# Effect of Surfactants on Percutaneous Absorption of Naproxen I: Comparisons of Rabbit, Rat, and Human Excised Skin

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**Abstract** □ The *in vitro* skin penetration model using excised skin from rats, rabbits, and humans was examined to evaluate the relative effect of surfactants on percutaneous absorption of naproxen. Differences in the magnitude of the effect of surfactants in promoting percutaneous absorption of naproxen through skins of different species were observed. For comparative evaluations of the formulation effects on percutaneous absorption, such as the effect of surfactants, *in vitro* studies with animal skin may not provide information in agreement with *in vitro* human skin.

**Keyphrases** □ Absorption, percutaneous—naproxen, effect of various surfactants, rabbit, rat, and human skin compared □ Naproxen—percutaneous absorption, effect of various surfactants, rabbit, rat, and human skin compared □ Surfactants, various—effect on percutaneous absorption of naproxen, rabbit, rat, and human skin compared □ Anti-inflammatory agents—naproxen, percutaneous absorption, effect of various surfactants, rabbit, rat, and human skin compared

Surfactants are important adjuvants in the formulation of vehicles for application to the skin. Nonionic surfactants generally have little effect in promoting percutaneous absorption, although numerous reports (1–5) discussed the influence of nonionic surfactants. Long chain alkyl methyl sulfoxides, which are nonionic surfactants, increased the permeability of guinea pig skin for sodium nicotinate and thiourea (6). Among the anionic surfactants, the laurate ion had the greatest effect on the penetration of other solutes (7, 8).

Excised animal (4, 9–11) and human (7, 8) skins have been used in studying the influence of surfactants on percutaneous absorption of water and organic solutes. A definite and reproducible relationship also exists between the excised skin permeabilities of different species. Rabbit skin is consistently more permeable than that of other rodents, pigs, or humans (12). Because of these differences, studies relating the effect of surfactants on percutaneous absorption may be confusing and sometimes contradictory.

This investigation evaluates the effect of surfactants on percutaneous absorption of naproxen through excised human skin. Cream formulations containing 5% naproxen were used to study the effect of selected surfactants on percutaneous absorption through excised human, rat, and rabbit skins. This study suggests that the data on excised skin of laboratory animals may not provide information in agreement with excised human skin concerning the effect of surfactants on percutaneous absorption of compounds similar to naproxen.

## EXPERIMENTAL

**Materials**—The surfactants hexadecylpyridinium chloride[1], triethylammonium lauryl sulfate[2], octylphenoxypolyoxyethanol[3], dioctyl sodium sulfosuccinate[4], sodium lauryl sulfate[5], sodium laurate[6], polysorbate 60[7], polyoxyethylene 23 lauryl ether[8], and methyldecyl sulfoxide[9] were used as received. Hydroxypropylcellulose[10] and carboxyvinyl polymer[11] were used in the preparation of gels. Thimerosal and glycerin were USP grade.

Nonlabeled naproxen[12] was at least 99% pure. The tritiated naproxen was purified by radiochromatography and was at least 98% pure. The two solvent systems used were hexane–ethyl acetate (85:15) and benzene–tetrahydrofuran–acetic acid (90:9:3). All other chemicals were analytical reagent grade unless otherwise indicated.

**Preparation of Vehicles**—The aqueous gels containing 1% hydroxypropylcellulose were prepared by dispersing the polymer in 5% glycerin and adding it to the aqueous portion containing 0.5% dissolved naproxen. The pH of the solution was adjusted to 6.5. The carboxyvinyl polymer (0.5%) gel was prepared by dispersing the polymer and naproxen in water, adding an equimolar quantity of sodium hydroxide, and adjusting the pH to 6.5.

The cream vehicles were prepared by dissolving 5% naproxen in an equimolar quantity of sodium hydroxide solution. The pH was adjusted to 7.5. The oil phase contained 6% cetyl alcohol, 6% stearyl alcohol, 3% mineral oil, and 4% polysorbate 60. The aqueous and the oil phases were heated to about 70°, and the aqueous phase was added to the oil phase with appropriate stirring. After the formation of the emulsion, the stirring was continued until the temperature of the cream reached 30°.

