71 Surfactants: Classification

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INTRODUCTION

Surfactants are chemical materials used by humans for centuries; a very remote use is reported as a kind of soap already prepared with ash and vegetal oils by Sumerian people (ca. 10,000 BC). Surfactants are used today in many practical applications in industry, home, and institutions. Cosmetics is an important application area among others (detergents, foods, fabric softeners, biocides, textiles industries, paints, inks, adhesives, dyes, herbicides, insecticides, fire extinguishers, etc.).

The term "surfactant" applies to a group of molecules having both a hydrophilic part and a hydrophobic (or lipophilic) part. Surfactants modify the interfacial properties of the liquids in which they are incorporated; this property stems from their tendency to concentrate at the interfaces separating immiscible phases.

This peculiar property favors the formation of micelles and structured liquid phases, which are involved in numerous facets of the cosmetic world: cleansing action, direct and inverse emulsions, gels, foam production, etc.

Depending on the nature of the hydrophilic moiety ensuring water affinity of the molecule, surfactants are distributed in anionic, cationic, amphoteric, and nonionic classes.

Regarding the hydrophobic moiety of the molecule, it is a hydrocarbon chain in most common surfactants; however, in some more specialized surfactants, this hydrophobic part can be a non-hydrocarbon chain such as a polydimethylsiloxane or a perfluorocarbon.

The selection of surfactants in the frame of cosmetic products development is a delicate task in which numerous factors have to be taken into account. Among others, one should consider those directly related to functions to be fulfilled (detergency, emulsification, foam quality, rinsability, mildness for skin, skin feel, etc.), and also those related to cost, toxicity, and biodegradability.

Although few surfactants are naturally produced such as saponins or lecithins, there is a trend to produce more and more surfactants issued from natural materials.

The aim of this chapter is to provide a classification of various commercially available surfactants.

IONIC SURFACTANTS

Anionic Surfactants

In aqueous solution, anionic surfactant molecules carry negative charges if the composition pH is not too low (slightly acidic, neutral, or alkaline). The ionized moiety can be a carboxylate, sulfate, sulfonate, or phosphate. Among the most frequently used surfactants in skin care products, the alkyl sulfates and alkyl ethoxylated sulfates can be mentioned for their high-foaming capacity. Anionics are generally used in association with other surfactants (nonionics or amphoterics), which bring improvements in the skin tolerance, foam quality, or product viscosity.

Other anionics are also used in personal products, however, as secondary surfactants, often for their milder profile and their low-foaming properties (isethionates, sulfosuccinates, taurates, sarcosinates, phosphoric acid esters, acylglutamates, etc.).

Carboxylates

Carboxylates salts. Surfactants belonging to this class generally derive from oleochemistry. Carboxylate salts (or soaps) are directly produced by the alkaline hydrolysis (or saponification) of animal and vegetable glycerides and result from the neutralization of fatty acids.

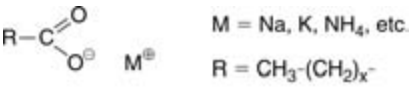
Saturated sodium soaps are extremely soluble in water up to C_8; those with chain lengths approaching C_{18} become less soluble, and they are insoluble above C_{20}.

Starting from C_{16} chain lengths, the fatty acids can be either saturated or unsaturated.

Unsaturated fatty acids are prone to undergo oxidation and form oxides and peroxides, which cause rancidity and yellowing.

Potassium soaps and salts of alkanolamines are more fluid and also more soluble than sodium salts.

The extremely low solubility of alkaline earth and heavy metals' fatty acid salts make this class of surfactants less appropriate for use in hard water.



Alkyl carboxylate

The main application of fatty carboxylates is found in the soap bars widely used in the world for fabric hand wash (generally based on tallow/coconut oil mixtures).

Water-soluble soaps are mainly used in skin cleansers (soap bars or liquids), shaving products (sticks, foams, or creams), and deodorant sticks.

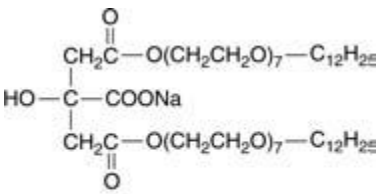
Mixtures of fatty acids and their salts are used in "acid soaps."

Water-insoluble soaps form gels in nonaqueous systems and, because of their hydrophobicity, they can be appropriate surfactants for w/o emulsions.

Ester carboxylates. This class of surfactants is a subcategory of the previously discussed surfactant group based on carboxylic acids; they are monoesters of di- and tricarboxylic acids.

These esters are produced by condensation reactions involving different types of molecules, either an alcohol with a polycarboxylic acid (e.g., tartric or citric acid) or a hydroxyacid (e.g., lactic acid) with a carboxylic acid.

The reacting alcohol may have been previously ethoxylated.



Sodium dilaureth-7 citrate

Because of their good foaming properties and substantivity on the hair, ester carboxylates are especially suitable in shampoos; in combination with alcohol ethoxy sulfates (AEOS), they provide reduced skin irritation.

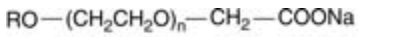
Short-chain lactylates (i.e., issued from lactyllactic acid) are substantive on the skin and show humectant properties.

Ether carboxylates. Alkyl polyglycol ether carboxylates are the best-known surfactants in this category.

These surfactants are formed by the reaction of sodium chloracetate with ethoxylated alcohols.

Because of the addition of ethoxylated groups, ether carboxylates are more soluble in water and less sensitive to water hardness compared with conventional soaps. Also, keeping the best properties of nonionic surfactants, they do not exhibit any cloud point and show good wetting and foam stability.

Ether carboxylates do not undergo hydrolysis in presence of alkali or acids.



Alkyl polyglycol ether carboxylate, sodium salt

Ether carboxylates are used as general emulsifier and emulsion stabilizers.

In personal care, they impart mildness, creamy foaming, skin feel, and hair-conditioning benefits. Therefore, they are especially suitable in shampoos in combination with alcohol ether sulfates and possibly with cationics.

More recently, a new generation of alkyl glucose carboxylates is emerging. These surfactants exhibit both the high mildness of alkyl polyglucoside (APG) surfactants and additional attributes such as foaming and sensory benefits. A typical surfactant of this class is the sodium lauryl glucose carboxylate.

Sulfates

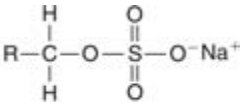
Alkyl sulfates. Alkyl sulfates are organic esters of sulfuric acid; they vary by the length of the hydrocarbon chain and by the selected counterion.

Alkyl sulfates are produced by sulfation of the corresponding fatty alcohols.

The properties of alkyl sulfates depend mainly on the chain length and on the degree of branching of the hydrocarbon chain as well as, to a smaller extent, on the nature of the counterions.

They are generally good foamers, more especially in hard water; best foam characteristics are obtained in the C_{12} to C_{14} chain length range.

Sodium lauryl sulfate (SLS) has a 12-carbon chain length and is one of the most common surfactants. It is not well tolerated by the skin. When the chain length increases, i.e., in the C_{14} to C_{18} range, surfactant penetrability through the stratum corneum decreases along with its irritation potential; but the foaming capacity is accordingly depressed. Chains with carbon number lower than 12 are better tolerated by the skin than SLS but exhibit more pronounced smell. Combination with other surfactants allows considerable improvement of the lauryl sulfate compatibility with skin while keeping a good foam. SLS is, however, less frequently used than its ethoxylated counterpart. Lauryl sulfate is available under the form of various salts: SLS, ammonium lauryl sulfate (ALS), magnesium lauryl sulfate [$Mg(LS)_2$] and triethanolamine lauryl sulfate (TEALS). Skin tolerance of lauryl sulfates is as follows: $Mg(LS)_2$ > TEALS > SLS > ALS



Sodium alkyl sulfate

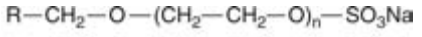
Alkyl sulfates are used in cosmetics and personal care areas [e.g., diethanolamine (DEA) lauryl sulfate in shampoos]; they are associated with other surfactants and improve foaming characteristics of detergent systems.

Pure SLS is also used in oral care and incorporated in dental creams, essentially as a foaming agent.

Alkyl ether sulfates. Alkyl ether sulfates (AESs), which are also identified as AEOS, result from the sulfation of an ethoxylated alcohol.

Compared with alkyl sulfates, the ether sulfates show higher water solubility, improved foam stability in hard water, and better skin tolerance. The viscosity of surfactant solutions of ether sulfates is much more sensitive to the presence of electrolytes than alkyl sulfates; formulators often take advantage of this opportunity to bring liquid formulations to the desired viscosity by simply adjusting the salt level (e.g., NaCl).

The higher the number of ethoxy groups (EOs) in the molecule, the lower the surfactant's ability to penetrate the stratum corneum, and the less irritant for skin it will be. Similar ranking is true for eye irritation. Also, the foaming capacity decreases as ethoxylation degree increases.

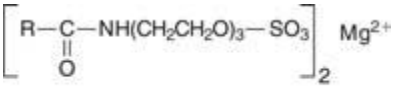


Sodium alkyl ether sulfate

AESs are extensively used in personal products such as liquid soaps, shower gels, foam baths, and, more especially, shampoos. Sodium lauryl ether sulfate (SLES) is today the most currently used primary tensioactive, and more especially, under the forms SLES-2 EO and SLES-3 EO, which combine good foaming and skin compatibility properties.

Amide ether sulfates. The amide ether sulfates are obtained by sulfation of the corresponding ethoxylated amide.

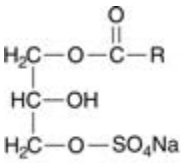
The magnesium salts foam well, and their skin compatibility is excellent.



Magnesium PEG-3 cocamide sulfate

Owing to their weak lipid removal effect, amide ether sulfates are used in very mild personal cleaners.

Alkyl glyceride sulfates. The best-known surfactant of this class is the cocomonoglyceride sulfate (CMGS). It is obtained by transesterification of coconut oil with glycerol followed by a sulfation with sulfur trioxide and a neutralization with sodium hydroxide.



Sodium alkyl monoglyceride sulfate

This surfactant is very well designed for cosmetic and personal care products. Compared with the corresponding AES, it shows similar foaming power. Because this surfactant acts as a foam booster, it can be advantageously combined with APG. Such mixtures also show a thickening ability induced by salt addition. CMGS is said to present a better skin compatibility profile than ether sulfate or other anionic surfactants.

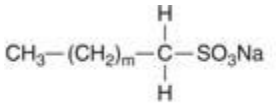
Sulfonates

On a chemical standpoint, there is an important difference between the previously discussed alkyl sulfates and the alkyl sulfonates: in the former, the sulfur atom is linked to the carbon chain via an oxygen atom, and in the latter, the sulfur atom is directly linked to the carbon atom.

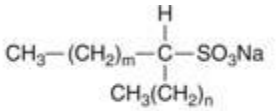
Alkyl sulfonates. Three major types of alkyl sulfonates must be considered: the primary and secondary paraffin sulfonates [PS and secondary alkyl sulfonate (SAS)] and the α-olefin sulfonates (AOSs).

The paraffin sulfonates are water-soluble surfactants, good foamers, and good o/w emulsifiers. Their solutions do not thicken easily upon salt addition. Therefore, they are particularly appropriate to formulate fluid liquids or highly concentrated products.

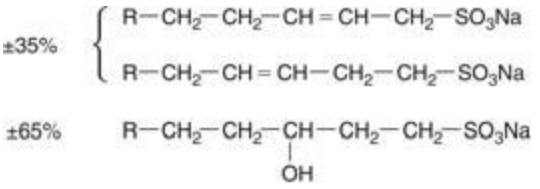
The AOS have general properties fully comparable to LAS (see sect. "Alkyl-Aryl Sulfonates"); they are good o/w emulsifiers, wetting, and foaming agents.



Primary sodium alkyl sulfonate



Secondary sodium alkyl sulfonate

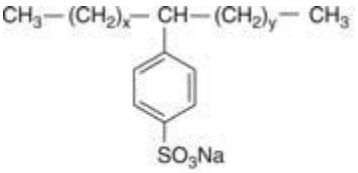


Constituants of α-olefin sulfonate: sodium alkene sulfonates and sodium hydroxy alkane sulfonate

Alkane sulfonates (PS and SAS) are mainly used in Europe in detergent products.
AOSs have been mainly used in Asia as surfactants for heavy- and light-duty laundry detergents, synthetic soap bars, and household products. Because they are less irritating than alkyl-aryl sulfonates, they have also been used in the United States in several personal products (liquid soaps, bubble baths, and shampoos) as alternatives to alcohol ether sulfates. They are also marginally used in oral care formulations.

Alkyl-aryl sulfonates. Today, LAS (linear alkylbenzene sulfonate) is the most important surfactant on a volume basis, but its use in personal care is very limited.

It is worth mentioning that some methyl or methyl-ethyl-substituted aryl sulfonates, i.e., sodium xylene, toluene, or cumene sulfonates (SXS, STS, or SCS, respectively), although not showing typical surfactant properties are used as hydrotropes (i.e., solubilizing agents, which decrease hydrophobic effects in aqueous systems).

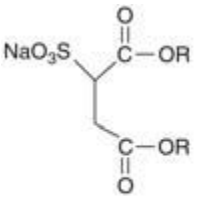


Sodium linear alkylbenzene sulfonate (LAS)

LAS is a very cost-effective surfactant that is extensively used in a broad variety of detergents for household, fabric care, institutional, and industrial products.

Because of its too high detersive action, LAS has a relatively low compatibility with skin and is only scarcely used in cosmetics except in some anti-seborrheic preparations.

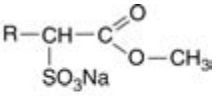
Sulfosuccinates. Sulfosuccinates are the sodium salts of alkyl esters of sulfosuccinic acid; they generally result from the condensation of maleic anhydride with a fatty alcohol, followed by a sulfonation with sodium bisulfite $NaHSO_3$. Some variants of sulfosuccinates are derived from other substituted fatty molecules such as fatty alcohol ethoxylates, fatty amines (yielding sulfosuccinamates), or fatty alkanolamides.



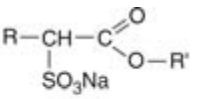
Sodium dialkyl sulfosuccinate

Monoesters disodium salts are the most common sulfosuccinates used in cosmetic applications. Monoesters of alkanolamines (sulfosuccinamates) are milder than monoesters of fatty alcohols (sulfosuccinates). Monoesters derived from ethoxylated alcohols or alkanolamides are extensively used in personal products and especially in shampoos; they are known for their mildness and skin irritation reduction when used in association with other anionic surfactants.

Sulfo fatty acid esters. These surfactants are sometimes known under their abbreviated names: FES, MES, and ASME, for fatty ester sulfonate, methyl ester sulfonate, and alpha sulfo (or α-sulfo) methyl ester, respectively. Most of α-sulfo fatty acid esters derive from fatty acid methyl esters.



Methyl ester of α-sulfo fatty acid, sodium salt.

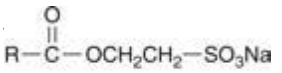


Alkyl ester of α-sulfo fatty acid, sodium salt.

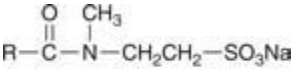
α-Sulfo methyl ester surfactants deriving from C_{16} to C_{18} fatty acid (e.g., ASMT, the tallowate) are appropriate for use in laundry detergents. ASME is also used in the formulation of syndet bars (laundry bars based on synthetic surfactants).

To our knowledge, these surfactants are not used in personal care.

