (Whatman, Atlanta, GA) and analyzed by HPLC methods described above. All experiments were repeated 3 times. Statistical analysis of the data was performed using analysis of variance (ANOVA, $\alpha = 0.05$) followed by a Dunnett's test if ANOVA indicated that a difference existed.

Skin Permeation Studies

In Vitro Percutaneous Absorption

Male hairless mice, 8 weeks old, SKH1 *br/br* were obtained from Charles River Laboratories (Wilmington, MA). Permeation of NSAIDs from the formulations was evaluated using modified Franz diffusion cells (Permeager, Riegelsville, PA), each having a receptor volume of 5.1 mL and diffusion area of 0.64 cm².^{18,19} Pieces of full-thickness, male, hairless mouse skin (2 cm²) were mounted between donor and receptor compartments of the Franz diffusion cells and allowed to equilibrate for 1 hour.²⁰ The receptor compartment was filled with phosphate buffer (pH 7.4)

and continuously stirred at 600 rpm to ensure uniform distribution and to maintain sink conditions. The temperature of the entire diffusion cell assembly was maintained at 37°C ± 0.5°C using a recirculating water jacket.^{18,21} The permeation studies were conducted using the infinite dose technique in which a large excess of permeant is applied to the skin in comparison to the amount permeating, so that an approximately constant concentration of the permeant is maintained in the donor compartment during the entire course of the experiment.^{22,23}

Five hundred microliters of drug formulation (control, drug-ASMs, drug-PEG, or drug-plur) was applied on the skin surface, and the cell donors were occluded with polyethylene. For the skin pretreatment (pt) studies, 500 µL of either ASM-containing solution (ASM-pt) or PEG-containing solution (PEG-pt) was applied for 60 minutes. Following 1 hour of exposure (ie, pretreatment), the ASM- or PEG-containing solution was removed from the donor compartment and replaced with 500 µL of drug aqueous suspension (Ind-sus, Pir-sus, or Ket-sus), and the cell donors were occluded with polyethylene. At predetermined time intervals, 300-µL samples were withdrawn from each receptor compartment and replenished with the same volume of fresh isotonic buffer after each sampling.^{22,23} Each experiment was repeated 4 times, and receptor samples were frozen at -30°C prior to HPLC analysis.^{19,23}

Data Analysis

Drug permeation was measured over 24 hours, and the data were plotted with the cumulative corrected amounts of drug (Q_{24} ; $\mu\text{g}/\text{cm}^2$) that permeated through the skin over time. The slope of the linear portion of the curve provided maximum flux values at steady state (flux; $\mu\text{g}/\text{cm}^2/\text{h}$). The permeation-enhancing activities were expressed as enhancement ratios of flux (ER flux) and enhancement ratios of cumulative amounts (ER Q_{24}), which were calculated using the following equations^{24,25}:

$$\text{ER flux} = \frac{A}{B} \quad (1)$$

Where A is the flux calculated from the sample, and B is the flux calculated for the control (aqueous suspension).

$$\text{ER } Q_{24} = \frac{C}{D} \quad (2)$$

Where C is Q_{24} calculated from the sample, and D is Q_{24} calculated from the control (aqueous suspension).

Controls were assigned the value of 1.00 and data are presented as the mean $\pm$ SD of 4 experiments. Statistical analyses of the effects of aqueous formulations on percutaneous permeation of NSAIDs were performed using 1-way ANOVA ($\alpha = 0.10$). A 2-sided Dunnett's test was followed if the ANOVA indicated that a difference existed.

In Vitro Release

Drug release studies from ASM formulations were performed using the same procedure as the skin percutaneous absorption studies. As controls, aqueous suspensions of model drugs (indomethacin, piroxicam, or ketoprofen) were used. Before the experiment, cellulose acetate membranes with molecular weight (MW) cut-off 10 000 (Spectrum, Laguna Hills, CA) were soaked in receptor medium for 12 hours. The membranes were blotted between 2 pieces of filter paper and then mounted between donor and receptor compartment of Franz diffusion cells.^{26,27} Each experiment was repeated 4 times. The data were expressed as mean $\pm$ SD and compared for statistical significance using a *t* test ($\alpha = 0.05$).