**Excised Human Skin Studies**—Whole abdominal skin, obtained at autopsy, was frozen on a glass plate with the epidermal surface flat in contact with the plate. Just prior to an experiment, the frozen skin was dislodged by warming the plate with water. A layer of skin, 0.76 mm thick, was removed[13] from the abdominal side. This procedure allowed removal of the subcutaneous fat without contamination of the intact epidermal surface.

Circular sections having a diameter of 2.2 cm were cut. The dermal side of each section was blotted with tissue[14] paper and then positioned between the two polytef disks of the diffusion cell. The cell design and operation were the same as those reported by Coldman *et al.* (13), except that the exposed surface area for penetration had a diameter of 1.4 cm and an area of 1.54 $cm^2$. The dose of formulation per cell was 1.4 ml. To prevent evaporation, a glass coverslip was placed over the vehicle and sealed with silicone grease.

A polytef-coated stirring bar attached to a polyethylene sail provided efficient mixing in the sampling chamber, which contained approximately 12 ml of 0.01 *M* phosphate buffer at pH 7.4 with 0.02% thimerosal. The sampling arm was stoppered with a cork to prevent evaporation. The samples (1 ml for radiochemical assay and 3 ml for the spectrophotometric assay) were withdrawn at appropriate times, and the sample volume was replaced by a fresh solution.

By the spectrophotometric method, the absorbance of the samples was recorded at 342 nm. The radioactive counts were measured by a liquid scintillation counter[15]. The composition of the scintillation fluid was 1.4 liters of scintillation grade toluene[16], 1.4 liters of spectrograde *p*-dioxane[1],

[1] Matheson, Coleman & Bell, Norwood, Ohio.
[2] Alcolac Chemical Corp., Baltimore, Md.
[3] Triton X-45, Rohm & Haas Co., Philadelphia, Pa.
[4] American Cyanamid Co., Pearl River, N.Y.
[5] E. I. du Pont de Nemours & Co., Menlo Park, Calif.
[6] Eastman Kodak Co., Rochester, N.Y.
[7] Tween 60, ICI America Inc., Atlas Chemical Division, Wilmington, Del.
[8] Brij 35, ICI America Inc., Atlas Chemical Division, Wilmington, Del.
[9] Institute of Organic Chemistry, Syntex Research, Palo Alto, Calif.
[10] Klucel HF, Hercules Inc., Wilmington, Del.
[11] Carbomer 940, B. F. Goodrich.
[12] *d*-2-(6′-Methoxy-2′-naphthyl)propionic acid, Syntex Research, Palo Alto, Calif.
[13] Model B Dermatome, Paddet-Hood, Division of Kansas City Assemblage Co., Kansas City, MO 64111.
[14] Kimwipes disposal wipers, Kimberly Clark Corp., Neenah, WI 54956.
[15] Unilux II, Nuclear-Chicago, Chicago, IL 60600.
[16] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.



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**Table I—Effect of Surfactants on Naproxen Flux through Excised Human Abdominal Skin from Aqueous Gels**

| Surfactant (Concentration) | Mean Flux, $\mu g/cm^2/hr$ | *SD* | Relative Flux |
|---|---|---|---|
| Experiment 1 | | | |
| No added surfactant (control) | 2.82 | 0.41 | 1.0 |
| Hexadecylpyridinium chloride (0.5%) | 1.26 | 0.22 | 0.44 |
| Triethylammonium lauryl sulfate (2%) | 2.00 | 0.81 | 0.71 |
| Octylphenoxypolyethoxy 5 ethanol (2%) | 2.30 | 0.24 | 0.82 |
| Dioctyl sodium sulfosuccinate (0.05%) | 2.94 | 0.21 | 1.04 |
| Experiment 2 | | | |
| No added surfactant (control) | 1.26 | 0.58 | 1.0 |
| Sodium lauryl sulfate (4%) | 10.45 | 0.18 | 8.29 |
| Sodium laurate (4%) | 3.17 | 1.29 | 2.51 |
| Polysorbate 60 (4%) | 1.22 | 0.11 | 0.97 |
| Polyoxyethylene 23 lauryl ether (4%) | 1.04 | 0.18 | 0.83 |