Fatty acid isethionates and taurides. Fatty acid isethionates are usually prepared by reaction of a fatty acid chloride with sodium isethionate ($HO-CH_2-CH_2-SO_3-Na$), itself resulting from the addition of sodium bisulfite to ethylene oxide (EO). These surfactants are insensitive to water hardness and show good wetting, foaming, and emulsifying properties. In addition, they are very mild and have excellent compatibility with the skin. Taurides (or taurates) are acyl–amino alkane sulfonates that have chemical structures close to isethionates. They can be used in association with other surfactants to increase the viscosity.



Fatty acid isethionate



Sodium methyl acyl tauride

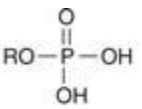
Acyl isethionates have been used in shampoos and personal cleansers. They are also incorporated in syndet bars together with various soaps. The most currently used isethionate is the cocoyl isethionate.

Taurides (or taurates), which have properties similar to soaps (except the sensitivity to water hardness), have been extensively used in shampoos but are now replaced by AEOS. Today they are limitedly used in cosmetics mainly in foam baths and toilet bars.

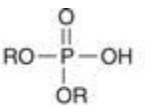
Taurides are also used in soap bars especially designed for laundering with seawater, in agriculture, and in textile dying.

Phosphates Esters

This class of surfactants includes alkyl phosphates and alkyl ether phosphates.



Alkyl phosphoric ester



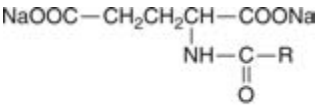
Dialkyl phosphoric ester

Phosphate esters as surfactants are especially useful in applications for which a particular tolerance to pH, heat, or electrolytes is required. They are also used in acidic cleaning products for household as well as industrial applications. Mild for the skin, alkyl phosphates sometimes enter the composition of facial and cleansing products.

Acyl–Amino Acids and Salts

Acyl glutamates. These surfactants are formed by acylation of a natural amino acid, the glutamic acid $HOOC-CH_2-CH_2-CH(NH_2)-COOH$ (or α-aminoglutaric acid).

These surfactants are mild for the skin and the eyes, deliver improved skin feel, but are poor foamers.



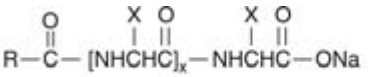
Sodium acyl glutamate

Acyl glutamates are mainly used in personal products such as shampoos.

Acyl peptides. These surfactants are formed from hydrolyzed proteins (e.g., animal collagen).

Depending on the protein hydrolysis process (chemical or enzymatic), the average polypeptide molecular weight can vary from about 350 to 2000, and some free amino acids may be present in the hydrolysate. An acylation reaction occurs on the amine terminal functions and, possibly, on some side groups (e.g., the hydroxyls) and thus leaves the carboxyl groups free, which must be neutralized.

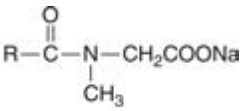
Products containing such surfactants are prone to be contaminated by various germs and have to be properly preserved.



Sodium acyl polypeptide (X = amino acids side groups)

Acyl peptides are mild surfactants designed for the personal care area; they are especially used in shampoos owing to their substantivity on the keratin of hair and, therefore, they effectively deliver the expected benefits of conditioning agents.

Acyl sarcosides. Sarcosinates (or salts of acyl–amino acids) are the condensation products of fatty acids with *N*-methylglycine $CH_3-NH-CH_2-COOH$ (or sarcosine).



Sodium acyl sarcosinate

Sarcosinates are good surfactants for cosmetic use because of their mildness to skin, substantivity on skin and hairs when incorporated in formulations around neutral pH, conditioning action, and foaming resistance in the presence of soaps or sebum. Incorporated in shampoos with alkyl sulfates, they boost the lather.

Sarcosinates are also used as corrosion inhibitors.

Cationic Surfactants

From a very general standpoint, cationic surfactants differ from anionic and nonionic ones by the fact that they carry a positive charge. Their major interest in cosmetic industry resides in hair care; in this frame, they are used as hair conditioners and antistatic agents.

Cationics are also found in the personal care area as emulsifiers in some cosmetic preparations and as bactericidal agents.

Alkylamines

Primary, secondary, and tertiary alkyl amines, and more especially their salts, are included in this surfactant class.

Alkyl amine | Dimethyl alkyl amine | Alkylamido | dimethy

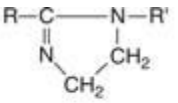
propylamine

Amines and their salts are mainly used in textile treatment and occasionally in rinse fabric softeners. Salts of amines are used in cosmetics together with other surfactants. Their usage is restricted to specialties; they exhibit conditioning and antistatic properties in hair care applications. Amidoamines are also used in cosmetic products.

Alkylimidazolines

Reaction of a fatty acid with a substituted ethylene diamine forms imidazoline. Heating the resulting amido-ethylamine yields the imidazoline with a five-member substituted ring.

The tertiary nitrogen atom can be quaternized.



$R' = CH_2CH_2NH_2$ ⇒ alkyl aminoethyl imidazoline

$R' = CH_2CH_2OH$ ⇒ alkyl hydroxyethyl imidazoline

Imidazolines are cationic o/w emulsifiers.

Considered to be irritating, they are scarcely used in cosmetics as substantive hair-conditioning agents.

Quaternary Ammonium Compounds

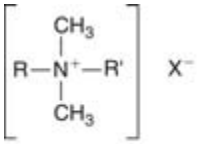
Quaternary ammonium compounds form a class of surfactants that contain a positively charged nitrogen atom linked to four alkyl or aryl substituents.

The positive charge is permanent, regardless of pH.

Tetra alkyl(-aryl) ammonium salts. Tetra alkyl ammonium salts have the structure $[R_1R_2R_3R_4N^+]\ X^-$ where R_1, R_2, R_3, and R_4 are alkyl or aryl groups and X^- represents an anion. The water solubility of quaternaries mainly depends on the nature of R substituents.

Low-solubility quaternaries can adsorb on various substrates and impart various useful conditioning effects (softening, antistat, corrosion inhibition, etc.).

With the exception of *N*-alkyltrimethyl ammonium salts, quaternary surfactants usually show poor detergency, wetting, and emulsifying capacities. Quaternaries are generally not compatible with anionics because of the formation of water-insoluble complexes.



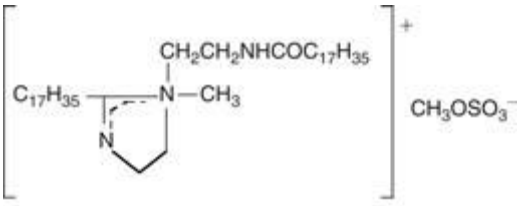
Quaternary compound

The major usage of quarternaries is related to their ability to adsorb on natural or synthetic substrates and fibers. They are widely used as softening agents in rinse fabric softeners.

Their softening and antistatic properties are similarly exploited in hair-conditioning shampoos or after-shampooing rinses.

It is worth to note that, in cosmetic applications, quaternaries may cause ocular and local irritation. Among quaternaries, some are used as germicides and disinfectants (e.g., didecyl dimethyl ammonium chloride and benzalkonium chloride).

Heterocyclic ammonium salts. Heterocyclic quaternaries are derived from heterocyclic aliphatic or aromatic compounds in which a nitrogen atom constitutive of the cycle is quaternized.



Imidazolinium quaternary compound

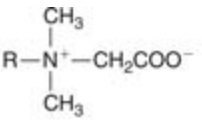
The quaternaries derived from imidazoline and morpholine are used as hair conditioners and antistatic agents. Those derived from aromatic heterocycles are used as germicides.

Alkyl betaines. Alkyl betaines, which are *N*-trialkyl derivatives of amino acids ($[R_1R_2R_3]N^+CH_2COOH$), are classified as cationics because they exhibit a permanent positive charge. Because they also have a functional group able to carry a negative charge in neutral and alkaline pH conditions, they are often regarded, although this position is questionable, as amphoterics.

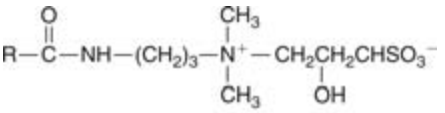
The positive charge is always carried by a quaternized nitrogen, while the anionic site can be a carboxylate (betaine), a sulfate (sulfobetaine or sultaine), or a phosphate (phosphobetaine or phostaine).

Betaines are good foaming, wetting, and emulsifying surfactants, especially in the presence of anionics. Alkylamido betaines deliver more stable foam and are better viscosifiers than alkyl dimethyl betaines. Betaines are compatible with other surfactants, and they frequently form mixed micelles; these mixtures often deliver unique properties that are not found in the individual constitutive surfactants.

Betaines have low eye irritation and skin irritation; moreover, the presence of betaines is known to decrease the irritation effect of anionics.



Alkyl dimethyl betaine



Alkylamidopropyl hydroxysultaine

Because of their ability to improve the skin's tolerance against irritating anionic surfactants, and also, because of their high price, betaines are usually used in association with other surfactants.

Betaines are especially suitable in personal care applications (e.g., shampoos, foam baths, liquid soaps, shower gels), fabric hand wash products, and dishwashing products.

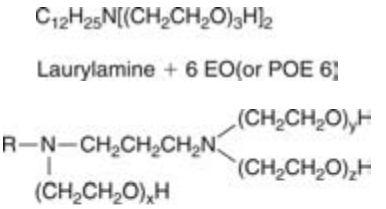
Quaternized APG. These surfactants, derived from natural sources, are recently available. They are made of alkyl polyglycoside with a cationic backbone. These very mild quaternary compounds are substantive to the skin and hair and provide a soft after feel. Thanks to the sugar moiety, they show low irritation potential, and therefore, they are particularly suitable

for personal care formulations. A major benefit is a longer-lasting effect associated with a reduction of irritation compared with traditional quaternary surfactants.

Some potential applications reside in hair care products (e.g., in wet combing benefit, flyaway hair control), w/o cationic emulsions, formulations in the presence of anionics, foam building in shampoo formulations, or surfactants for baby wipes.

Ethoxylated Alkylamines

These surfactants can be considered as cationic or nonionic depending on the degree of ethoxylation and the pH at which they are used. Polyethoxylated amines are formed by ethoxylation of primary or secondary fatty amines.



Alkyl propanediamine ethoxylate

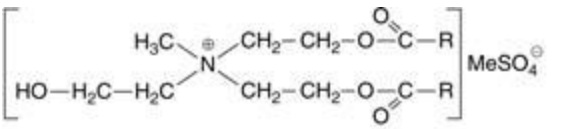
The ethoxylated alkylamines have various application fields; they are generally exploited for their capacity of adsorbing on surfaces.

In personal care, ethoxylated alkylamines are used as emulsifiers and hair-conditioning agents.

Ethoxylated amidoamines find applications in rinse fabric softeners.

Esterified Quaternaries

Esterified quaternaries (or esterquats) are produced by the esterification of the hydroxyl group (s) of secondary or tertiary amino-alcohols with selected fatty acids.



Esterquat: *N*-Methyl-*N*,*N*-bis[$C_{16/18}$-acyloxy)ethyl]-*N*-(2-hydroxyethyl)ammonium-methosulfatesalt

The esterquats are suitable substitutes for straight quaternaries; they present improved environmental profile and comparable softening properties compared with straight quaternaries.

Amphoteric Surfactants

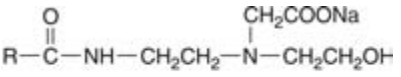
Amphoteric surfactants are characterized by the fact that these surfactants can carry both a positive charge on a cationic site and a negative charge on an anionic site. The use of amphoteric terminology is still more restrictive: the charge of the molecule must change with pH, showing a zwitterionic form at intermediate pH (i.e., around the isoelectric point).

The surfactant properties are accordingly influenced by pH: around the isoelectric point, the zwitterionic form takes place, exhibiting the lowest solubility; in alkaline conditions, the anionic form is predominant, delivering foam and detergency; and in acidic conditions, the cationic form prevails, providing surfactant substantivity.

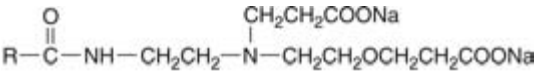
Although betaines are commonly classified among amphoterics, this classification is improper because these surfactants never exhibit in single anionic form.

Amphoteric surfactants are generally used as secondary tensioactives for their foam-stabilizing effect, their thickening capacity, and their skin irritation reduction capacity on alkyl sulfates and alkyl ethoxy sulfates.

Acyl Ethylenediamines and Derivatives
These surfactants are made by the reaction of an alkyl imidazoline with chloroacetic acid (yielding amphoglycinates) or with acrylic acid (yielding amphopropionates).



Acylamphoacetate



Acylamphodipropionate

Amphoterics of this class are mainly used in personal products (e.g., coco amphocarboxy glycinate). Incorporated in baby shampoos, they reduce eye irritation.

Other applications are fabric softeners, industrial cleaners, and car cleaners.

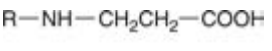
N-Alkyl Amino Acids or Imino Diacids
These molecules are chemical derivatives of amino acids that can be produced by the reaction of chloroacetic acid or acrylic acid with an alkyl amine.

Their compatibility with other surfactants is excellent.

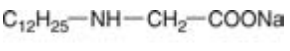
These surfactants are good emulsifiers and show optimal wetting and detergency under alkaline pH. They are good foamers at neutral and alkaline pH but lose their foaming properties under acidic conditions.

They are substantive to surfaces and provide antistatic effects.

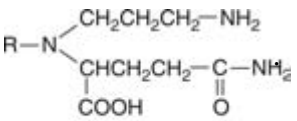
They provide skin and eye irritancy reduction in combination with anionics.



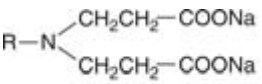
Alkyl aminopropionic acid



Sodium coco glycinate



Aminopropyl alkylglutamide



Sodium alkyliminodipropionate

Amphoterics of this class are mainly used in personal products.

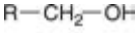
Polycarboxylates deliver reduced eye irritation and provide hair-conditioning benefits. Their zwitterionic forms are substantive on the hairs.

NONIONIC SURFACTANTS

Nonionic surfactants do not dissociate into ions in aqueous medium. They generally deliver a weak to moderate foam. They are appreciated for their good skin and eye compatibility as well as for their anti-irritant potential when they are combined with anionics in appropriate concentration ratio. Therefore numerous products for sensitive skin, babies, or the face incorporate nonionics as major surfactant.

Fatty Alcohols

Fatty alcohols are primarily used as a chemical precursor for the production of several other surfactants.



Fatty alcohol

Because they are not water soluble, the use of fatty alcohols is very limited in liquid products. They are mainly used as opacifiers, thickening agents, and foam depressors (e.g., lauric alcohol).

Ethers

Alkoxylated Alcohols

This class of surfactants mainly covers ethoxylated or propoxylated alcohols.

Ethoxylated alcohols (also called "polyethyleneglycol ethers" or "PEG ethers") are produced from the reaction of fatty alcohols with EO.

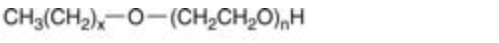
Similarly, propoxylated alcohols (also called "polypropyleneglycol ethers" or "PPG ethers") are obtained with propylene oxide (PO).

The hydrophilic-lipophilic balance (HLB) of ethoxylated alcohols can be adjusted by properly balancing the hydrophilic ethoxylated chain and the hydrophobic fatty chain.

Ethoxylate nonionics are compatible with all surfactants. Some beneficial associations with ionic surfactants are often shown.

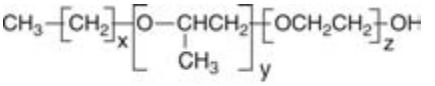
In the frame of personal care applications, ethoxylated alcohols often result from the transformation of natural lipids. The nomenclature specific to cosmetic chemicals [i.e., international nomenclature of cosmetic ingredients (INCI) names[a]] is applied to these nonionics: they are denominated by using the root of the fatty acid name terminated by the suffix "-eth" (contraction of "ethoxylated"), directly followed by the ethoxylation degree (e.g., laureth-4, oleth-5, and myristeth-7).