RESULTS

Drug Encapsulation Efficiency

Liu et al demonstrated that hydrophobic drugs can be physically encapsulated inside the hydrophobic core of ASMs using the precipitation and dialysis bag method.^{10,12} In this article, we describe a coprecipitation technique to encapsulate NSAIDs into the ASMs. In this process, the drug is encapsulated when ASMs collapse during the precipitation step. Drug loading was determined by dissolving the drug-polymer complexes in methanol, hence completely releasing all encapsulating drug, and then measuring the drug content by HPLC.⁹ The drug loading was calculated as follows:

$$\text{Drug loading} = \left(\frac{\text{mass of encapsulated drug}}{\text{initial mass of polymer}} \right) \times 100 \quad (3)$$

Drug loading studies clearly demonstrated that sparingly soluble drugs such as indomethacin, piroxicam, and ketoprofen could be incorporated into the hydrophobic interior of the ASMs. Drug loading for Ind-ASM was 14.1 ± 0.7 wt%; Pir-ASM, 8.0 ± 2.1 wt%; and Ket-ASM, 24.4 ± 1.5 wt%. Each drug evaluated belongs to a different chemical group (**Figure 2**): indomethacin is an aryl acetic acid derivative; ketoprofen belongs to the related arylpropionic acids; and piroxicam belongs in the oxycam group.²⁸

Solubility Measurements

A distinguishing feature of micelle systems is their ability to enhance the solubility of compounds that otherwise have low solubility in pure solvent (typically water for medical applications). This phenomenon occurs because the core of the micelle, which is incompatible with water, provides a more suitable microenvironment for the solubilize. Consequently, the extent of dissolution of the solubilize in micelle solution is enhanced when compared with that in the pure solvents.²⁹

The solubilities of indomethacin, piroxicam, and ketoprofen in investigated samples are listed in **Table 2**. Generally, addition of PEG (5 wt%), Pluronic P-85 (5 wt%), and ASMs (5 wt%) to drug-containing solutions increased the drug's solubility in water ($P < .05$). By addition of the micelle-forming Pluronic P-85, indomethacin solubility was increased 1.7-fold; piroxicam, 1.3-fold; and ketoprofen, 1.2-fold, whereas with addition of hydrophilic PEG, solubility was increased 1.5-fold, 1.4-fold, and 1.0-fold, respectively. Aqueous solubility of indomethacin was most pronounced (2.1-

Figure 2. Chemical structures of indomethacin, ketoprofen, and piroxicam.

fold) upon encapsulation into the ASMs ($P < .05$). Negligible increases in solubility upon ASM encapsulation were observed for ketoprofen (1.0-fold) and piroxicam (1.0-fold).

In Vitro Release Studies

When drugs are topically applied, the pharmacologically active agent must be released from its carrier (ie, delivery vehicle) before it can contact the epidermal

Table 2. Solubility of NSAIDs in Various Formulations*

Sample	Solubility ($\mu\text{g/mL}$)
Ind-sus	9.7 ± 0.4
Ind-PEG	14.5 ± 0.6
Ind-plur	16.9 ± 0.4
Ind-ASM	20.5 ± 0.3
Pir-sus	30.2 ± 0.7
Pir-PEG	42.3 ± 1.7
Pir-plur	40.1 ± 1.5
Pir-ASM	30.8 ± 1.3
Ket-sus	113 ± 9.0
Ket-PEG	116 ± 4.0
Ket-plur	136 ± 7.0
Ket-ASM	116 ± 3.0

*ASM indicates amphiphilic star-like macromolecule; Ind, indomethacin; Ket, ketoprofen; NSAIDs, nonsteroidal anti-inflammatory drugs; PEG, poly(ethylene glycol); Pir, piroxicam; plur, Pluronic P-85; and sus, suspension. Solubility values are expressed as mean $\pm$ SD.

surface and permeate through the stratum corneum and lower layers of the skin. Thus, a topical formulation is a fairly complex drug delivery system; the dynamics of drug release from a vehicle have been a subject of debate and investigation for many years.³⁰ A simple and reproducible method, generally applicable to all topical formulations, has been developed to measure in vitro release of the drug from a delivery vehicle using a diffusion cell and synthetic membrane.³¹ This methodology employs a static diffusion cell system, commonly referred to as a Franz cell, a commercially available cellulose acetate synthetic membrane, and an aqueous receptor phase.^{32,33} To determine in vitro drug release, an infinite amount of drug was applied on the donor chamber. Aliquots of the receptor media were removed after specific time intervals and analyzed, and the cumulative amount of drug released (expressed in $\mu\text{g/cm}^2$; Q_{24}) was plotted against time.^{33,34} The release profiles for all 3 drugs are shown in **Figures 3-5**, whereas the release parameters are provided in **Table 3**.

