



The diffusion barrier within the unhairing process

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Hair-saving processes for bovine hides are being applied in tanneries in several countries to replace traditional hair burning systems. The factors that are taken into account when using this unhairing technique are:

- the need for a pelt that is suitable for the production of different types of leather
- a reduction in the effluent load arising from the beamhouse
- the recovery of the partially degraded hair as a solid waste with potential industrial applications

It is well known that the organic matter, the sulfide ion content and the presence of suspended solids in the liquid effluent is greatly dependant on the unhairing process employed. Within the sulfide unhairing system there are problems associated with toxicity, odour and effluent treatment.

Hydrogen sulfide is a highly toxic gas, and can be liberated from within the unhairing process and from effluent treatment. In extreme cases it has led to the death of workers, and in the wider environment creates an odour within the tannery surroundings which causes associated complaints. The acceptable maximum concentration in the work environment, in which a worker can remain for 8 hours/day and 5 days/a week, is 10 cm³ H₂S/m³ (15 mg/m³) without suffering physical damage.

The enzymatic removal of intact hair offers a suitable alternative to destructive sulfide unhairing. However, interest in the enzyme unhairing process comes and goes. This is because of difficulties in managing the process, mainly because the proteolysis cannot be accurately focused and controlled. Essentially, the enzyme activity is not restricted to the function of hair removal, but also affects the structure of the dermis. The grain layer is therefore partially digested hence there is a decrease in the quality of the grain structure in the final leather. Enzyme unhairing remains a fascinating concept, but is full of difficulties.

Nevertheless, the problems and costs of treating effluent to acceptable standards are considerable. If hair could be removed intact without using sulfide, then there would be a significant easing of techniques, capital equipment and power requirements to achieve discharge limits. In addition, the volume of sludge would be considerably reduced, with the hair remaining as a new clean raw material with many potential outlets.

The epidermis as a diffusion barrier

When mechanisms of enzyme unhairing and diffusive processes are discussed, the epidermis (*stratum corneum*) is

usually presented simply as a barrier against the penetration of unhairing agents. There is no appropriate attention to the components of this barrier, its structure, or how it may be bridged during the depilation process.

As a step in the design of an ideal enzyme depilation process the epidermal layers need careful consideration, and a study of ways to improve enzyme access to the basement membrane for hair loosening is required.

A modification of the epidermis layer, and the generation of more accessible routes for enzyme diffusion would enable unhairing agents to quickly reach these target sites. This would minimise any detrimental enzyme effects within the main collagen structure.

The role of lipids within the epidermis

During the keratinisation process, part of the epidermis is transformed into chemically and physically resistant scales or squames called corneocytes. These corneocytes are flat, packed mainly with keratin filaments, and surrounded by a cell envelope composed of cross-linked proteins and covalently bound lipids^[1]. In turn these structures are held together as conglomerates of cells by *desmosomes* which have a central core of glycoproteins. These are tripsin sensitive, and can be slowly disintegrated.

Lipids also play a major role in holding the corneocytes together. These lipids are located in the spaces between the squames, and mainly comprise of ceramides, free fatty acids and cholesterol. The intercellular spaces are almost filled, forming the diffusion barrier within the stratum^[2].

A barrier is therefore formed as a two-component system of lipid-depleted corneocytes embedded in a lipid enriched extracellular matrix. The lipid matrix (*a keratin-phospholipid complex*) plays a significant role in the permeability of the epidermis, hence the diffusion of chemicals and auxiliaries into the skin structure^[3].

The diffusion of substances across the epidermis

In order to overcome the diffusion barrier, the macro and micro routes for permeation through the grain layer need consideration. There are three routes:

- 1) the hair follicles with their associated sebaceous glands
- 2) the sweat glands
- 3) the trans-epidermal route, that is, through the continuous *stratum corneum*. ➤



THE DIFFUSION BARRIER WITHIN THE UNHAIRING PROCESS

Penetration across the *stratus corneum* requires the transport of chemicals via intracellular and intercellular spaces^[4]. These possible macro and micro routes through the epidermis are shown respectively as *Panels 1 and 2*.