As some raw materials yield on hydrolysis various fatty chain lengths, the names of the derived nonionics are either drawn from the natural source (e.g., laneth-16 for a lanolin-derived nonionic) or from the combined abbreviations of the constitutive fatty chains (e.g., ceteareth-20 for a combination of cetyl and stearyl).



Alkyl polyethyleneglycol ether or alcohol ethoxylate

(e.g., laureth 20 for $x = 11$ and $n = 20$)



EO/PO alkyl ether

(e.g., propyleneglycol capreth-4 for $x = 9$, $y = 1$, and $z = 4$)

Applications of ethoxylated alcohols are numerous in industrial as well as in household products.

When properly selected, alkoxylated alcohols are also useful for personal products as good emulsifiers and solubilizers. The cosmetic applications remain, however, limited because of their rather weak foaming capacity.

Because they are prone to undergo degradation by oxidation, the following precautions can greatly improve the stability of ethoxylate nonionics: storage in the dark, minimal air contact, low-temperature storage, avoiding storage of diluted products, and the addition of an antioxidant.

EO/PO Block Polymers

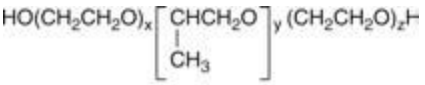
These polymeric surfactants have some similarity with the previously discussed alkoxylated alcohols. They consist in the combination of the assembly of PPG (hydrophobic part) and PEG chains (hydrophilic part). Such surfactants are known under the denomination "poloxamers" (INCI name) and are called EO/PO block copolymer nonionics.

A major property of EO/PO nonionics is their low-foaming profile.

As straight EO nonionics, EO/PO copolymers exhibit the cloud point phenomena.

[a]The International Cosmetic Ingredient Dictionary provides a nomenclature of conventional names for cosmetic ingredients that are defined by the CTFA (The Cosmetic, Toiletry, and Fragrance Association).

EO/PO nonionics are also mild surfactants.



Ethoxylated PPG ether

These surfactants are especially useful for applications in which foaming must be significantly depressed, such as automatic dishwashing detergents, laundry detergents, and rinse aids.

Owing to their mildness, EO/PO block polymers also find applications in cosmetic products. They are generally used as emulsifying, solubilizing, or fluidizing agents.

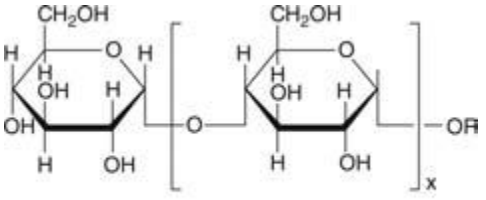
APGs

Alkyl polyglycosides are most often known by the simple abbreviation APGs.

APGs are produced by the alkylation of short-chain glucosides resulting from acidic alcoholysis of polysaccharides such as starch. Commercial products consist of mixtures of mono-, di-, and triglucosides. Accordingly the glucosidic average chain length varies between 1.2 and 3 glucose units, depending on the production conditions.

Surfactants of this class are good emulsifiers and provide good wetting and foam profile.

APGs are compatible with all other surfactants. They show good chemical stability at neutral and alkaline pH, and are impaired under acidic conditions (pH < 5).



Alkylpolyglucoside

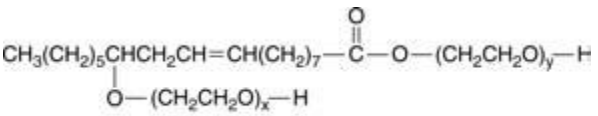
APGs are used in detergents and personal care cleansers (e.g., shampoos). They are claimed to be very mild for skin as well as to reduce the skin irritation potential of anionics. Additionally, they impart an excellent skin feel.

Their thickening effect in the presence of anionics and their foam stabilization capacity are also exploited in personal care applications.

APGs made from fully natural and renewable sources are now available, the glycosidic chain being made from corn, potato, or wheat starch and the alkyl chain from coconut and/or palm oil. Such surfactants are readily biodegradable and approved for eco labels (e.g., EU Flower, Nordic Swan, and Bra Miljöval).

Ethoxylated Oils and Fats

This class of surfactants essentially covers ethoxylated derivatives of lanolin (i.e., aliphatic alcohols and sterols and fractionation products of wool fat) and of castor oil (i.e., fatty acids with a high ricinoleic acid fraction extracted from ricinus seeds).



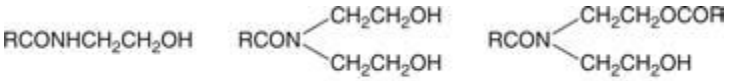
PEG castor oil derivative

Ethoxylated products of lanolin and castor oil are good and excellent emulsifiers, respectively. These surfactants are mainly used in the cosmetic industry; their major interest is to allow claims based on the natural origin of the constitutive surfactant systems.

Alkanolamides

Straight Alkanolamides

Alkanolamides are *N*-acyl derivatives of monoethanolamine and diethanolamine.



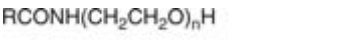
Monoalkanolamide dialkanol amide ester-amide

Alkanolamides have been largely used in household detergent products; their consumption has now significantly declined because of the extensive use of alkyl ethoxylated detergent products.

Because of their foam boosting and viscosity-enhancing capacity in the presence of anionics, alkanolamides are also usefully incorporated in personal care, especially in shampoos.

Ethoxylated Alkanolamides

Reaction of an alkanolamide with EO leads to an ethoxylated amide.



Polyethoxylated monoalkanolamide

It is more expensive than its corresponding ethoxylated alcohol and has therefore restricted usage. The benefits of thickening, foam stabilization, and dispersibility are exploited in personal care cleansers.

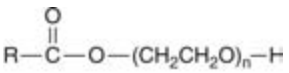
Esters

In this surfactant class, there are five major subcategories to be considered:

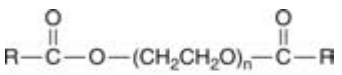
1. Ethoxylated fatty acids
2. Glycol esters, glycerol esters, and ethoxylated derivatives
3. Sorbitan esters and ethoxylated derivatives
4. Alkyl carbohydrates esters
5. Triesters of phosphoric acid

Ethoxylated Fatty Acids

This class of surfactants comprises mono- and diester that result from the reaction of fatty acids with either EO or polyethylene glycol.



PEG fatty acid ester



PEG fatty acid diester

Given their outstanding emulsifying properties, ethoxylated fatty acids are useful in domestic and industrial detergents, more especially in degreasing compositions.

If properly balanced, combinations of esters with low and high ethoxylation provide excellent emulsifiers for creams and lotions. They are also used as mild cleaners or viscosifying agents (e.g., PEG-150-distearate).

In cosmetics (shampoos), less water-soluble grade (i.e., ethylene glycol monostearate) is used as a pearlescent agent.

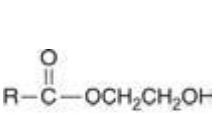
Glycol Esters, Glycerol Esters, and Ethoxylated Derivatives

A common point among the surfactants grouped in this class and the following two classes (sorbitan esters and alkyl carbohydrates esters) is that they all derive from the condensation reaction of a polyhydroxyl compound (e.g., glycol, glycerol, sorbitol, sucrose) with a fatty acid. Some of them can be directly extracted from natural sources.

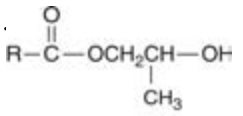
The resulting esters can be additionally ethoxylated to increase their HLB value and, thereby, their solubility in water.

These surfactants show poorer wetting and foaming properties in comparison with alcohol-derived nonionics. Emulsifying properties are excellent.

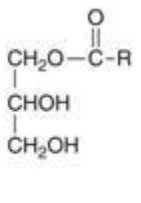
In general, esters and lower ethoxylates are appropriate for w/o dispersions, whereas higher ethoxylates are more suitable emulsifiers for o/w dispersions.



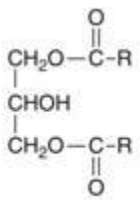
Ethylene glycol ester



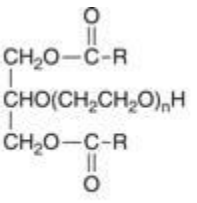
Propylene glycol ester



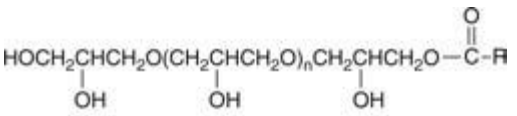
Monoglyceride



1.3-diglyceride



Polyethoxylated 1.3-diglyceride



Polyglyceryl monoester

Because of their high compatibility, these surfactants are widely used in the cosmetic and food industry.

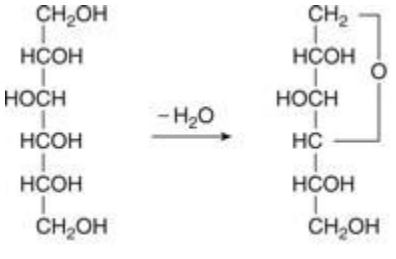
Glycol and glycerol esters are used in the pharmaceutical and cosmetic industries either as emulsifying agents or as oily compounds, refatting agents, emollients and skin conditioners in various products such as creams, lotions, ointments, and gels.

Stearate derivatives also deliver thickening and opacifying properties (e.g., the glyceryl stearate). Some of them are also used as pearlescent agents (i.e., glycol stearate and distearate).

Ethoxylated derivatives are used as solubilizing agents, emulsifiers, and even as emollients. Some show effective thickening effect when combined with other surfactants (e.g., PEG-200 glyceryl stearate)

Sorbitan and Sorbitol Esters and Ethoxylated Derivatives

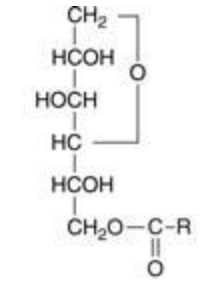
Sorbitan molecule is generated from the dehydration of the sorbitol molecule, which results in an internal ether bond.



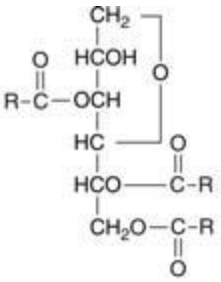
Sorbitol

1,4-Sorbitar

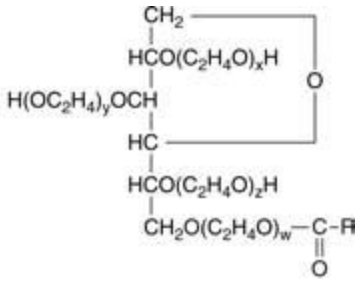
Sorbitol and sorbitan esters are obtained by acylation of hydroxyl groups, using most frequently natural fatty acids such as lauric, palmitic, stearic, or oleic. These surfactants can be optionally ethoxylated. Acylation (or ethoxylation) can occur on almost all hydroxyl groups present in the original polyol molecule.



1,4-Sorbitan monoester



1,4-Sorbitan triester



Polyethoxylated Sorbitan monoester

The field of application of sorbitan esters and their ethoxylated derivatives is identical to the one of glycol and glycerol esters (see the sect. "Glycol Esters, Glycerol Esters, and Ethoxylated Derivatives").

The sorbitol esters with a higher degree of ethoxylation (e.g., sorbitol septaoleate 40 EO) are also used as spreading aids in emollient bath oils.

Alkyl Carbohydrates Esters

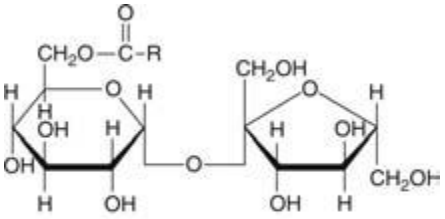
Surfactants of this class are better known as "sugar esters" or "sucrose esters."

The sucrose esters are obtained by transesterification of sucrose with fatty acid methyl esters or triglycerides.

Surfactants of this class are good emulsifiers.

Of great interest about such surfactants is their natural origin and good biodegradability.

It is worth noting that some glucosides surfactants, e.g., the so-called *saponins*, are already present in nature and directly available from vegetal sources.



Saccharose fatty acid monoester

Sucrose esters are food grade ingredients and have similar uses as the previously described glycol, glycerol, and sorbitan esters in the food and cosmetic industries.

They are very mild surfactants and can be used as emulsifiers or as cleansing agents with emollient properties.

Amine Oxides

Amine oxides are produced by the oxidation of tertiary amines using a 35% hydrogen peroxide solution as the oxidizing agent.

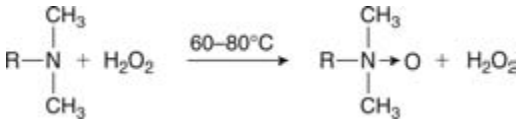
Amine oxides remain mainly nonionic in neutral and alkaline conditions (pH >7) but can become weakly cationic under acidic conditions.

In current amine oxides, the initial reactive are alkyl dimethyl amines with chain lengths ranging from C_{12} to C_{18}.

Amine oxides are compatible with all other surfactants.

Amine oxides are also known to increase the skin compatibility of detergent products.

A small amount of amine oxide increases the cloud point of nonionics.



Incorporated in shampoos, amine oxides contribute to impart viscosity, reduce eye and skin irritancy, and enhance foam properties (more creamy). They are especially suitable in slightly acidic or neutral formulas.

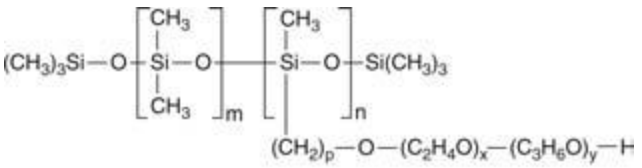
NON-HYDROCARBON SPECIALTY SURFACTANTS

Alkoxylated Polysiloxanes

Surfactants, which can be classified in the chemical group of organosilicones, are structurally derived from polydimethylsiloxanes in which some methyl are replaced by hydrophilic groups that can be of anionic, cationic, or nonionic nature.

The nonionic derivatives are mostly represented by the polyether-polydimethylsiloxane copolymers.

The general structure of these surfactants is illustrated below. The hydrophilic chain(s) generally contains EO/PO block copolymers.



Polysiloxan-polyether copolymer (*p* generally equals 0 or 3)

These surfactants are specialty ingredients and are used in very different fields (e.g., painting, foam control, phytosanitary products).

They are also used in cosmetics and hair care:

- In cosmetic or personal care products as emulsifiers (e.g., in protective creams, hydrating body milks, liquid soaps, and shave creams) and
- In hair care products (e.g., shampoos, conditioners, gels, lotions, and foams) to act as combing out auxiliaries, reduce the irritancy of surfactant system, provide improved skin feel, or control the foam. The CTFA-adopted name of these surfactants is dimethicone copolyol.

Fluorosurfactants

Fluorosurfactants form a distinct group of surfactants besides the conventional surfactants based on hydrocarbon chains.

Fluorosurfactants differ from hydrocarbon surfactants by the hydrophobic moiety of the molecule, which is made of perfluoroalkyls chains $F–(CF_2–CF_2)_n–$, in which n ranges from about 3 to 8.

As for conventional surfactants, a broad variety of hydrophilic functional groups (e.g., ethoxylated chains, sulfonates, quaternaries, and betaines) can be grafted on fluorosurfactants.

Depending on their nature, these surfactants show variable emulsifying and foaming characteristics.

Although fluorosurfactants have some potential prospects in personal care (e.g., improved hair conditioning), we are not aware of any significant application in this field. We can, however, report their use in barrier creams that require good spreading and stable o/w emulsions.