Compared with the control (aqueous suspension), drugs encapsulated within the ASMs were released more slowly during the 24-hour experiment (**Figures 3-5**). It is well known that drug release rates depend on the total drug concentration in the topical formulations. For

Figure 3. Release profiles of indomethacin from Ind-sus and Ind-ASM formulations (n = 4).

Figure 4. Release profiles of piroxicam from Pir-sus and Pir-ASM formulations (n = 4).

Figure 5. Release profiles of ketoprofen from Ket-sus and Ket-ASM formulations (n = 4).

example, higher drug loading decreases the diffusion rate up to 50%, whereas lower drug loading leads to the formation of drug-enriched shell particles that promote faster release.^{35,36} The enhancement ratios (**Table 3**) were lowered for drugs encapsulated within the ASMs: indomethacin-loaded and piroxicam-loaded ASMs have ER flux and ER Q_{24} values in the range of 0.31 to 0.33. For ketoprofen, the effect of ASMs was even more pronounced, ER flux and ER Q_{24} values were significantly lowered (~ 0.10).

In Vitro Skin Permeability Studies

Percutaneous Absorption of Indomethacin

The permeation profiles of indomethacin from all investigated formulations are shown in **Figure 6** as the cumulative amount of indomethacin that permeated across full-thickness skin over time. The profiles represent an average of 4 experiments for each sample and are compared with the permeation of indomethacin from the control sample (Ind-sus).

Indomethacin slowly but continuously permeated through the skin over the 24-hour time period for each sample. Permeation profiles from all formulations except Ind-plur were lower than that of the indomethacin control. **Table 4** summarizes the effects of various delivery vehicles on the permeation parameters of the investigated drugs, including indomethacin. Indomethacin flux through the skin increased in formulation Ind-plur (1.63-fold), whereas other formulations decreased indomethacin flux compared with the control ($P < .10$). The increase in Q_{24} was also observed from the Ind-plur (1.57-fold) formulation, whereas all other formulations displayed decreased Q_{24} relative to the control ($P < .10$). In addition, pretreatment of the skin with PEG and ASM solutions before drug exposure decreased both Q_{24} and flux of indomethacin through the skin ($P < .10$).

Percutaneous Absorption of Piroxicam

Figure 7 shows the permeation profiles of piroxicam across full-thickness mouse skin from various delivery vehicles. The permeation parameters obtained from the permeation profiles are listed in **Table 4**. As displayed in **Figure 7**, all formulations decreased both piroxicam flux and Q_{24} compared with the control ($P < .10$). Pretreatment of the skin with ASM solutions resulted in a decrease in both flux and Q_{24} of piroxicam ($P < .10$), whereas pretreatment with PEG-containing solution decreased Q_{24} ($P < .10$) but did not have a statistically significant effect on piroxicam flux ($P > .10$).

Table 3. Release Parameters of the Investigated Samples*

Sample	Flux† ($\mu\text{g}/\text{cm}^2/\text{h}$)	ER Flux	Cumulative Amount† ($\mu\text{g}/\text{cm}^2$)	ER Q_{24}
Ind-sus	138 ± 1	1.00	3230 ± 445	1.00
Ind-ASM	43.2 ± 4.4	$0.313 \ddagger$	1070 ± 75	0.331
Pir-sus	250 ± 24	1.00	5790 ± 560	1.00
Pir-ASM	81.3 ± 37.9	$0.325 \ddagger$	1910 ± 160	0.329
Ket-sus	714 ± 115	1.00	17000 ± 2300	1.00
Ket-ASM	43.9 ± 10.9	$0.0615 \ddagger$	1150 ± 260	0.0676

* ASM indicates amphiphilic star-like macromolecule; ER, enhancement ratio; ER Q_{24} , enhancement ratio of cumulative amounts; Ind, indomethacin; Ket, ketoprofen; Pir, piroxicam; and sus, suspension.

†Mean $\pm$ SD.

‡Statistical significance according to paired analysis.

Figure 6. Permeation profiles of indomethacin from the investigated samples ($n = 4$).

Percutaneous Absorption of Ketoprofen

The permeation profile for ketoprofen across full-thickness mouse skin, as compared with the control sample (Ket-sus) is shown in **Figure 8**. Similar to the results from the permeation studies of indomethacin and piroxicam, the permeation profiles of the ketoprofen formulations showed that the total amount of ketoprofen permeated is significantly lower for formulations of Ket-PEG and Ket-ASM samples ($P < .10$) and slightly higher for Ket-plur formulation.