0.84 liter of methanol[17], 18.2 g of 2,5-diphenyloxazole[18], 0.364 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene[18], 219.2 g of naphthalene[16], and 0.350 g of 2,6-di-*tert*-butyl-4-methylphenol[19].

**Excised Rat Skin Studies**—Male Sprague–Dawley rats[20], 250–300 g, were employed. Hair was removed[21] between the forelegs and hindlegs on both sides of the spine 1 day prior to the experiment. The rats were killed by exposure to carbon dioxide generated in a chamber from dry ice and water. A 5 × 6-cm piece of skin was excised, and the connective tissue below the dermis was removed. Circular sections having a diameter of 2.21 cm were cut and used in the skin cell. The rest of the procedure was exactly the same as that used for the human skin studies.

**Excised Rabbit Skin Studies**—Male albino rabbits[22], ~2 kg, were employed. Hair was removed[21] between the forelegs and hindlegs on both sides of the spine 1 day prior to the experiment. The rabbits were killed with an intraperitoneal injection of 2 ml of pentobarbital[23], and a 7 × 8-cm piece of skin was excised. The connective tissue below the dermis was removed. Circular sections having a diameter of 2.21 cm were cut and used in the skin cell. The rest of the procedure was exactly the same as that used for the human skin studies.

## RESULTS

The effect of surfactants on percutaneous absorption of naproxen through excised human skin was studied using a simple aqueous gel system. Four skin cells, taken from the same piece of skin, were used for the control and for each surfactant under study. The results of these experiments are given in Table I. For comparison, the mean flux of control in each experiment was taken as one, and the relative flux was then calculated. The concentration of all surfactants was above the critical micelle concentration (CMC).

The effect of nonionic, cationic, and amphoteric surfactants on the relative flux of naproxen was small. The lower relative flux with certain surfactants may be due to the lowering of the thermodynamic activity of naproxen by complexation or other interactions with the skin. Above the CMC, proportionately less surfactant is available for interaction. The anionic surfactants, sodium laurate and sodium lauryl sulfate, increased the *in vitro* flux of naproxen appreciably.

Similar experiments were conducted to study the influence of two surfactants, sodium lauryl sulfate and methyldecyl sulfoxide, at different concentrations. The mean *in vitro* flux was used to calculate the relative flux similarly. The relative flux *versus* surfactant concentration plots are given in Fig. 1. A large increase in flux of naproxen was seen up to 0.2% methyldecyl sulfoxide, above which the relative flux essentially remained constant. This effect appears to be limited by the surfactant solubility in aqueous gel. The effect of sodium lauryl sulfate on the *in vitro* flux of naproxen was less pronounced compared to methyldecyl sulfoxide and appeared linear in the concentration range studied.

The results of the influence of surfactants on excised human skin in a simple aqueous gel vehicle indicated that polysorbate 60 at 4% did not change the *in vitro* flux of naproxen (Table I). For studying selected surfactants from oil-in-water cream vehicles, it was assumed that polysorbate 60 did not alter the *in vitro* flux of naproxen by interaction with the surfactant under study. Sodium lauryl sulfate, sodium laurate, and methyldecyl sulfoxide were studied using excised human, rat, and rabbit skins.