FURTHER READING

Rieger MM. Surfactant Encyclopedia. 2nd ed. Carol Stream: Allured Publishing, 1996.
Ash M, Ash I. Handbook of Industrial Surfactants. An international Guide to More Than 16.000 Products by Trade Name, Application, Composition and Manufacturer. Aldershot: Gower, 1993.
Falbe J. Surfactants in Consumer products. Theory, Technology and Application. Berlin/New York: Springer, 1987.
Lange KR. Detergents and Cleaners: A Handbook for Formulators. München: Hanser Publishers, 1994.
Porter MR. Handbook of Surfactants. London: Blackie Academic & Professional, 1991.

72 Encapsulation to Deliver Topical Actives

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INTRODUCTION

The insertion of active ingredients into protective boundaries that can isolate, disperse, mobilize, enhance activity, and transport these ingredients in different barriers has been and is a very important tool for the cosmetics industry.

The effects of encapsulation on the performance of ingredients can be rated by the following criteria:

- Transport them across biological media,
- Increase their active lifetime,
- Improve ingredients' solubility in desired media,
- Protect the body from possible poisonous side effects of ingredients, and
- Concentrate active ingredients in the desired biological target area.

Various encapsulation systems have been studied successfully in the past 30 years, with the aim of controlling the drug release and improving the efficacy and selectivity of the formulations. Among the techniques, nanotechnology is one of the most prominent factors of the scientific revolution we are witnessing. Good reviews, including topical applications, are made in literature (1–6). As for biotechnology, nanotechnology is the outcome of an interdisciplinary, new approach to old technological issues ranging from device manufacturing to drug design.

Nanotechnology is considered by many as the logical step in science, integrating engineering with biology, chemistry, and physics (7). Nanometric systems have a great surface area, which renders them highly satisfactory for application on the skin (8). In the last couple of years, the term "nanotechnology" has been inflated and has almost become synonymous for things that are innovative and highly promising. On the other hand, it is also the subject of considerable debate regarding the open question on toxicological and environmental impact (9,10).

The various nano formulations currently being used in cosmetics are

- nanoemulsions (submicron-sized emulsions)
- nanosuspensions (submicron-sized suspensions)
- nanospheres (drug nanoparticles in polymer matrix)
- nanotubes (sequence of C60 atoms arranged in a long thin cylindrical structure)
- nanopowders
- nanocapsules (encapsulated drug nanoparticles)
- lipid nanoparticles (lipid monolayer enclosing a solid lipid core)
- dendrimers (nanoscale three-dimensional macromolecules of polymer)

In this chapter, an overview of various methods of encapsulation including microparticles, nanotechnology, vesicles and special emulsions are given, as well as their advantages and limitations for topical applications.

MICROPARTICLES

Microencapsulation is a process in which very thin coatings of inert natural or synthetic polymeric materials are deposited around micro-sized particles of solids or droplets of liquids.

Commercial microparticles typically have a diameter between 1 and 1000 μm and contain 10 to 90 wt.% core. Most capsule shell materials are organic polymers, but fat and wax are also used. Various types of physical structure of the product of microencapsulation such as mononuclear spheres, multinuclear spheres, multinuclear irregular particles, etc., can be obtained depending on the manufacturing process.

A thorough description of the formation of microparticles is given by several reviews (11–14).

The efficacy of microparticles into the human skin depends on the size and the type of the formulation with which they are topically applied. Microparticles with a diameter of >1 μm do not penetrate the human skin. They are located on the skin surface and form a film, which can be used for protection against ultraviolet (UV) radiation in sunscreens, among other examples. While the penetration of the microparticles in the lipid layers of the stratum corneum is limited, they penetrate efficiently into the hair follicles up to a depth of >2 μm (15,16).

Concerning microsponge systems, each microsphere is composed of thousands of small beads wrapped together to form a microscopic sphere capable of binding, suspending, or entrapping a range of substances.

Skin absorption of benzoyl peroxide from a topical lotion containing freely dispersed drug was compared with that of the same lotion in which the drug was entrapped in a controlled-release styrene-divinylbenzene polymer system (Microsponge®). The studies done by Wester et al. (17) showed (*i*) in vivo, less benzoyl peroxide was absorbed through rhesus monkey skin from the polymeric system, (*ii*) reduced skin irritation in cumulative irritancy studies in rabbits and human, and (*iii*) when the experimental formulations were evaluated for antimicrobial activity in vivo, their efficacy was in line with that of conventional products.

Jelvehgari et al. (18) showed that the drug-polymer ratio influenced the particle size and drug release behavior of microsponges. An increase in the ratio resulted in a reduction of the release rate of benzoyl peroxide attributed to the microsponge's decreased internal porosity.

The cidofovir distribution in porcine skin, after topical application of microparticles and drug solution, was determined by horizontal slicing of the skin. The amount of cidofovir found in the epidermis was higher with microparticles than with the control solution, and the quantity retained decreased with depth (19).

Scalia et al. (20) loaded lipid microparticles with the sunscreen agent 4-methylbenzylidene camphor (4-MBC). The influence of the microparticle's carrier system on percutaneous penetration was evaluated after its introduction in a topical formulation (emulsion). The amount of sunscreen penetrating the stratum corneum was greater for the emulsion containing nonencapsulated 4-MBC compared with the formulation with the sunscreen-loaded microparticles.

Lipid microparticles loaded with butyl methoxydibenzoylmethane (BMDBM) were prepared and evaluated for skin permeation in vivo and in vitro. Following in vivo human skin application of an oil-in-water (O/W) emulsion containing 2% of BMDBM loaded in lipospheres, 15% of the applied sunscreen was found accumulated in the uppermost layers of the stratum corneum (21).

On the basis of this literature, it can be concluded that the general release of active substances from microparticulate systems is directly or indirectly related to some factors:

- The presence of pores in the microparticulate system; a good example is provided by microsponges.
- Particle size: particles with >1 μm are retained in the skin surface or deposited on the surface of the hair follicles.
- Intrinsic characteristics of delivery systems being directly related to the process and materials used.

NANOPARTICLES

Nanoparticles can be defined as submicron (<1 μm) colloidal systems, generally, but not necessarily made of polymers (biodegradable or not). According to the preparation process used, nanocapsules or nanospheres can be obtained. Nanocapsules are vesicular systems in

which the drug is confined to a cavity surrounded by a unique polymeric membrane. Nanospheres are matrix systems in which the drug is dispersed throughout the particles.

There are many methods for preparing nanoparticles, whereas the most common methods are the required polymerization reaction or from a preformed polymer.

Good reviews with methods of preparation for nanoparticles can be found in the literature (6,22–25). Other reviews (26,27) reports that the system of solid lipid nanoparticles (SLNs) is one of the most attractive systems for encapsulating cosmetic ingredients.

Drug release from colloidal carriers is dependent on both the type of carrier and the loading mechanisms involved.

Nanospheres

Release from nanospheres may be different according to the drug entrapment mechanism involved. When the drug is superficially adsorbed, the release mechanism can be described as a partitioning process (rapid and total release if sink conditions are met). When the drug is entrapped within the matrix, diffusion plus bioerosion will be involved with a biodegradable carrier, whereas diffusion will be the only mechanism if the carrier is not biodegradable. The entrapment rate within the matrix of nanospheres may lead to a sustained release, which may be related to the polymer's biodegradation rate.

Nanocapsules

Release from nanocapsules is related to partitioning processes within immiscible phases. The equilibrium between the carrier (loaded drug) and the dispersing aqueous medium (free drug) is dependent both on the partition coefficient of the molecule between the oily and aqueous phases, and on the volume ratio of these two phases. This means that the amount released is directly related to the dilution of the carrier and that release is practically instantaneous when sink conditions exist.

Because of the increasing use of nanotechnology, the number of papers on topical nanoparticles has increased proportionally. Following is a short summary of some of these works.

Shim et al. (28) evaluated the effect of size of self-assembled nanoparticles on skin penetration of minoxidil in vitro and in vivo. Self-assembled 40 and 130 nm nanoparticles were prepared with poly (caprolactone)-block-poly(ethyleneglycol) and applied onto the skin of both hairy and hairless guinea pigs in the Franz diffusion cell. In hairy guinea pig skin, the permeation of the minoxidil incorporated in 40 nm nanoparticles was 1.5-fold higher in the epidermal layer and 1.7-fold higher in the receptor solution than that of 130 nm nanoparticles.

Lombardi et al. (29) describe techniques to characterize drug loading to carrier systems and skin penetration profiles by using the lipophilic dye nile red as a model agent. Nile red was incorporated into the lipid matrix of SLNs and nanostructured lipid. Nile red concentrations were followed by image analysis of vertical sections of pigskin treated with dye-loaded nanoparticular dispersions and an O/W cream for four and eight hour in vitro. Following the SLNs dispersions, dye penetration increased about over fourfold. Nanostructured lipid carriers turned out to be less potent (<3-fold increase), and penetration appeared even more reduced when applying nanoemulsions.

Trimethylpsoralen (TMP) permeates moderately the skin barrier. Three formulations were performed. Each form (liposomes, nanospheres, and EtOH solution) contained 0.05% of TMP. The results indicated that the controlled release of TMP by incorporation into PLG nanospheres [poly (DL-lactide-co-glycolide)] may increase drug content in the skin, while maintaining a minimal percutaneous absorption (30).

The nature of the vehicle used can enhance or block the percutaneous absorption of UV filters. Luppi et al. (31) suggested that polymeric nanoparticles hydrophobically modified (polyvinyl alcohol with fatty acids) were able to prevent benzophenone-3 movement toward the skin. Nanoparticles prepared with a high degree of substitution (fatty acids) prevented benzophenone-3 percutaneous absorption.

Santos Maia et al. (32) incorporated prednicarbate [(PC), 0.25%] into SLNs of various compositions. Conventional PC cream of 0.25% and ointment served as reference. Local tolerability as well as drug penetration and metabolism were studied in excised human skin and reconstructed epidermis. The drug recovery from the acceptor medium was about 2% of the applied amount following PC cream and ointment, but 6.65% following nanoparticle dispersion.

The influence of cross-linked pullulan nanoparticles on human dermal fibroblasts in vitro has been assessed in terms of cell adhesion, cytotoxicity, and light microscopy. Results from cell adhesion/viability assay suggest that the pullulan nanoparticles are nontoxic to cells and do not cause any distinct harm to cells. Fibroblasts were healthy and maintained their morphology and adhesion capacity (33).

Wissing (34) showed that the crystalline cetylpalmitate in SLNs has the ability of reflecting and scattering UV radiation on their own, thus leading to photoprotection without the need for molecular sunscreens. The photoprotective effect after the incorporation of the molecular sunscreen 2-hydroxy-4-methoxybenzophenone (Eusolex® 4360) into the SLN dispersion was increased threefold compared with a reference emulsion. Further, film formation on the skin was investigated by scanning electron microscopy, showing particle fusion due to water evaporation and formation of a dense film.

In another study (35), the same team showed the influence of the carrier on the release rate in vitro. It could be decreased by up to 50% with the SLN formulation. In vivo, penetration of oxybenzone into stratum corneum on the forearm was investigated by the tape-stripping method. It showed that the rate of release is strongly dependent upon formulation and could be decreased by 30% to 60% in SLN formulations.

SLN dispersions with different crystallinity indices of the lipid matrix were produced. Their characterization and occlusion factor were determined after 6, 24, and 48 hours. It was shown that the occlusion factor depends strongly on the degree of the crystallinity of the lipid matrix (36).

The actual sunscreens contain nanoparticles of titanium dioxide (TiO_2) or zinc oxide (ZnO), which are colorless and reflect UV. Most available data suggest that insoluble nanoparticles do not penetrate human skin. In vitro cytotoxicity studies on TiO_2 report uptake by cells, oxidative cell damage, or genotoxicity that should be interpreted with caution, since such toxicities may be secondary to phagocytosis of the cells exposed to high concentrations of insoluble particles.

There is little evidence that smaller particles have greater effects on the skin. Overall, the current knowledge suggests that nanomaterials such as nano-sized vesicles or TiO_2 and ZnO nanoparticles currently used in cosmetic preparations or sunscreens pose no risk to human skin or health, although other nanoparticles may have properties that warrant safety evaluation on a case-by-case basis before human use (37).

Other nanoscale materials such as carbon *fullerene* have been used in some cosmetic products because of their antioxidative properties. Bianco et al. (38) showed a good review on functionalized carbon nanotubes that are emerging as new tools in the field of nanobiotechnology and nanomedicine. They can be easily manipulated and modified by encapsulation with biopolymers or by covalent linking of solubilizing groups to the external walls and tips.

Certain chemical forms of fullerenes have been reported to elicit oxidative damage to cell in culture experiments (39). Adverse effects have not been reported following the application of fullerenes in formulations such as topically applied lotions or creams. Two studies are often cited about fullerenes, Oberdorster (40) and Zhu et al. (41) reported toxicity in many aquatic species treated with fullerenes in water. It is important to mention that the toxicological potential of nanoscale materials such as fullerenes can be evaluated through current safety evaluation processes, and these materials should be only used in products once their safety is confirmed.

Dendrimers are artificial macromolecules, which have the structure of a tree. They are hyperbranched and monodispersed three-dimensional molecules with defined molecular weights, large numbers of functional groups on the surface, and well-established host-guest entrapment properties. Dendrimers may be engineered to meet the specific needs of biologically active agents, which can either be encapsulated within dendrimers or chemically attached to these units. Recently, dendrimers have successfully proved themselves as promising nanocarriers for drug delivery because they can render drug molecules a greater water solubility, bioavailability, and biocompatibility (42,43).

Finally, *DNA vaccines* have been shown to elicit both broad humoral and cellular immune responses. Recent human clinical studies with needle-free injection devices and the gene gun have validated the direct targeting concept of dendritic cells (Langerhan's cells) in the viable epidermis of the skin. Cui and Mumper (44) showed that several different chitosan-based nanoparticles containing plasmide DNA (pDNA) resulted in both quantifiable levels of luciferase expression in mouse skin 24 hours after topical application as well as significant

antigen-specific Immunoglobulin G (IgG) titers at 28 days. In another study (45), the same authors showed that pDNA-coated nanoparticles, especially the mannan-coated pDNA nanoparticles with DOPE (dioleoyl phosphatidyl ethanolamine), resulted in significant enhancement in both antigen-specific IgG titers (16 fold) and splenocyte proliferation over "naked" pDNA alone.

Regarding the action mode of nanoparticles, we can say that they are associated with the skin surface, facilitating drug transport by changing the vehicle/stratum corneum partition coefficient.

NANOEMULSIONS

The main characteristic of nanoemulsions is the droplet size that must be inferior to 1 μm. Usually, the average droplet size is between 100 and 500 nm. The particles can exist as water-in-oil (W/O) and O/W forms where the core of the particle is either water or oil, respectively. The terms "submicron emulsion" (SME) and "miniemulsion" are used as synonyms. Usually, nanoemulsions contain 10% to 20% oil stabilized with 0.5% to 2% of an emulsifyng agent.

Emulsions prepared by use of conventional apparatus, e.g., electric mixers and mechanical stirrers, show large droplet sizes and wide particle distribution. The techniques usually employed to prepare nanoemulsions involve the utilization of ultrasound, evaporation of solvents (46), PIT (phase-inversed temperature) method (47), two-stage homogenizer (48,49), and the microfluidizer (50,51).

In recent years, nanoemulsions have been gaining more and more attention in the cosmetic industry, which has rapidly grasped its benefits. The patents assert that these systems penetrate through the skin to a greater extent compared with usual topical compositions.

Nanoemulsions are so strongly compressed that they become ultralight and, like vesicular systems, constitute a new form that could prove extremely fruitful for the release of substances.

Nanoemulsions have a much higher surface area and free energy than macroemulsions, which make them an effective transport system. They can be formulated in a variety of formulations such as foams, creams, liquids, and sprays.

Many studies have shown that nanoemulsion formulation processes improved both transdermal and dermal delivery properties in vitro (52–55) as well as in vivo (56,57).