ER flux values show that Ket-plur formulations enhance ketoprofen flux by 1.10-fold ($P < .10$), whereas all other formulations decrease ketoprofen flux compared with the control ($P < .10$). Similarly, the increase in the Q_{24} was also observed from Ket-plur (1.09-fold) formulation, whereas all other formulations have de-

creased Q_{24} relative to the control ($P < .10$). The results from pretreatment studies revealed that pretreatment of the skin with ASM solutions decreased both flux and Q_{24} of ketoprofen ($P < .10$), whereas pretreatment with the PEG-containing solution decreased Q_{24} ($P < .10$) but had no statistically significant effect on ketoprofen flux ($P > .10$).

DISCUSSION

The overall efficacy of a topical formulation depends on the release characteristics of the delivery vehicle and the pharmacokinetics of the drug as it diffuses through the skin. The driving force for transport is the chemical potential gradient, and within most single-phase systems, this force reduces to the concentration gradient.³⁶ In addition, the penetration or bioavailability rate of topically applied drugs is dependent on the thermodynamic activity of the drug in the vehicle if specific penetration enhancers are absent.³⁷ However, in formulations in which penetration enhancers are used, penetration enhancement and thermodynamic effects are both expected to influence drug flux.³⁸ Only aqueous suspensions lead to equivalent, or in this case, maximum thermodynamic activity.^{36,39} Therefore, suspensions were used in this study to maintain equal or maximum thermodynamic activity. Changes in the vehicle composition usually alter the drug solubility and, therefore, its thermodynamic activity. However, if drug concentrations above the solubility limit are used, then the concentration of unassociated, nonionized drug in solution remains constant; therefore, the thermodynamic activity remains constant and reaches its maximum.^{40,41} Thermodynamic activities of the 3 drugs used in this study were calculated (data not shown) and

Table 4. Effect of the Investigated Samples on Percutaneous Parameters of NSAIDs*

Sample	Flux [†] (μg/cm ² /h)	ER Flux	Q ₂₄ [†] (μg/cm ²)	ER Q ₂₄
Ind-sus	0.5 ± 0.2	1.00	11.8 ± 4.8	1.00
Ind-ASM	0.4 ± 0.1	‡0.78	10.3 ± 1.9	0.87
Ind-PEG	0.2 ± 0.1	‡0.43	5.8 ± 0.8	0.49
Ind-plur	0.9 ± 0.3	‡1.63	18.6 ± 6.0	1.57
Ind-ASM-pt	0.4 ± 0.2	‡0.79	9.6 ± 4.3	0.81
Ind-PEG-pt	0.2 ± 0.1	‡0.46	8.5 ± 4.2	0.72
Pir-sus	0.8 ± 0.4	1.00	27.6 ± 9.6	1.00
Pir-ASM	0.6 ± 0.2	‡0.78	20.2 ± 5.8	0.73
Pir-PEG	0.3 ± 0.1	‡0.30	15.9 ± 3.9	0.57
Pir-plur	0.3 ± 0.7	‡0.45	23.0 ± 15.8	0.83
Pir-ASM-pt	0.2 ± 0.3	‡0.26	24.3 ± 2.8	0.88
Pir-PEG-pt	0.8 ± 0.2	1.03	17.8 ± 5.6	0.65
Ket-sus	53.9 ± 30.3	1.00	1220 ± 670	1.00
Ket-ASM	15.4 ± 6.9	‡0.28	3480 ± 150	0.28
Ket-PEG	25.0 ± 14.5	‡0.46	7990 ± 470	0.65
Ket-plur	59.3 ± 13.2	‡1.10	1340 ± 290	1.09
Ket-ASM-pt	38.3 ± 12.4	‡0.71	1330 ± 490	1.08
Ket-PEG-pt	52.1 ± 35.2	0.96	1010 ± 260	0.83

* ASM indicates amphiphilic star-like macromolecule; ER, enhancement ratio; ER Q₂₄, enhancement ratio of cumulative amounts; Ind, indomethacin; Ket, ketoprofen; NSAIDs, nonsteroidal anti-inflammatory drugs; PEG, poly(ethylene glycol); Pir, piroxicam; plur, Pluronic P-85; pt, pretreatment; and sus, suspension.