Molecules that carry a charge mainly diffuse through a pathway that consists of bound water within the structure, whereas the non-polar molecules dissolve and diffuse through the non-aqueous lipid matrix^[5,6].

Enhancers for penetration, or promoters for reactions

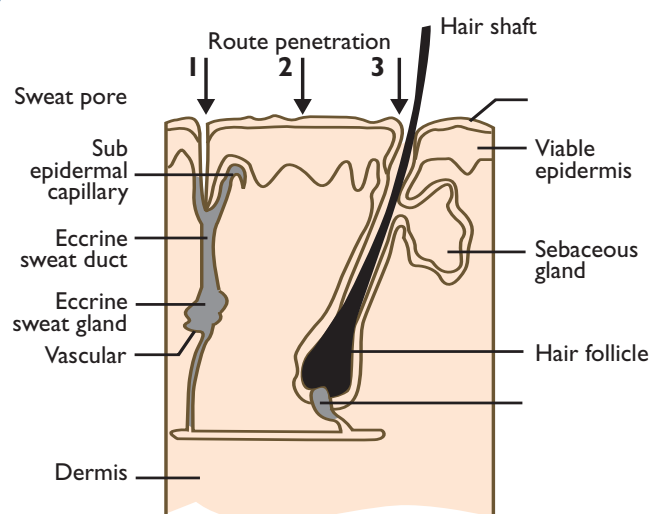
SODIUM SULFITE:

Disulfide bonds in the cystine residue of keratin react with sodium sulfite giving S-sulfocysteine (RS-SO_3^-) and cysteine (RS^-) (sulfitolysis reaction)^[7]. The net charge on the disulfide molecule was found to have a considerable effect on the rate of reaction with the SO_3 ion. Positively charged compounds react quickly and completely under conditions in which negative charges are non-reactive. This behaviour is also observed in proteins with many S-S bonds, including keratin fibrous protein^[8].

SURFACTANTS:

Over and above the ability to emulsify and disperse fats and oils, some surfactants can interact with proteins through electrostatic and hydrophobic interactions. Surfactants, such as

Panel 1



Possible macro routes for substance penetration across human skin via intact horny layer or hair follicles or eccrine sweat glands. Taken, with permission, from Panchagnula R., "Transdermal delivery of drugs", Indian Journal of Pharmacology, 1997, 29, 140-156.



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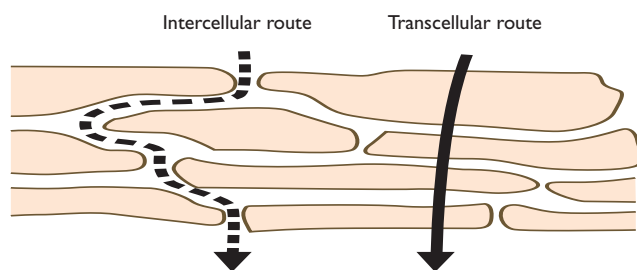
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FOR LEATHER AND THE ENVIRONMENT.....QUALITY FROM START TO FINISH



Panel 2



Possible micro routes for substance penetration across human skin intercellular or transcellular. Taken, with permission, from Panchagnula R., "Transdermal delivery of drugs", Indian Journal of Pharmacology, 1997, 29, 140-156.

sodium dodecylsulfate, can bind to both fibrous and globular proteins. In particular, this surfactant has a very high affinity with proteins such as keratin.

Anionic surfactants also show a specific lyotropic effect on proteins. When sodium dodecylsulfate is applied as 5% solution it causes hide swelling, lowers the shrinkage temperature, and disorganises the collagen structure^[8]. This is because the resistance of the *stratum corneum* is reduced due alterations in the

structure of keratin within the layer^[9].