The determination of silicones and hydrogenated didecenes deposited on human hair from shampoo applications is described by Haake et al. (58). A transparent shampoo containing 1.8% of hydrogenated didecenes delivered via a nanoemulsion showed a good performance profile.

The penetration of octyl methoxycinnamate formulated in nanocapsules was compared with one obtained from a nanoemulsion and one from a conventional O/W emulsion. The percutaneous penetration, assessed by the tape-stripping technique, demonstrated that nanoemulsion increased the extent of octyl methoxycinnamate penetration. The accumulation in the skin was significantly greater with nanoemulsion than with emulsion or nanocapsules (59).

Calderilla-Fajardo et al. (60) evaluated the influence of sucrose laureate and sucrose oleate on in vivo percutaneous penetration of octyl methoxycinnamate. The sunscreen agent was formulated in nanoemulsions, nanocapsules, and conventional O/W emulsions. The results showed that nanoemulsions of sucrose laureate exhibited the highest stratum corneum penetration compared with the other formulations.

The skin transport of inulin incorporated in O/W nanoemulsions was found to be significantly higher (5–15-fold) than that obtained with micellar dispersions or aqueous controls. The results suggest that W/O nanoemulsions are more compatible with the lipophilic sebum environment of the hair follicle (61,62).

VESICLES

In the 1960s, Bangham et al. (63) clearly demonstrated that the dispersion of natural phospholipids in aqueous solutions leads to the formation of "closed vesicles structures," which morphologically resemble cells. Since 1975 (64), vesicles have been prepared from

surfactants. Mezei and Gulasekharam (65) published the first paper to report the effectiveness of liposomes in the skin.

In 1986, the first commercial product incorporating liposomes appeared on the market (Capture®). At the same time, a synthetic one made by nonionic surfactants (66) was also launched (Niosomes®).

These microscopic vesicles contain from one to several concentric lipid bilayers with intercalated aqueous compartments. Transepidermal penetration of the content of vesicles is proportional to the "fluidity" of their lipids and negative charge. Several drugs and cosmetics in this form are already commercially available and successfully used, presenting a better dose-effect ratio and provoking less side effects.

In the 1990s, transfersomes, i.e., lipid vesicles containing large fractions of fatty acids, were introduced. Transfersomes (67–69) consist of a mixture of a lipidic agent with a surfactant. In consequence, their bilayers are much more elastic than those of most liposomes.

Niosomes can be prepared from various classes of nonionic surfactants, e.g., polyglycerol alkyl ethers (66,70), glucosyl dialkyl ethers (71), crown ethers, and polyoxyethylene alkyl ethers and esters (72).

The ethosomes are soft phospholipid vesicles, whose size can be modulated from tens of nanometers to microns. These vesicular systems have been found to be very efficient for enhanced delivery of molecules with different physicochemical characteristics to/through the skin. They can be modulated to permit enhancement into the skin strata as far as the deep dermis or to facilitate transdermal delivery of lipophilic and hydrophilic molecules (73).

Transfersomes have been shown to be versatile carriers for the local and systemic delivery of various steroids, proteins, and hydrophilic macromolecules (69). The mechanism proposed by the author for transfersomes is that they are highly deformable, thus facilitating their rapid penetration through the intercellular lipids of the stratum corneum. The osmotic gradient, caused by the difference in water concentrations between the skin surface and skin interior, has been proposed as the major driving force for transfersome penetration (68).

The effectiveness of vesicles has been investigated by several research groups. Liposomes, in particular, have received a great deal of attention (74–77).

In several studies, the diffusion of a drug was facilitated or achieved certain selectivity into human and nonhuman skin by vesicle encapsulation (85% of the papers). Other studies show that the influence of vesicles on drug transport is negligible (10%). Only a few papers claimed that the vesicles have no effect on the skin (5%).

OTHER ENCAPSULATION SYSTEMS

Cyclodextrins (CDs) are cyclic oligosaccharides containing at least 6 D–(+) glucopyranose units attached by α-(1, 4) glucosidic bonds. The three natural CDs, α-, β-, and γ-CDs (with 6, 7, or 8 glucose units, respectively) differ in their ring size and solubility.

CDs have been used to optimize local and systemic dermal drug delivery. Applications of CDs in transdermal drug delivery include enhancement of drug release and/or permeation, drug stabilization in formulation or at absorptive site, alleviation of drug-induced local irritation, sustaining of drug release from vehicle, and alteration of drug bioconversion in the viable skin (78).

CDs increase topical availability of drugs by solubilizing lipophilic drugs and thus increasing availability of the drug at the skin surface. The drugs are therefore solubilized without modifying their intrinsic property of lipophilicity. Polymers, such as hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), and carboxymethylcellulose sodium (CMC), may be added to increase permeability. The type and amount of CDs are two important parameters for maximizing the permeability of the drug in formulation. Finally, use of other permeation enhancers has been seen to aid the CD-mediated delivery of drugs, such as prostaglandin E1 through the skin (79).

Microemulsions are stable dispersions in the form of spherical droplets, whose diameter is in the range of 10 to 100 nm. They are composed of oil, water, and usually surfactant and cosurfactant.

These systems show structural similarity to micelles and inverse micelles, resulting in O/W or W/O microemulsions, respectively. They are highly dynamic systems showing fluctuating surfaces caused by forming and deforming processes.

The main characteristics of microemulsions are the low viscosity associated with a Newtonian-type flow, a transparent or translucid appearance, isotropic, and thermodynamic stability within a specific temperature setting. Certain microemulsions may thus be obtained without heating, simply by mixing the components as long as they are in a liquid state.

One of the conditions for microemulsion formation is a very small, rather than a transient negative, interfacial tension. This is rarely achieved by the use of a single surfactant, almost always necessitating the addition of a cosurfactant. The presence of a short chain alcohol, e.g., can reduce the interfacial tension from about 10 mN/m to a value less than 10^{-2} mN/m. Exceptions to this rule are provided by nonionic surfactants which, at their phase inversion temperature, also exhibit very low interfacial tensions.

Since microemulsions were discovered approximately six decades ago, their applications in several fields, including cosmetics, have been increased because of their good appearance, thermodynamic stability, high solubilization power, and ease of preparation (80).

Liquid crystals are defined as the intermediary state between solid and liquid and also called mesomorphous or crystalline phase, presenting characteristics of the mentioned physical states. That intermediary phase, in simple emulsions, can act as forms of encapsulation of drugs providing its controlled liberation and can increase cutaneous hydration. These characteristics evidence the differentiation of the developed formulations and the use of the same ones in the release of new cosmetic vehicles (81).

Multiple emulsions are emulsions in which the dispersion phase contains another dispersion phase. Thus, water-in-oil-in-water (W/O/W) emulsion is a system in which the globules of water are dispersed in globules of oil, and the oil globules are themselves dispersed in an aqueous environment. A parallel arrangement exists in oil-in-water-in-oil (O/W/O) type.

The first commercial use of a W/O/W-type multiple emulsion is unique moisturizing by Lancaster, which was introduced on the market in 1991.

Cosmetic applications of multiple emulsions have been protected in the patents issued for their composition. The patents show that multiple emulsions are highly recommended for all kinds of cosmetic applications: sunscreens, makeup removers, cleansers, and nutritive, hydrating, and cooling products.

Ferreira (82,83) showed three emulsions types (W/O/W, O/W, and W/O) containing a water-soluble molecule (glucose). The release of glucose from the O/W emulsion was the fastest. From the W/O emulsion, it was the slowest, while the one from the W/O/W emulsion was intermediate. The W/O/W emulsion showed some tendency toward steady state during the first 3 to 12 hours, and the flux was found to be 1.7 times greater than that from the W/O emulsion.

CONCLUSION

Encapsulation is now part of our everyday life and should be used to improve our life. However, much care needs to be taken to decipher completely and exhaustively the effects of nanomaterials before they may be commercialized.

Molecular biology has provided us with tools to identify and build genetic materials that can be used for treatment of hereditary diseases. Developing a carrier for gene therapy is one of the main challenges that the encapsulation field faces today.

What will the future bring us? We have already indicated where, on the basis of our present knowledge, encapsulation in many different vectors offer a rational advantage as active carrier systems to the skin. Therefore, efforts should be made to obtain a better understanding concerning the mechanisms of these systems at the molecular and supra-molecular level. This could lead to new formulation processes and open new prospects in the area of active delivery by means of encapsulated systems.

REFERENCES

1. Couvreur P, Barrat G, Fattal E, et al. Nanocapsule technology: a review. Crit Rev Ther Drug Carrier Syst 2002; 19:99–134.
2. Müller RH, Radtkeb M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev 2002; 54(suppl 1):S131–S155.

3. Vauthier C, Dubernet C, Fattal E, et al. Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications. Adv Drug Deliv Rev 2003; 55(4):519–548.
4. Champion JA, Katare YK, Mitragotri S. Particle shape: a new design parameter for micro- and nanoscale drug delivery carriers. J Control Release 2007; 121(1–2):3–9.
5. Dutta RC. Drug carriers in pharmaceutical design: promises and progress. Curr Pharm Des 2007; 13(7): 761–769.
6. Guterres SS, Alves MP, Pohlmann A. Polymeric nanoparticles, nanospheres and nanocapsules, for cutaneous applications. Drug Targ Insights 2007; 2:147–157.
7. Lehn JM. Toward self-organization and complex matter. Science 2002; 295:2400–2403.
8. Bouchemal K, Brianon S, Perrier E, et al. Synthesis and characterization of polyurethane and poly (ether urethane) nanocapsules using a new technique of interfacial polycondensation combined to spontaneous emulsification. Int J Pharm 2004; 269(1):89–100.
9. Donaldson K, Stone V, Tran CL, et al. Nanotoxicology. Occup Environ Med 2004; 61:727–728.
10. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005; 113:823–839.
11. Deasy P. Microencapsulation and Related Drug Processes. New York: Marcel Dekker, 1984.
12. Kondo A. Microcapsule Processing and Technology. New York: Marcel Dekker, 1979.
13. Thies C. A survey of microencapsulation processes. In: Benita S, ed. Microencapsulation, Methods and Industrial Applications. New York: Marcel Dekker, 1996:1–9.
14. Jacobs IC, Mason NS. Polymeric delivery systems. In: Elnolkaly MA, Piatt DM, Charpentier BA, eds. ACS Symposium Series 520, American Chemical Society. 1993: 1–17
15. Rolland A, Wagner N, Chatelus A, et al. Site-specific drug delivery to pilosebaceous structures using polymeric microspheres. Pharm Res 1993; 10(12):1738–1744.
16. Lademann J, Schaefer H, Otberg N, et al. Penetration of microparticles into human skin. Hautarzt 2004; 55(12):1117–1119.
17. Wester RC, Rajesh P, Nacht S, et al. Controlled release of benzoyl peroxide from a porous microsphere polymeric system can reduce topical irritancy. J Am Acad Dermatol 1991; 24(5):720–726.
18. Jelvehgari M, Siahi-Shadbad MR, Azarmi S, et al. The microsponge delivery system of benzoyl peroxide: preparation, characterization and release studies. Int J Pharm 2006; 308(1–2):124–132.
19. Santoyo S, Ga de Jalón E, Ygartua P, et al. Optimization of topical cidofovir penetration using microparticles. Int J Pharm 2002; 242(1–2):107–113.
20. Scalia S, Mezzena M, Iannuccelli V. Influence of solid lipid microparticle carriers on skin penetration of the sunscreen agent, 4-methylbenzylidene camphor. J Pharm Pharmacol 2007; 59(12): 1621–1627.
21. Iannuccelli V, Coppi G, Sergi S, et al. In vivo and in vitro skin permeation of butyl methoxydibenzoylmethane from lipospheres. Skin Pharmacol Physiol 2007; 21(1):30–38.
22. Kreuter J. Nanoparticles—Preparation and applications. In: Donbrow Med. Microcapsules and Nanoparticles in Medicine and Pharmacy. Boca Raton, Florida: CRC Press, 1992:125–148.
23. Couvreur P, Coarraze G, Devissaguet JP, et al. Nanoparticles: preparation and characterization. In: Benita S, ed. Microencapsulation, Methods and Industrial Applications. New York: Marcel Dekker, 1996:183–211.
24. Villalobos-Hernández JR, Müller-Goymann CC. Artificial sun protection: sunscreens and their carrier systems. Curr Drug Deliv 2006; 3(4):405–415.
25. Parekh HS. The advance of dendrimers-a versatile targeting platform for gene/drug delivery. Curr Pharm Des 2007; 13(27):2837–2850.
26. Müller RH. Particulate systems for the controlled delivery of active compounds in pharmaceutics and cosmetics. In: Diederichs JE, Müller RH, eds. Future Strategies for Drug Delivery with Particulate Systems. Stuttgart: CRC Press, 1998:73–90.
27. Müller RH, Mehnert W, Dingler A, et al. Solid lipid nanoparticles (SLNTM, LipopearlsTM). Proceed Int'l Symp Cont Rel Bioact Mater, 24. 1997; 923–924.
28. Shim J, Seok Kang H, Park WS, et al. Transdermal delivery of mixnoxidil with block copolymer nanoparticles. J Control Release 2004; 97(3):477–484.
29. Lombardi Borgia S, Regehly M, Sivaramakrishnan R, et al. Lipid nanoparticles for skin penetration enhancement: correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. J Control Release 2005; 110(1):151–163.
30. Lboutounne H, Guillaume YC, Michel L, et al. Study and development of encapsulated forms of 4, 5, 8-trimethylpsoralen for topical drug delivery. Drug Dev Res 2004; 61(2):86–94.
31. Luppi B, Cerchiara T, Bigucci F, et al. Polymeric nanoparticles composed of fatty acids and polyvinylalcohol for topical application of sunscreens. J Pharm Pharmacol 2004; 56(3):407–411.
32. Santos Maia C, Mehnert W, Schaller M, et al. Drug targeting by solid lipid nanoparticles for dermal use. J Drug Target 2002; 10(6):489–495.
33. Gupta M, Gupta AK. In vitro cytotoxicity studies of hydrogel pullulan nanoparticles prepared by AOT/N-hexane micellar system. J Pharm Pharm Sci 2004; 7(1):38–46.