[†] Mean ± SD.

‡Statistical significance according to paired analysis.

shown to be similar, indicating that thermodynamic activity was not a factor in our measurements.

For drug carrier design, it is attractive to obtain a simple polymer that can form stable polymeric micelles with a high efficiency to physically entrap a wide variety of hydrophobic drugs.⁸ Encapsulation studies showed that by utilizing the coprecipitation technique, NSAIDs can be incorporated in the ASMs with a substantial loading level (8.0-24.4 wt%). More polar hydrophobic compounds are more easily incorporated into micelle cores compared with nonpolar compounds.⁴² Of the 3 drugs studied, ketoprofen is more polar than piroxicam and indomethacin. Thus, the highest loading of ketoprofen (24 wt%) may be motivated by its more polar character as well as its lower melting point.

Among the numerous applications of micelle systems, an important advantage is their ability to enhance the

Figure 7. Permeation profiles of piroxicam from the investigated samples (n = 4).

Figure 8. Permeation profiles of ketoprofen from the investigated samples ($n = 4$).

solubility of hydrophobic small molecules by trapping them in the micelle core (if those small molecules are energetically compatible with the monomers of the insoluble block).⁴³ From our solubility results (**Table 2**), drug encapsulation into the ASMs as well as the addition of PEG and Pluronic P-85 increased the drug solubility in aqueous media ($P < .05$), thus improving the diffusible form of the drug species at the skin-vehicle interface. Because of the high MW of polymers, drug-polymer complexes are unlikely to penetrate the skin. Most likely, the drug encapsulated into the core is in rapid dynamic equilibrium with the drug in the aqueous phase, and continuously supplying the drug molecules to the skin surface in a diffusible form.^{44,45} The ability of Pluronic P-85 to encapsulate and solubilize drugs was previously explored.^{46,47} These block copolymers form polymeric micelles above a critical micelle concentration (CMC) (~ 0.03 wt%) in which drug molecules are solubilized in the inner hydrophobic core, and the hydrophilic block forms an outer shell to induce water solubility.⁴⁸ Our studies revealed that addition of Pluronic P-85 caused a statistically significant ($P < .05$) increase in aqueous solubility of all 3 NSAIDs. ASMs were anticipated to have a similar mechanism of action as Pluronic P-85: they encapsulate and solubilize lipophilic drugs within the inner core, while the PEG hydrophilic chain induces water solubility. However, the ASMs do not possess a CMC and therefore can be used in any concentration in solution.^{10,12} Similar to Pluronic P-85 and PEG, ASMs caused a statistically significant ($P < .05$) increase in indomethacin water solubility. From the 3 drugs studied, the most pronounced effect on aqueous solubility was observed for indomethacin.

In vitro release studies demonstrated that drug encapsulation into the ASMs delays drug release ($P < .05$) as

shown in **Figures 3-5**. From the ER flux and ER Q_{24} values, the most pronounced effect of delayed drug release was observed from ketoprofen-loaded ASMs (Ket-ASM). Generally, diffusion through the carrier is the main mechanism of controlled release as described by Fick's law of diffusion.³³ In the case of Ket-ASM, the high drug loading (24.4 wt%) may result in decreased diffusion because too many molecules are trying to diffuse, thus limiting their own permeation (ie, hindering effects).⁴⁹ After drug is released from ASMs, it partitions onto the skin and finally permeates through the skin.⁵⁰ However, the rate of transport across the stratum corneum is dependent on the nature of the solute (eg, size, lipophilicity, ionization, H-bonding ability) as well as the nature of the vehicle, which may determine NSAID release and influence barrier properties of the stratum corneum.^{1,20}

As stated in the introduction, the main objective of this study was to evaluate ASMs as a carrier for topical drug delivery, and to characterize its release mechanism to optimize the formulations. The permeation studies showed that drug encapsulation into the ASMs displayed decreased flux and Q_{24} values for all drugs studied ($P < .10$), indicating that the ASM formulations do not possess the necessary intrinsic activity to dramatically promote skin permeation. The observed decrease in drug permeability may be attributed to the sustained release of NSAIDs from ASMs (**Figures 3-5**) as well as the effect of ASMs on skin conditions.