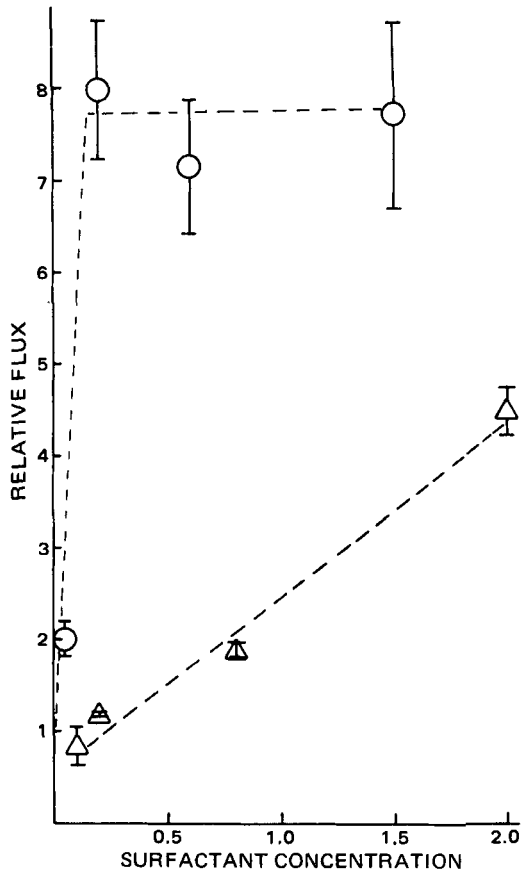


**Figure 1**—*Effect of surfactant concentration on the* in vitro *relative flux of naproxen (micrograms per milliliter per centimeter hour) through excised human skin from aqueous gels. Key:* ○, *methyldecyl sulfoxide; and* △, *sodium lauryl sulfate. Experimental points are averages of four skin cells, and vertical bars are standard deviations.*

The *in vitro* mean flux of naproxen through excised human, rat, and rabbit skins is given in Table II. The *in vitro* mean flux of the control experiments indicates that the excised human skin was the least permeable and that the excised rabbit skin was most permeable to naproxen. These results are in agreement with the results reported earlier for other solutes (12). Another important difference in the three types of excised skin was in the lag time. The lag time for the human skin was approximately 100 hr; for the rat and rabbit skins, it was about 14 hr. Although excised rat skin was less permeable than excised rabbit skin, the lag time was similar.

For comparing the effect of surfactants on percutaneous absorption through excised human, rat, and rabbit skins, flux relative to the control is given in Table II and Fig. 2. Excised human skin, which was least permeable in control experiments, showed a larger increase in the *in vitro* relative flux due to the presence of these surfactants. On the other hand, excised rabbit skin, which was most permeable in control experiments, showed a moderate increase in the relative flux of naproxen when creams containing these surfactants were tested. The excised rat skin showed a relatively small increase in naproxen flux from creams containing the same surfactants. For example, the influence of 1% methyldecyl sulfoxide on excised human skin in increasing the relative flux was approximately 10-fold, compared to a fourfold increase on excised rabbit skin and only about a 50% increase on excised rat skin.

## DISCUSSION

Excised animal skin has been used in studies on percutaneous absorption of drugs and other solutes, possibly because of its ready availability, although it differs significantly from human skin. The skin of

[17] Mallinckrodt Chemical Works, St. Louis, MO 63100.
[18] Arapahoe Chemicals, Division of Syntex Corp., Boulder, CO 80301.
[19] Aldrich Chemical Co., Milwaukee, WI 53210.
[20] Simonsen Laboratories, Gilroy, CA 95020.
[21] Model-A2 animal electric clipper with size 40 blade, John Oster Manufacturing Co., Milwaukee, WI 53200.
[22] L.I.T. Rabbitery, Aptos, CA 95003.
[23] Euthenol.