34. Wissing SA, Müller RH. Solid lipid nanoparticles (SLN)-a novel carrier for UV blockers. Pharmazie 2001; 56(10):783–786.
35. Wissing SA, Müller RH. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J Control Release 2002; 81(3):225–233.
36. Wissing S, Müller R. The influence of the crystallinity of lipid nanoparticles on their occlusive properties. Int J Pharm 2002; 242(1–2):377–379.
37. Nohynek GJ, Lademann J, Ribaud C, et al. Grey good on the skin? Nanotechnology, cosmetic and sunscreen safety. Crit Rev Toxicol 2007; 37(3):251–277.
38. Bianco A. Carbon nanotubes for the delivery of therapeutic molecules. Expert Opin Drug Deliv 2004; 1(1):57–65.
39. Sayes CM, Gobin AM, Ausman KD, et al. Nano-C60 cytotoxicity is due to lipid peroxidation. Biomaterials 2005; 26(36):7587–7595.
40. Oberdorster E. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. Environ Hlth Perspect 2004; 112(10): 1058–1062.
41. Zhu S, Oberdorster E, Haasch ML. Toxicity of an engineered nanoparticle (fullerene C60) in two aquatic species. Environ Res 2006; 62(suppl):S5–S9.
42. Jain NK, Asthana A. Dendritic systems in drug delivery applications. Expert Opin Drug Deliv 2007; 4(5):495–512.
43. Cheng Y, Wang J, Rao T, et al. Pharmaceutical applications of dendrimers: promising nanocarriers for drug delivery. Front Biosci 2008; 13:1447–1471.
44. Cui Z, Mumper RJ. Chitosan-based nanoparticles for topical genetic immunization. J Control Release 2001; 75(3):409–419.
45. Cui Z, Mumper RJ. Topical immunization using nanoengineered genetic vaccines. J Control Release 2002; 81(1–2):173–184.
46. Yu W, Tabosa do Egito ES, Barrat G, et al. A novel approach to the preparation of injectable emulsions by a spontaneous emulsification process. Int J Pharm 1993; 89:139–146.
47. Tadros T, Izquierdo P, Esquena J, et al. Formation and stability of nano-emulsions. Adv Colloid Interface Sci 2004; 108–109:303–318.
48. Hansrani PK, Davis SS, Groves MJ. The preparation and properties of sterile intravenous emulsions. J Parenter Sci Technol 1983; 37:145–150.
49. Yalabik-Kas HS, Erylmaz S, Hincal AA. Formation, stability and toxicity studies of intravenous fat emulsions. STP Pharm 1985; 1:12–19.
50. Washington C, Davis SS. The production of parenteral feeding emulsions by microfluidizer. Int J Pharm 1988; 169–176.
51. Lidgate DM, Fu RC, Fleitman JS. Using a microfluidizer to manufature parenteral emulsions. Pharm Technol 1990; 14:30–33.
52. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterization and transdermal drug delivery potentials of microemulsion systems. J Control Rel 2000; 69:421–433.
53. Alvarez-Figueroa MJ. Blanco-mendez.transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int J Pharm 2001; 215:57–65.
54. RheeYS, Choi JG, Park ES, et al. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm 2001; 228:161–170.
55. Lee PJ, Langer R, Shastri VP. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm Res 2003; 20:264–269.
56. Kreilgaard M. Dermal pharmacokinetics of microemulsion formulations determined by in-vitro microdialysis. Pharm Res 2001; 18:367–373.
57. Kreilgaard M, Kemme MJB, Burggraaf J, et al. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res 2001; 18:593–599.
58. Haake HM, Lagren ÃH, Brands A, et al. Determination of the substantivity of emollients to human hair. J Cosmet Sci 2007; 58(4):443–450.
59. Olivera-Martinez BI, Cázares-Delgadillo J, Calderilla-Fajardo SB, et al. Preparation of polymeric nanocapsules containing octyl methoxycinnamate by the emulsification-diffusion technique: penetration across the stratum corneum. J Pharm Sci 2005; 94(7):1552–1559.
60. Calderilla-Fajardo SB, Cázares-Delgadillo J, Villalobos-Garcia R, et al. Influence of sucrose esters on the in vivo percutaneous penetration of octylmethoxycinnamate formulated in nanocapsules, nanoemulsion, and emulsion. Drug Dev Ind Pharm 2006; 32(1):107–113.
61. Wu H, Ramachandran C, Bielinska AU, et al. Topical transfection using plasmid DNA in a water-in-oil nanoemulsion. Int J Pharm 2001; 221(1–2):23–34.
62. Wu H, Ramachandran C, Weiner ND, et al. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm 2001; 220(1–2):63–75.
63. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965; 13:238–252.

64. Gebicki JM, Hicks M. Preparation and properties of vesicle enclosed by fatty acid membranes. Chem Phys Lipids 1975; 16:142–160.
65. Mezei M, Gulasekharam V. Liposomes—a selective drug delivery system for the topical route of administration. Life Sci 1980; 26:1473–1477
66. Handjani-Vila RM, Ribier A, Rondot B, et al. Dispersions of lamellar phases of non-ionic lipids in cosmetic products. Int J Cosmet Sci 1979; 1:303–314.
67. Planas ME, Gonzalez P, Rodriguez L. Non invasive percutaneous induction of topical analgesia by a new type of drug carriers and prolongation of the local pain-insensitivity by analgesic liposomes. Anesth Analg 1992; 95:614–621.
68. Cevc G, Blume G. Lipid vesicles penetrate into the skin owing to the transdermal osmotic gradients and hydration force. Bioch Biophys Acta 1992; 1104:226–232.
69. Cevc G, Gebauer D, Stieber J, et al. Ultraflexible vesicles, transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Bioch Biophys Acta 1998; 1368:201–215.
70. Baillie AJ, Florence AT, Hume LR, et al. The preparation and properties of niosome non-ionic surfactant vesicles. J Pharm Pharmacol 1985; 37:863–868.
71. Van Hal DA, Bowstra JA, Junginger HE. Preparation and characterization of new dermal dosage form for antipsoriatic drug, dithranol, based on non ionic surfactant vesicles. Eur J Pharm Biopharm 1992; 38:47s.
72. Hofland HEJ, Bowstra JA, Ponec M, et al. Interactions of non-ionic surfactant vesicles with cultured keratinocytes and human skin in vitro. J Contr Rel 1991; 16:155–168.
73. Touitou E, Dayan N, Bergelson L, et al. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release 2000; 65(3):403–418.
74. Bowstra JA, Junginger HE. Non-ionic surfactant vesicles (niosomes) for oral and transdermal administration of drugs. In: Puisieux F, Couvreur P, Dellatre J, Devissaguet JP, eds. Liposomes, New Systems and New Trends in their Applications. 1995: 101–121.
75. Posner R. Liposomes. J Drugs Dermatol 2002; 1(2):161–164.
76. Redziniak G. Liposomes and skin: past, present, future. Pathol Biol (Paris) 2003; 51(5):279–281.
77. El Maghraby GM, Williams AC, Barry BW. Can drug-bearing liposomes penetrate intact skin? J Pharm Pharmacol 2006; 58(4):415–429.
78. Matsuda H, Arima H. Cyclodextrins in transdermal and rectal delivery. Adv Drug Deliv Rev 1999; 36:81–99.
79. Uekama K, Adachi H, Irie T, et al. Improved transdermal delivery of prostaglandin E1 though hairless mouse skin: combined use of carboxymethyl-ethyl-b-cyclodextrin and permeation enhancers. J Pharm Pharmacol 1992; 44:119–121.
80. Boonme P. Applications of microemulsions in cosmetics. J Cosmet Dermatol 2007; 6(4):223–228.
81. Massaro RC, Zabagli MS, Souza CR, et al. O/w dispersions development containing liquid crystals. Boll Chim Farm 2003; 142(7):264–270.
82. Ferreira LAM, Doucet J, Seiller M, et al. In vitro percutaneous absorption of metronidazole and glucose: comparison of o/w, w/o/w and o/w systems. Int J Pharm 1995; 121:169–179.
83. Ferreira LAM, Seiller M, Grossiord JL, et al. Vehicle influence on in vitro release of glucose: w/o, w/o/w and o/w systems compared. J Cont Rel 1995; 33:349–356.

73 Elastic Vesicles as Topical/Transdermal Drug Delivery Systems

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INTRODUCTION

The topical/transdermal (TT) delivery route for drug administration has many advantages over other pathways, including avoiding the hepatic first-pass effect, continuous drug delivery, fewer side effects, and improved patient compliance (1). A major obstacle to TT drug delivery is the low penetration of drugs through skin. The stratum corneum (SC) provides a principal barrier to TT delivery of applied drugs and consists of corneocytes that are embedded in an intercellular lipid matrix composed of ceramides, free fatty acids, and cholesterol (CHOL) (2). Several approaches have been used to weaken this skin barrier and to improve TT drug delivery (3–8).

One possibility for increasing the penetration of drugs is the use of vesicular systems such as liposomes. Because of their biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs, liposomes have been investigated as parenteral drug and antigen-carrier systems and more recently as TT drug delivery systems (9–18). Despite improvements in TT delivery, conventional liposomes were not efficient at delivering transdermally across the skin because they do not deeply penetrate the skin, but rather remain confined to the upper layer of the SC. Thus, several investigators developed novel elastic vesicles to deeply and easily penetrate across the skin (19–21).

In the early 1990s, novel series of liquid-state vesicles have been developed, and these vesicles could better facilitate drug transport across the skin as compared with conventional vesicles. Novel types of vesicular systems have been recorded to penetrate intact skin if applied nonocclusively in vivo by virtue of their very high and self-optimizing deformability. Elastic vesicles are classified with phospholipid and detergent-based types (Table 1). Because of high flexibility, elastic vesicles squeeze through small pores in SC less than the vesicle sizes. Elastic vesicles were more efficient at delivering a low- and high-molecular-weight drug to the skin in terms of quantity and depth (24–26,33–38). The precise quantity and depth of elastic vesicles' penetration through skin depends on the carrier type, the total mass applied, the entrapment efficiency, and the detailed application conditions such as occlusion, pretreatment of vesicles, duration, and application volume. Elastic vesicles prolonged the release and showed better biological activity in comparison with conventional liposomes and ointments.

This review focuses on the effect of elastic vesicles for enhancing the penetration chemicals, and it defines action mechanism and optimal condition of elastic vesicles.

ELASTIC VESICLES–SKIN INTERACTION

Vesicle–skin interactions can occur either at the skin surface or in the deeper layers of the SC. Hofland et al. (39) and Abraham and Downing (40) have shown fusion and adsorption of vesicles onto the SC surface, forming stacks of lamellae and irregular structures on top of the skin. Vesicle–skin interactions are strongly influenced by the composition of the vesicles, resulting in differences in their phase, state, and elasticity. Liquid-state vesicles have been shown to have superior to gel-state vesicles (41). When comparing the interactions of elastic and rigid vesicles with hairless mouse skin in vivo, only the elastic liquid-state vesicles affected the ultrastructure of the viable tissue. No changes in the ultrastructure were observed with any of the conventional liposomes (42).

Table 1 Drug Skin Penetration from Phospholipid- and Detergent-Based Elastic Vesicles

Drug (comments)	Animal	Composition	Enhancing factor	Reference
Dipotassium Glycyrrhizinate (KG)	Pig	PC:KG (4:1)	5.9	22
		HPC:KG (4:1)	5.5	
Methotrexate	Pig	PC:KG (2:1)	5.2	23
		HPC:KG (2:1)	5.9	
Dexamethasone	Rat	PC:CHOL (7:3)	1.0	24
		PC:deoxycholate (85:15)	2.2	
		PC:Tween-80 (85:15)	1.9	
		PC:Span-80 (85:15)	2.3	
Diclofenac	Rat	Commercial form	1.0	25
		Lotion-like transfersomes	30–100	
Gap junction protein (Antibody production)	Mouse	Soybean PC	1.0	26
		PC/sodium cholate/SDS	4.7	27
Insulin (decrease of blood glucose)	Mouse	PC liposomes or micelle	No change	
		PC/cholate (8.7:1.3)	20–30%	
Cyclosporin A	Mouse	PC/cholate (10:2.8)	16.2	28
Estradiol	Human	PC/cholate (84:16)	18	29.30
		PC/Span 80 (84:16)	16	
		PC/Tween 80 (84:16)	15	
		PC/oleic acid (84:16)	13	
5-Fluorouracil	Human	PC/cholate (84:16)	6.9–13.2	31
Rotigotine	Human	L-595/PEG-8-L (50:50)	30.6	32
Pergolide	Human	L-595/PEG-8-L (50:50)	2.7	33

Abbreviations: PC, phosphatidylcholine; KG, dipotassium glycyrrhizinate; HPC, hydrogenated lecithin; CHOL, cholesterol; SDS, sodium dodecyl sulfate.
Source: From Refs. 22–33.

Cevc and Blume (19) suggested that elastic vesicles (Transfersomes) were able to penetrate through intact SC under the influence of a transepidermal osmotic gradient. Although the transport of most compounds is increased during occlusive application, Cevc and Blume (19) have suggested that elastic vesicles (Transfersomes) are most efficient under nonocclusive conditions. Nonocclusive conditions are necessary to create a transepidermal osmotic gradient, which is believed to be the driving force for elastic transport into the skin. The osmotic gradient is caused by the difference in water concentrations between the skin surface and skin interior. Transfersomes are highly deformable, and this property facilitates their rapid penetration through the intercellular lipid pathway of the SC. Schatzlein and Cevc (43) reported the existence of irregularities within the intercellular lipid packing of murine SC that can act as virtual channels through which Transfersomes could penetrate.

In the case of detergent-based elastic vesicles, Van den Bergh et al. (42,44) investigated the interaction of elastic and rigid vesicles with murine skin in vivo and with human skin in vitro. Unlike Transfersomes, these studies did not show any evidence that elastic vesicles could penetrate through the SC. However, Honeywell-Nguyen et al. (45) demonstrated a fast penetration of intact elastic vesicles into human SC, and these vesicles were localized within channel-like regions. They also investigated the in vivo interaction of elastic vesicles with human skin, using the tape-stripping technique in combination with freeze–fracture electron microscopy, and demonstrated a fast penetration of intact elastic vesicles into human SC (46). They proposed that the channel-like regions represent imperfections within the intercellular lipid lamellae in the areas with highly undulating cornified envelopes. Taken together, intact elastic vesicles may penetrate into human SC via channel-like regions.

Ethosomes are also phospholipid elastic vesicles having a high content of ethanol. Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the melting point of SC lipid, increasing their fluidity. The interaction of ethanol into the polar head group environment can result in an increase in membrane permeability. This is followed by a fusion of ethosomes with cell membranes. In addition to the effects of ethanol on SC structure, ethosome itself interacts with the SC barrier and then can penetrate the disturbed SC bilayer (47,48).

TRANSFERSOMES

Skin has small virtual pores (20–40 nm), and this limits passing through intercellular passages in the outer skin layers (20,49). To overcome this problem, Cevc developed a new liposomal system with more deformable aggregates called Transfersomes (20). Transfersomes differ from more conventional liposomes in several respects. Transfersomes resemble liposomes in morphology, but not in function (50). The most important is the extremely high and stress-dependent adaptability of such mixed lipid aggregates. Because of high deformability, Transfersomes squeeze through pores in SC that are less than one-tenth in the liposomes's diameter. Thus, sizes up to 200 to 300 nm can penetrate intact skin (27). Transfersomes contain phosphatidylcholine (PC) and a surfactant (edge activator) and also consist of at least one inner aqueous compartment surrounded by a lipid bilayer. Sodium cholate, Span 80, Tween 80, oleic acid, and dipotassium glycyrrhizinate (KG) (20,22,29,30) were employed as edge activators. For transdermal DNA delivery, DOTAP (positive-charged molecule), as a component, was used in producing Transfersomes instead of PC (38). Subsequent studies have documented that Transfersomes were more effective than conventional liposomes or ointment in the enhancement of small and large drug molecules across mouse and human skin (24–26,28,31,35–38).

Several investigators reported that Transfersomes prolonged the release and improved the biological activity in vivo (24,25,35,51). The in vivo performance of Transfersomes was studied by a carrageenan– and arachidonic acid–induced edema model with dexamethasone and triamcinolone acetonide (TRMA). Jain et al. (24) showed that dexamethasone Transfersomes could provide a maximum of 82.32% inhibition of paw edema, whereas conventional liposomes and ointments prevented approximately 38.32% and 25.35% of paw edema, respectively. With TRMA, the drug dose of 0.2 mg cm^{-2} suppressed 75% inhibition of ear edema for 48 hours. In contrast, a conventional formulation of TRMA required a 10-fold higher drug dosage to achieve a similar effect (35). A similar result was obtained with hydrocortisone and diclofenac Transfersomes (25,35,51). Diclofenac association with Transfersomes prolonged the effect and reached 10-fold higher concentrations in the tissue under the skin in comparison with the drug from a commercial hydrogel (25).