In addition to these effects, appropriate mixtures of polar and non-polar surfactants/solvents may alter the lipid envelopes within the epidermis, thus substantially reducing the barrier.

PROTEOLYTIC ENZYMES - TRYPSIN:

Proteolytic enzymes can bring about the breakdown of specific epidermal components (*desmosomes*) that hold the corneocytes together. This can affect the dermis /epidermis union^[10].

However, trypsin is a serine protease. It specifically catalyses the peptide hydrolysis of amino acid esters and amides at the carbonyl group of the amino acids arginine and lysine^[11]. This enzyme acts on the basement membrane (*it has been used to separate epidermal sheets from human skin.*)^[12]

Histological studies on human and other skin types with different proteases indicate dermal-epidermal and cell separation, followed by epidermal cell destruction. In association with moderate mechanical action, Trypsin's activity enables the release of micro-particles from desmosomal spaces^[10]. In this event, trypsin must penetrate the epidermal layer via the hair follicle, and can be assisted by a moderate alkaline and surfactant-solvent action.

Preliminary results obtained at CITEC indicate that tryptical action combined with sodium sulfite, generates an epidermis hydrolysis which is 10 times higher than the produced by trypsin alone. ➤

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UREA:

Urea is well known as a denaturing agent within the protein structure. It competes for the active sites of the hydrogen bridges. The lyotropic effect of concentrated solutions of urea (>6M) on the hide collagen structure has been convincingly established^[8].

Epidermal cellular proteins within different stratum behave differently when urea is present. In the lower strata, the proteins are readily dispersed in 6M urea, but in the outer parts this does not happen. In the *stratum corneum*, the most important effect of urea is to cause the separation of the cells^[13].

Urea also promotes the sulfitolysis reaction. In the presence of urea the time taken to reach the maximum reaction rate at a given pH is about one-quarter of the time when urea is absent^[14].

ORGANIC SOLVENTS - TERPENES

Lipids occupy almost all of the space within the *stratum corneum*, hence they play a fundamental role as a hydrophobic barrier. Terpenes undermine this barrier, enhance skin penetration, and promote sorption^[15].

Terpenes are comprised of carbon, hydrogen and oxygen atoms, and are found in essential oils. Their structure can be as linear or cyclical molecules. These may be composed of many units of hydrocarbons of 5-carbon structures, isoprene, or 2-methyl 1,3-butadiene. Compounds can be formed by two, three, four, six and eight units of isoprene^[16].

An increase in the permeability of the *stratum corneum* as a result of applying terpenes implies one mechanism whereby drug portioning into the tissue has been improved^[15].

Phases of the unhairing process

With reference to the information set out in the previous sections, a model for an ideal unhairing system is proposed. This model applies irrespective of the technique of unhairing, but is seen as an important prerequisite for a successful unhairing process using depilatory enzymes.

- Phase I: Chemical-mechanical modification of the epidermis to decrease barrier resistance.
- Phase II: Rapid diffusion of depilatory enzymes, mainly through the modified epidermis.
- Phase III: Depilatory activity on the basement membrane, separation of epidermis and hair from dermis, slight action on the collagen structure.
- Phase IV: Opening-up of the fibrous structure by alkaline swelling and gentle enzymatic activity.

Within this model the emphasis is placed on actions for the modification of the epidermis. The objective is to make full use of all three routes for the fastest diffusion of products through the epidermis to the basement membrane. Moreover, the ideal unhairing^[17], would be to:

- Rapidly loosen the attachment of the bulb to the papilla.
- Quickly bring about the separation of the outer root sheath from the underlying connective tissue.
- Cause sufficient cell destruction and dispersion of the outer root sheath to permit the easy withdrawal of the hair.
- A gentle enzyme activity on collagen structure with no alteration to the valuable grain enamel.

To satisfy these requirements it is essential that the unhairing

enzymes reach the active sites in the basement membrane via fast diffusion through the epidermis tissue. Modification of the epidermis is essential to maximise this diffusion. 

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