**Table II—Effect of Surfactants on the *In Vitro* Flux of Naproxen through Excised Human, Rat, and Rabbit Skins from an Oil-in-Water Cream Formulation**

| Surfactant (Concentration) | Excised Human Skin | | | Excised Rat Skin | | | Excised Rabbit Skin | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean Flux, $\mu g/cm^2/hr$ | *SD* | Relative Flux | Mean Flux, $\mu g/cm^2/hr$ | *SD* | Relative Flux | Mean Flux, $\mu g/cm^2/hr$ | *SD* | Relative Flux |
| No added surfactant (control) | 1.66 | 0.49 | 1 | 3.75 | 0.60 | 1 | 5.86 | 0.55 | 1 |
| Sodium lauryl sulfate (2%) | 5.12 | 1.86 | 3.09 | 4.91 | 0.79 | 1.31 | 10.18 | 4.78 | 1.74 |
| Sodium laurate (2%) | 7.32 | 3.69 | 4.41 | 6.53 | 0.18 | 1.74 | 20.01 | 9.00 | 3.41 |
| Methyldecyl sulfoxide (1%) | 17.18 | 4.32 | 10.35 | 5.47 | 0.54 | 1.46 | 24.11 | 10.34 | 4.11 |

rodents lacks sweat glands and abounds in hair and hair follicles in contrast to human skin. The thickness of the stratum corneum also varies from species to species. In addition, the absorption of medicaments by way of sweat ducts of human skin has been reported to be negligible as compared to the transepidermal routes (14–16).

The effect of surfactants on percutaneous absorption of several solutes using excised human and animal skin was reported previously (17). Anionic surfactants, sodium laurate and sodium lauryl sulfate, caused continuous damage to the stratum corneum as long as the surfactant solutions remained in contact (17). These surfactants were thought to bind strongly with $\alpha$-protein, thus causing a reversible denaturation and an uncoiling of the filaments. Membrane expansion, likely hole formation, and loss of water binding capacity are consistent with the reversible $\alpha \rightleftarrows \beta$ conversion of keratin, induced by the strong binding of the surfactant with the protein (18).

Several methods have been used to study the dermatitic effect of nonionic surfactants on rabbit skin (19–22). These studies suggested that nonionic surfactants change the content, composition, and biosynthesis rate of epidermal phospholipids. These results probably indicate changes in epidermal membrane structure since phospholipids are major components of biological membranes.

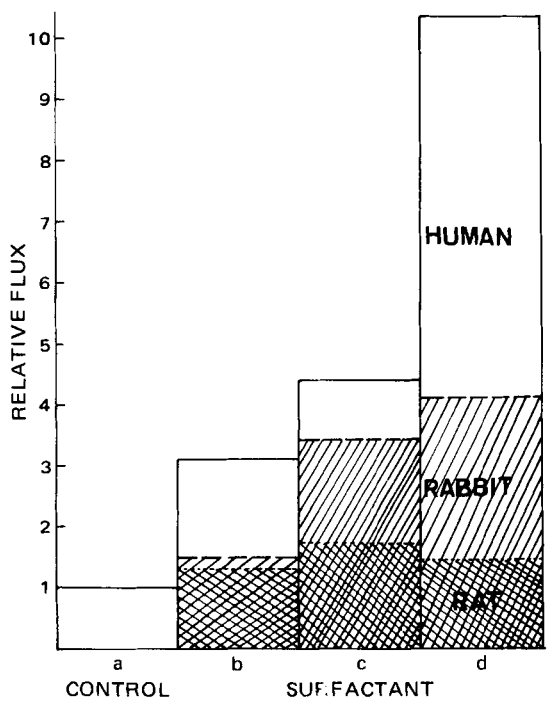


**Figure 2**—*Relative flux of naproxen through excised human, rabbit, and rat skins, showing the effect of surfactants. Key: a, control; b, 2% sodium lauryl sulfate; c, 2% sodium laurate; and d, 1% methyldecyl sulfoxide.*

The manner in which methyldecyl sulfoxide increases skin permeability is not known. The exact mechanism by which nonionic and anionic surfactants induce permeability changes may differ considerably. However, the net results are damage or disruption of the membrane and permeability changes. Although the mechanism by which a certain class of surfactants changes the permeability of organic solutes may be similar for skins of different species, the magnitude of morphological changes due to damage or disruption of the skin may be different for skins of different species. The results of this study indicate that the excised skin from laboratory animals may lead to erroneous conclusions regarding the effect of surfactants on excised human skin in altering percutaneous absorption of compounds similar to naproxen.

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