In addition to chemical drugs, Transfersomes could deliver large molecules into the body through intact skin. Paul et al. (26) investigated the effect of Transfersomes on the transdermal immunization with protein antigen. They applied Transfersomes to the intact skin surface with gap junction proteins (GJP) and showed that the specific antibody titers were higher than those elicited by subcutaneous injection of GJP in Transfersomes, mixed micelles, or conventional liposomes. Hofer et al. (37) reported the formulation of IL-2 and IFN-α containing Transfersomes for transdermal application. They showed that IL-2 as well as IFN-α were trapped by Transfersomes (75–80%) in biologically active form and in sufficient concentration for immunotherapy. Cevc (36) also reported the transdermal delivery of insulin with Transfersomes. Transfersomes could deliver insulin through skin barrier with a reproducible drug effect that resembled closely to that of insulin injected under the skin. In addition, Kim et al. (38) investigated the effect of Transfersomes (DOTAP and cholate) on the transdermal application of DNA in mice-intact skin. They reported that the GFP expression was detected in some organs, such as liver and lungs, when green fluorescent protein (GFP) was complexed with Transfersomes, whereas the GFP mixed only with phosphate buffered saline (PBS) did not observe with GFP expression. Transfersomes were capable of penetrating DNA into intact skin of mice when topically applied. Thus, Transfersomes may be developed further as a noninvasive protein and gene delivery system.

Transfersomes have several advantages on the TT drug delivery. They can be entrapped and delivered with small and large molecules through TT delivery. When applied on the intact skin, Transfersomes are not detrimental to the skin. Phospholipid, as a component of Transfersomes, even seems to improve the hydration of the aged skin (25). The advantages include a faster onset of drug effect, longer times of action, a biological action that is unaffected by mechanical abrasion, and the ability to reduce the necessary dosage needed to achieve therapeutic effects. Thus, the use of Transfersomes on the skin offers unprecedented opportunities for well-controlled and modern topical medication, not only just for low-molecular weight, but also for a variety of macromolecular therapeutics.

EFFECT OF TYPE SURFACTANTS AND CONCENTRATION

To prepare Transfersomes vesicles, edge activators (surfactants) were incorporated into the vesicular membranes; sodium cholate or sodium deoxycholate, Span 80, and Tween 80 have been used for this purpose. El Mghraby et al. (29,30) investigated the effect of surfactants on the formation of Transfersomes and permeation into the skin using cholate, Span 80, Tween 80, and oleic acid. Also, they investigated the effect of surfactant concentration on the skin permeation.

Transfersomes significantly improved the epidermal delivery of estradiol compared with the aqueous solution. The maximum flux increased by 18-, 16-, 15-, and 13-fold for Transfersomes containing sodium cholate, Span 80, Tween 80, and oleic acid compared with control. The skin deposition also increased by eight-, seven-, and eight-fold for cholate, Span 80, and Tween 80, respectively, compared with control. The efficiencies of Span 80 and Tween 80 were comparable with that of sodium cholate, but efficiency of oleic acid was less than that of sodium cholate. With dexamethasone Transfersomes, Span 80 was more effective as compared with sodium deoxycholate and Tween 80 as edge activators on penetration and edema inhibition assay.

With respect to drug delivery from vesicles, J_{max} first increased with increasing surfactant concentration, then decreased. These results suggested that too low or too high concentrations of surfactants are not beneficial in vesicular delivery of estradiol through skin and also indicated that the possible penetration enhancing effect of surfactants is not mainly responsible for improved estradiol skin delivery from deformable vesicles. The surfactant concentrations in the refined formulation were assessed to be 14.0%, 13.3%, and 15.5% w/w for sodium cholate, Span 80, and Tween 80, respectively (29). A possible explanation for lower drug delivery at high surfactant concentrations may be that surfactant at high concentrations decreased the entrapment efficiency and disrupted the lipid membrane so that it became more permeable to the entrapped drug. This will in turn reduce the delivery. The overall results suggested that there may be an optimum concentration of surfactant in lipid vesicles for maximum skin delivery of drug using Transfersome vesicles.

In addition, KG is used in preparing elastic vesicles. KG has emulsifying properties and good solubility. Thus, it is widely used in cosmetics. Trotta et al. (22) evaluated the ability of KG to produce elastic vesicles with soya lecithin (PC) or hydrogenated lecithin (HPC). They compared KG permeation with elastic vesicles and aqueous solution in the pigskin. All systems showed a negligible flux, but differed in the residual amount of KG in the skin. PC and HPC elastic vesicles promote the transfer of KG into the pigskin (60 ± 8 to 71 ± 10 mg cm^{-2}), while the KG solution failed to achieve transport (12 ± 3 mg cm^{-2}). There were no significant differences between elastic vesicles containing PC or HPC. The skin deposition increased by fivefold compared with 0.25% KG control solution.

Trotta et al. (23) also reported the effect of KG elastic vesicles on the dermal penetration of methotrexate (MTX) in pigskin. In an estimation of the cumulative amount of MTX permeated after 24 hours through pigskin, aqueous solution and conventional liposomes are quite similar in terms of MTX delivery through skin, whereas elastic vesicles show an increase in the amount of MTX permeated. In a skin deposition after 24-hour application, PC and HPC elastic vesicles promoted the transfer of MTX into pigskin. Skin deposition increased by a factor of 3 compared with either aqueous solution or conventional liposomes. Thus, KG acts as a good edge activator to produce elastic vesicles for TT drug delivery.

Which detergents are the best choices in Transfersomes formulation? The effect of detergents was different from other formulation conditions. The use of cholate seems to be better than other detergents in the case of skin delivery of large molecules. In addition to cholate, KG is a good candidate. KG has chemical stability, good solubility, emulsifying property, and anti-inflammatory activity. Thus, the use of elastic vesicles containing KG creates new opportunities for the well-controlled and modern topical medication. However, a more extensive study about compositions should be undertaken to define the optimal formulation regardless of drug types.

NONPHOSPHOLIPID-BASED ELASTIC VESICLES

In addition to phospholipid-based elastic vesicles (Transfersomes and ethosomes), Van den Bergh et al. (21) developed a series of elastic vesicles, consisting of the bilayer-forming surfactant L595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L

(octaoxyethylene laurate ester). L595 consisted of 100% sucrose laurate ester (30% monoester, 40% diester, and 30% triester). Surfactant-based elastic vesicles consisted of L595, PEG-8-L, and sulfosuccinate as stabilizers in the molar ratio 50:50:5. Several investigators reported that L595–PEG-8-L elastic vesicles were effective in enhancing the skin permeation of various drugs (32,33,52,53). However, drug transport was influenced by fluidity of elastic vesicles. The most rigid vesicles consisting of L595/PEG-8-L (100:0) significantly reduced the flux by 50% compared with the buffer control ($p < 0.05$). However, increasing the PEG-8-L content—and thereby increasing the vesicle elasticity—resulted in a significant higher flux for the L595/PEG-8-L (70/30 or 50/50) elastic vesicles ($p < 0.01$). This was a threefold increase to the buffer treatment and a sixfold increase to the most rigid vesicle treatment (33). Similar results were obtained from rotigotine elastic vesicles (32).

Elastic vesicle transport and the appearance of vesicle material in human SC can be affected by several factors, including pH, entrapping efficiency, pretreatment of vesicles, occlusive volume, and duration of application (32,45,46). The optimal pH differs depending on the drugs. The optimal pH was found to be 5.0 in case of pergolide, giving the highest drug incorporation as well as the highest drug transport. There was more than a fourfold difference between the highest flux at pH 5.0 (371.0 ± 51.7 ng cm^{-2}) and the lowest flux at pH 7.0 (89.3 ± 9.1 ng cm^{-2}) (33). Unlike pergolide, optimal pH was found to be 9.0, giving the highest drug incorporation ($99.8\% \pm 0.02\%$), in case of rotigotine elastic vesicles. At pH 5.0, the entrapment efficiency is very low ($22.1\% \pm 9.6\%$). The flux and cumulative amount of vesicle solution at pH 9.0 with high drug entrapment efficiency was 2.7-fold higher than those resulting from the vesicle solution at pH 5.0. Vesicles solution at pH 9.0 (3251 ± 902 ng cm^{-2}) gave rise to enhancement effect of factor 80 as compared with the corresponding buffer solution (42 ± 29 ng cm^{-2}). In contrast, vesicles solution at pH 5.0 (1072 ± 160 ng cm^{-2}) did not significantly enhance the drug transport as compared with its corresponding buffer control (1133 ± 241 ng cm^{-2}) (32). Table 2 summarizes the effect of elastic vesicles on pergolide and rotigotine delivery into the skin. As shown in Table 2, enhancement of rotigotine was much higher than that of pergolide. Entrapment efficiency and drug properties may result in this difference.

Nonocclusive co-treatment with elastic vesicles improved the skin delivery of pergolide compared with nonocclusive buffer control by more than twofold. Occlusion improved drug transport from both elastic vesicles as well as buffer solutions because of the fact that water is an excellent penetration enhancer for pergolide (45). In contrast to nonocclusive application, occlusive treatment with elastic vesicles showed a lower flux compared with occlusive treatment with the buffer control. A higher volume of application could increase the partitioning of vesicles into the skin, thereby increasing the enhancement effect. Pergolide transport from the 40- and 100-mL application were much higher within the first 20 hours as compared with that from the 20-mL application. However, there were no significant differences in the total cumulative amounts of drugs transported (306 ± 47.4, 462.0 ± 112.0, and 509.7 ± 141.9 in 20, 40, and 100 μL application, respectively) (45). They also investigated the effect of co-application and pretreatment on the rotigotine transport. Co-application (3483 ± 1067 ng cm^{-2}) significantly enhanced the drug transport by many folds, whereas pretreatment (126 ± 18 ng cm^{-2}) clearly had no effect on the drug transport as compared with buffer control (133 ± 27 ng cm^{-2}) (32). Similar result was obtained from pergolide elastic vesicles.

Table 2 Comparison of Pergolide and Rotigotine Skin Delivery with Elastic Vesicles

Factors	Rotigotine	Pergolide
Use	Dopamine agonist	Dopamine agonist
Compositions	L595/PEG-8-L-sulfosuccinate (50/50/5)	L595/PEG-8-L-sulfosuccinate (50/50/5)
Enhancement ratio	30.6-fold	2.7-fold
Occlusion		Increase penetration
Optimal pH	9.0	5.0
Entrapment	99.8% ± 0.02% at pH 9.0	2.5 mg/mL at pH 5.0 (Saturated)
Average size	117 ± 6 nm	100 ± 5 nm
Pka	7.9	5–6
Lipophilicity	Lipophilic at pH 9.0	Lipophilic and positive charge

From these results, detergent-based elastic vesicles were found to be powerful drug delivery systems across the skin. Co-application (co-treatment) and entrapment efficiency were essential factors for an optimal drug delivery by elastic vesicle formulations. The drug entrapment efficiency is strongly dependent on the pH of the drug–vesicular system. Thus, pH was also an important factor to deliver drugs with TT pathway.

ETHOSOMES

Ethosomes are phospholipid liposome carriers containing high content of ethanol (20–45%) (34). However, due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulation. Touitou developed ethosomes for transdermal drug delivery (54). Currently, ethanol can only be found in relatively low concentrations in liposome formulations: 7% to 10% for Transfersomes, 14% for Mibelle, and 16% for Natipide II. But, high content of ethanol was used in case of proniosomes (30–50%) (55,56). Ethosomes are soft, malleable vesicles tailored for enhanced delivery of various drugs to/through the skin and cellular membranes. Unlike conventional liposomes, which are known mainly to deliver drugs to the outer layers of the skin, ethosomes were shown to enhance permeation through the SC barrier. They penetrate skin and enhance drug delivery to deep skin SC (34,47,54,57). They are noninvasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation.

Touitou et al. (34) investigated the effect of phospholipid and ethanol concentration on the size distribution of ethosome vesicles. In the ethanol concentration range of 20% to 45%, the size of the vesicles increased with decreasing ethanol concentration, with the largest sizes in preparation containing 20% ethanol (193 $\pm$ 8 nm) and the smallest in preparations containing 45% ethanol (103 $\pm$ 9 nm). The dependence of vesicle size on phospholipid content was determined for ethosomes containing 30% ethanol and PC concentration ranging from 0.5% to 4%. An eightfold increase in PC concentration (from 0.5% to 4%) resulted in a twofold increase in ethosome size (from 118 $\pm$ 2 to 249 $\pm$ 24 nm). Also, ethanol imparted a negative charge to the vesicles and improved the vesicle stability. Hydrophilic and hydrophobic chemicals can be entrapped into ethosomes, and entrapment efficiency of hydrophobic chemicals was higher than that of hydrophilic chemicals. Entrapment efficiencies of minoxidil, testosterone, trihexylphenidyl (THP), and bacitracin were 83% $\pm$ 6%, 90% $\pm$ 3.5%, 75% $\pm$ 0.8%, and 77.4% $\pm$ 2.9%, respectively (34,47,58).

Enhanced delivery of chemicals from the ethosomal carrier was observed in permeation experiments with fluorescent probes (for detection of penetration depth) and drugs to nude mouse skin. Hydrophilic calcein penetrated the skin to a depth of 160, 80, and 60 mm from ethosomes, hydroethanolic solution (30% ethanol in water), and liposomes, respectively. Lipophilic rhodamine red (RR) penetrated the nude mouse skin to a depth of 140 mm from both the ethosomal system and from hydroethanolic solution. The probe into the fluorescence intensity was significantly greater from the ethosomal system [150 arbitrary unit (AU) for ethosomes and 40 AU for hydroethanolic solution]. Fluorescence was still visible at the deepest skin layers (20 AU at 260-mm depth) in case of ethosomal system. Deep penetration from liposomes was almost negligible (20 AU at 40-mm depth) (59).

Touitou et al. (34) also investigated the ability of ethosomes to deliver minoxidil to the deep layers of the skin. When it permeated the skin, it was 45 and 35 times higher from the ethosomal system than 30% ethanolic solution and absolute ethanol, respectively; the amount of minoxidil in the skin was also seven and five times greater than control systems, respectively. Similar results were obtained using acyclovir®, testosterone, and ionic molecules such as propranolol and THP (34,47,60). Horwitz et al. (60) reported that acyclovir delivered from an ethosomal system performed significantly better than Zovirax® (GlaxoSmithKline, Middlesex, U.K.). The amount of testosterone permeated in the rabbit skin in 24 hours was 30 times greater from the ethosomal system than from Testoderm® (848.16 $\pm$ 158.38 mg vs. 27.79 $\pm$ 16.23 mg). The amount of testosterone in the skin was also almost seven times greater when the drug was delivered from ethosomal system (130.76 mg vs. 18.32 mg) (34). THP flux from ethosomes (0.21 mg/cm^2/hr) was 87, 51, and 4.5 times higher than that from liposomes, buffer, and hydroethanolic solution, respectively (47). These data indicate that the ethosomal system is a more effective permeation enhancer than ethanol and hydroethanolic solution. Table 3

Table 3 Drug Skin Permeation from Ethosomal Vs. Control System

Drugs	Animal model	System	Qr[a] ($\mu g\ cm^{-2}$)	Qs[a] ($\mu g\ cm^{-2}$)	References
Minoxidil®	Nude mouse	Ethosomes	673.0 ± 92.0	69.6 ± 11.0	34
		30% EtOH	13.1 ± 3.5	10.0 ± 2.3	
Trihexyphenidyl®	Nude mouse	Ethosomes	1750 ± 250	586 ± 77	47
		30% EtOH	500 ± 50	415 ± 21	
Cannabidiol®	Nude mouse	Ethosomes	110.07 ± 24.15	–	61
Bacitracin®	Human	Ethosomes	12.0 ± 1.0	–	58
Testosterone®	Rabbit	Ethosome patch	848.16 ± 158.38	130.76 ± 20.23	34
		Testoderm®	27.79 ± 16.23	18.32 ± 8.34	

[a]The quantity of drugs that permeated the skin (Qr) and the quantity of drugs in the skin (Qs) in 24 or 18 hours (trihexyphenidyl) were measured in diffusion cells from systems, each containing 0.5% minoxidil, 0.1% bacitracin, 1% trihexyphenidyl, 0.25 mg cm^{-2} testosterone.
Source: From Refs. 34, 47, 58, and 61.

summarizes the ethosomal drug delivery to skin and cell membranes. Lodzki et al. (61) also reported the cannabidiol® transdermal delivery in a murine model. The flux of cannabidiol differs depending on skin sites. After a 24-hour application, it permeated 37.43 ± 13.58 and 110.07 ± 24.15 mg cm^2 into the hip skin and abdominal skin, respectively. Cannabidiol was also detected in the muscle, liver, pancreas, and blood. Godin and Touitou (58) evaluated the skin-depth penetration from bacitracin® ethosomes in vivo in rats. After an eight-hour topical application to rat abdomen, bacitracin penetrated more deeply into the skin from ethosomes than 30% ethanolic solution and liposomes. Taken together, the ethosomal system is effective in delivering drugs deeply into and through the skin.