PEGs are nonionic, water-soluble polymers that are limited by their poor skin permeability and limited sensitivity of detection. Thus, PEGs are not frequently utilized as skin penetration markers except in facilitated transport such as iontophoresis.^{51,52} PEG was included in our experiments, because it represents approximately 80 wt% of the ASMs. The skin permeation studies showed that the PEG formulations decreased both the flux and Q_{24} for all 3 drugs studied ($P < .10$). The condition of the stratum corneum, such as hydration/dehydration, influences percutaneous permeation of various drugs. PEG is known to be hygroscopic, dehydrating the skin upon application. It is generally accepted that most substances are absorbed through the stratum corneum more rapidly if this tissue is in a hydrated state rather than in its dehydrated state.^{21,52} From our results, the PEG formulations have lowered skin permeation potential for all 3 NSAIDs, likely due to skin dehydration.

Pluronic P-85 is a block copolymer that forms polymeric micelles above a CMC. When the concentration drops below CMC, the micelle structure dissociates into free chains, leading to the burst release of en-

trapped drugs.^{48,49} The effect of Pluronic P-85 on percutaneous permeation was evaluated because it has a similar amphiphilic structure to the ASMs. In the skin permeation studies, except for piroxicam, flux values were higher in formulations containing Pluronic P-85 relative to formulations containing PEG and ASM ($P < .10$).

We hypothesized that slower skin permeation of NSAIDs encapsulated within ASMs occurred by 2 mechanisms: (1) ASMs slowed partitioning of the drug from the ASM core to the ASM shell, and (2) PEG chains comprising the ASM shell were dehydrating the stratum corneum. To clarify the mechanism of action of ASMs on the skin, the skin was first pretreated with an ASM-containing aqueous solution, then the NSAID was applied onto the skin and percutaneous permeation of the NSAIDs was observed.⁵³ Pretreatment of the skin with ASM solutions for 1 hour decreased the flux of 3 drugs studied ($P < .10$) and Q_{24} of the indomethacin and piroxicam (Table 4). Skin was also pretreated with aqueous solutions of PEG to elucidate whether PEG has a similar effect on NSAID skin permeation following pretreatment with ASMs. Pretreatment of full-thickness skin with PEG solutions generally had similar effects on NSAID permeation as pretreatment with ASM solution. Overall, skin pretreatment with ASM solutions decreased flux of all 3 drugs studied and Q_{24} values for indomethacin and piroxicam, indicating some possible effect of ASMs on skin structure and/or condition.^{53,54}

The influence of skin pretreatment with ASM solutions before drug application is similar to the observed permeation of drug that was encapsulated within the ASMs. Thus, the influence of ASMs on drug permeation through full-thickness skin is likely a combination of their sustained-release capability and dehydrating effect on the skin. Despite numerous studies highlighting the advantages of vesicular systems as drug carriers in skin, little is known about the mechanisms by which the permeation of vesicular lipophilic and hydrophilic drugs is promoted or decreased, and how the vesicle structure affects the mechanism. Intact vesicles or vesicle constituents are mainly confined to the horny layer and do not penetrate deeper but still influence the penetration of lipophilic drugs.⁵⁵⁻⁵⁷ From the results of our investigations, ASMs appear to have a similar dehydrating effect on the skin as PEGs, but further experiments evaluating the ASM/skin interface will be performed using confocal light microscopy to further establish the mechanism of action.

CONCLUSION

Because of a specific core-shell structure, ASMs have the ability to incorporate sparingly water-soluble NSAID drugs (indomethacin, piroxicam, and ketoprofen) into the core to enhance the drugs' aqueous solubility ($P < .05$). In vitro release experiments showed that drug release rates were decreased by encapsulation into the ASMs ($P < .05$). Full-thickness skin permeation experiments revealed that ASMs decreased drug permeation through the skin—regardless of the drug used ($P < .10$). PEG formulations also decreased percutaneous permeation of NSAIDs, likely due to their dehydrating effect on the skin. Pluronic P-85 had different effects on percutaneous permeation of NSAIDs than ASMs; for indomethacin and ketoprofen, an increase in flux and Q_{24} was observed, whereas the opposite effect was observed for piroxicam. Skin pretreatment studies indicate that the effect of ASMs on skin permeation may be attributed to the slowed drug partitioning from the ASM core and to the dehydrating capability of the ASM shell. Because encapsulation of the NSAID drugs (indomethacin, piroxicam, and ketoprofen) into the ASMs decreased their ability to permeate the stratum corneum, ASMs are currently under investigation for delaying and/or preventing permeation of compounds (eg, sunscreen agents, N,N-diethyl-m-toluamide [DEET], and chemical warfare agents) through the skin.

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