Ethosomal systems are easy to prepare, nonirritant, and composed mainly of phospholipids and ethanol; compounds commonly found in pharmaceutical preparations. Ethosomes, because of their unique structure, are able to entrap and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil as well as cationic drugs such as propanolol and trihexyphenidil. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

ACTION MECHANISM OF ELASTIC VESICLES ON PENETRATION

How do phospholipid- and detergent-based elastic vesicles enhance drug penetration into the skin? Two mechanisms can be proposed. First, the elastic vesicles can act as penetration enhancers, whereby vesicle bilayers enter the SC and subsequently modify the intercellular lipid matrix. This will facilitate the penetration of free drug molecules into and across the SC (mechanism 1). Second, the elastic vesicles can act as drug-carrier systems, whereby intact vesicles can enter the SC-carrying vesicle-bound molecules into the skin (mechanism 2) (32,45). In order to assess whether a drug-carrier mechanism of action is involved or whether elastic vesicles simply act as penetration enhancers, two important questions should be answered: (*i*) Is pretreatment of the skin with empty vesicles sufficient (for mechanism 1), or is it essential to incorporate drugs into the vesicle solution (for mechanism 2)? (*ii*) What is the effect of the entrapment efficiency on the drug transport? Does higher entrapment efficiency result in a higher drug transport?

Cevc et al. (19) proposed that Transfersomes are drug-carrier systems that can deliver across the intact skin. It is believed that the successful passage of such carriers is based on two important factors: the high elasticity (deformability) of the vesicle bilayers and the existence of an osmotic gradient across the skin. Because of high deformability, Transfersomes could—under influence of the transepidermal osmotic gradient—squeeze themselves between the cells in the SC and carry large amounts of drugs across the intact skin. Fang et al. (18) investigated the mechanism of vesicular system across the skin with soybean PC liposomes containing enoxacin®. After a 12-hour pretreatment, drug permeation across PC-treated skin was higher

than that across nontreated skin. Also, Verma et al. (17) reported that PC liposomes carry not only the entrapped hydrophilic drug but also the nonentrapped drug into the SC and possibly into the deeper skin layers. These results indicated that PC liposomes could serve as permeation enhancers for drug delivery via the skin. With 5-fluorouracil Transfersomes, the percentage of drug penetrated (13.5%) was higher than the drug entrapment efficiency (8.8%) of Transfersomes (31). This strongly suggested that Transfersomes components may have altered the skin structure, as a penetration enhancer. Taken together, Transfersomes may have two functions to enhance drug transport across the skin, as a carrier system as well as a penetration enhancer.

Several investigators reported that pretreatment of detergent-based elastic vesicles did not improve the transport of pergolide and rotigotine, whereas higher entrapment efficiency resulted in higher drug transport (32,46). These data suggest that a penetration-enhancing process is not the main or the only mechanism of action, and detergent-based elastic vesicles may act as a carrier system. Honeywell-Nguyen and Bouwstra (45) proposed that detergent-based elastic vesicles facilitate drug transport by a fast partitioning in the SC, thereby carrying vesicle-bound drug molecules into SC. The vesicles remain in the SC and do not penetrate into the deeper skin layers. Hence, there are four major steps determining the effectiveness of the elastic vesicles system: (*i*) the drug association to the vesicle bilayers, (*ii*) the partitioning of vesicles into the SC, (*iii*) the drug release from the vesicles once in the SC, and (*iv*) the diffusion of free drugs in the SC and partitioning into the viable skin tissue, and subsequently into the systemic circulation (45). Taken together, they proposed that a penetration-enhancing effect of the individual surfactant component is not the main or the only mechanism of action for the elastic vesicles, and it is essential to apply drug molecules together with the vesicles.

Touitou et al. (34) proposed the action mechanism of ethosomal systems. First, ethanol disturbs the organization of the SC lipid bilayer and enhances its lipid fluidity. The flexible ethosome vesicles can then penetrate the disturbed SC bilayers. The release of the drug in the deep layers of the skin and its transdermal absorption could then be the result of a fusion of ethosomes with skin lipids, and drug release at various points along the penetration pathway. Unlike other elastic vesicles, occlusion slightly increased the skin penetration of ethosomes. This result indicated that the existence of an osmotic gradient across the skin was not an important factor (58). These data differ from that observed with elastic vesicles, where permeation enhancement occurred only in nonocclusive conditions, and point toward different mechanisms of action of the two carriers.

To further investigate mechanism of ethosomal skin permeation, Godin and Touitou (58) used double-staining methods; ethosomes co-loaded with two fluorescent probes, RR and FITC-bacitracin (FITC-Bac). Both probes were delivered from ethosomes to a maximal possible depth of 200 mm. When the two probes were observed separately at the skin depth of 90 mm, it was clearly seen that the delivery of FITC-Bac from ethosomes was followed by the delivery of ethosomal components in the same area. Skin penetration profile date indicated that penetration of ethosomal vesicles into the skin peaked at approximately 40 mm, while depth of maximum bacitracin penetration was approximately 90 mm, suggesting that the release of the drug in deep skin layers occurred. In a double-staining study, the bacitracin, delivered from ethosomes, entered the skin between the coreocytes through the intercellular lipid domain. High content of ethanol fluidizes the ethosomal membranes to produce highly deformable vesicles, and subsequently ethosomes squeeze drugs between the cells in the SC and carry large amounts of drugs across the intact skin (31,48). Additionally, we cannot exclude the possibility that ethosomes can be trapped in follicles and delivered to deep layers of the skin.

CONCLUSIONS

Highly deformable elastic vesicles (Transfersomes, ethosomes, detergent-based elastic vesilces) improve the transdermal delivery of low and high molecules in vitro and in vivo systems. The use of elastic vesicles as a vesicular drug carrier could overcome the limitation of low penetration ability of conventional liposomes or commercial ointment across the skin. Penetrating-enhancing effects of phosphlipid- and detergent-based elastic vesicles act as a drug carrier system as well as penetration enhancers. For optimal drug delivery, it is essential

that drug molecules are associated with vesicles and applies with co-application (co-treatment) conditions. In in vivo study, Transfersomes and ethosomes showed better biological activity in comparison with conventional liposomes or commercial ointment. Thus, many topical drugs may be developed using elastic vesicles. However, a more extensive study about vesicular type and compositions should be undertaken to fully establish the optimal condition, for which elastic vesicles are the most suitable vehicles.

REFERENCES

1. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001; 14:101–114.
2. Schurer NY, Elias PM. The biochemistry and function of stratum corneum lipids. Adv Lipid Res 1991; 24:27–56.
3. Banga AK, Bose S, Ghost TK. Iontophoresis and electroporation: comparison and contrasts. Int J Pharm 1999; 179:1–19.
4. Regnier V, Preat V. Localization of a FITC-labeled phosphorothioate oligonucleotide in the skin after topical delivery by iontophoresis and electroporation. Pharm Res 1998; 15:1596–1602.
5. Mezei M, Gulasekharam V. Liposomes: a selective drug delivery system for the topical route administration. Part I. Lotion dosage forms. Life Sci 1980; 26:1473–1477.
6. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters 9 (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int J Pharm 1994; 105:1–6.
7. Williams AC, Barry BW. Penetration enhancers. Adv Drug Del Rev 2004; 56:603–618.
8. Elias PM, Tsai J, Menon GK, et al. The potential of metabolic interventions to enhance transdermal drug delivery. J Invest Dermatol Sym Proc 2002; 7:79–85.
9. Lasic DD. Doxorubicin in sterically stabilized liposomes. Nature 1996; 380:561–562.
10. Abra RM, Bankert RB, Chen F, et al. The next generation of liposome delivery system: recent experience with tumor-targeted, sterically stabilized immunoliposomes and active-loading gradients. J Liposome Res 2002; 12:1–3.
11. Clemons KV, Stevens DA. Comparative efficacies of four amphotericin B formula-tions – Fungizone, amphotech (Amphocil), AmBisome, and Abelcet — against systemic murine aspergillosis. Antimicrob Agents Chemother 2004; 48:1047–1050.
12. Choi MJ, Maibach HI. Topical vaccination of DNA antigens: topical delivery of DNA antigens. Skin Pharmacol Appl Skin Physiol 2003; 16:271–282.
13. Chang JS, Choi MJ, Cheong HS, et al. Development of Th1-mediated CD8þ effector T cells by vaccination with epitope peptides encapsulated in pH-sensitive liposomes. Vaccine 2001; 19:3608–3614.
14. Chang JS, Choi MJ, Kim TY, et al. Immunogenecity of synthetic HIV-1 V3 loop peptides by MPL adjuvanted pH-sensitive liposomes. Vaccine 1999; 17:1540–1548.
15. Chang JS, Choi MJ. pH-Sensitive liposomes as adjuvants for peptide antigens. Methods Enzymol 2003; 373:127–136.
16. Touitou E, Junginger HE, Weiner ND, et al. Liposomes as carriers for topical and transdermal delivery. J Pharm Sci 1994; 83:1189–1203.
17. Verma DD, Verma S, Blume G, et al. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm Biopharm 2003; 55:271–277.
18. Fang JY, Hong CT, Chiu WT, et al. Effect of liposomes and niosome on skin permeation of enoxacin. Int J Pharm 2001; 219:61–72.
19. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta 1992; 1104:226–232.
20. Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst 1996; 13:257–388.
21. Van den Bergh BAI. Elastic liquid state vesicles as a tool for topical drug delivery. Thesis. Leiden University, The Netherlands, pp. 49–80(1999).
22. Trotta M, Peira E, Debernardi F, et al. Elastic liposomes for skin delivery of dipotassium glycyrrhizinate. Int J Pharm 2002; 241:319–327.
23. Trotta M, Peira E, Carlotti ME, et al. Deformable liposomes for dermal administration of methotrexate. Int J Pharm 2004; 270:119–125.
24. Jain S, Jain P, Umamaheshwari RB, et al. Transferosmes—a novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. Drug Dev Ind Pharm 2003; 29:1013–1026.
25. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochim Biophys Acta 2001; 1514:191–205.

26. Paul A, Cevc G, Bachhawat BK. Transdermal immunization with an intergral membrane component, gap junction protein, by means of ultradeformable drug carriers, Transfer-somes. Vaccine 1998; 16:188–195.
27. Cevc G, Gebauer D, Stieber J, et al. Ultraflexible vesicles, Transfer-somes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochim Biophys Acta 1998; 1368:201–215.
28. Guo J, Ping Q, Sun G, et al. Lecithin vesicular carriers for transdermal delivery of cyclosporin A. Int J Pharm 2000; 194:201–207.
29. El Maghraby GMM, Williams AC, Barry BW. Oestradiol skin delivery from deformable liposomes: refinement of surfactant concentration. Int J Pharm 2000; 196:63–74.
30. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of oestradiol from lipid vesicles: importance of liposome structure. Int J Pharm 2000; 204:159–169.
31. El Maghraby GMM, Williams AC, Barry BW. Skin delivery of 5-fluorouracil from ultra-deformable and standard liposomes in-vitro. J Pharm Pharmacol 2001; 53:1069–1077.
32. Honeywell-Nguyen PL, Arenja S, Bouwstra JA. Skin penetration and mechanisms of action in the delivery of the D2-agonist rotigotine from surfactant-based elastic vesicle formulation. Pharm Res 2003; 20:1619–1625.
33. Honeywell-Nguyen PL, Frederik PM, Bomans PHH, et al. Transdermal delivery of pergolide from surfactant-based elastic and rigid vesicles: characterization and in vitro transport studies. Pharm Res 2002; 19:992–998.
34. Touitou E, Dayan N, Bergelson L, et al. Ethosomes-novel vesicular carriers: characterization and delivery properties. J Control Release 2000; 65:403–418.
35. Cevc G, Blume G. Biological activity and characteristics of triamcinolone-actonide formulated with the self-regulating drug carriers, Transfersomes. Biochim Biophys Acta 2003; 1614:156–164.
36. Cevc G. Transdermal drug delivery of insulin with ultradeformable carriers. Clin Pharmacokinet 2003; 42:461–474.
37. Hofer C, Gobel R, Deering P, et al. Formulation of interleukin-2 and interferon-alpha containing ultradeformable carriers for potential transdermal application. Anticancer Res 1999; 19:1505–1507.
38. Kim A, Lee EH, Choi SH, et al. In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome. Biomaterials 2004; 25:305–313.
39. Hofland HEJ, van der Geest R, Bodde HE, et al. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. Pharm Res 1994; 11:659–664.
40. Abraham W, Downing DT. Interaction between corneocytes and stratum corneum lipid liposomes in vitro. Biochim Biophys Acta 1990; 1021:119–125.
41. Van Kuijk-Meuwissen MEMJ, Junginger HE, Bouwstra JA. Interactions between liposomes and human skin in vitro, a confocal laser scanning microscopy study. Biochim Biophys Acta 1998; 1371:31–39.
42. Van den Bergh BA, Bouwstra JA, Junginger HE, et al. Elasticity of vesicles affects hairless mouse skin structure and permeability. J Control Release 1999; 62:367–379.
43. Schätzlein A, Cevc G. Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). Br J Dermatol 1998; 138:583–592.
44. Van den Bergh BAI, Vroom J, Gerritsen H, et al. Interactions of elastic and rigid vesicles with human skin in vitro: electron microscopy and two photon excitation microscopy. Biochim Biophys Acta 1999; 1461:155–173.
45. Honeywell-Nguyen PL, Bouwstra JA. The in vitro transport of pergolide from surfactant-based elastic vesicles through human skin: a suggested mechanism of action. J Control Release 2003; 86:145–156.
46. Honeywell-Nguyen PL, Wouter Groenink HW, de Graaff AM, et al. The in vivo transport of elastic vesicles into human skin: effects of occlusion, volume and duration of application. J Control Release 2003; 90:243–255.
47. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials 2000; 21:1879–1885.
48. Valjakka-Koskela R, Kirjavainen M, Mönkkönen J, et al. Enhancement of percutaneous absorption of naproxen by phospholipids. Int J Pharm 1998; 175:225–230.
49. Cevc G, Blume G, Schätzlein A, et al. The skin: a pathway for systemic treatment with patches and lipid-based agent carriers. Adv Drug Del Rev 1996; 18:349–378.
50. Planas ME, Gonzalez P, Rodriguez L, et al. Noninvasive percutaneous induction of topical analgesia by a new type carrier and prolongation of local pain insensitivity by anesthetic liposomes. Anesth Analg 1992; 75:615–621.
51. Cevc G, Blume G, Schätzlein A. Transfersomes-mediated transepidermal delivery improves the regiospecificity and biological activity of corticosteroids in vivo. J Control Release 1997; 45:211–226.
52. Honeywell-Nguyen PL, van den Bussche MH, Junginger HE, et al. The effect of surfactant-based elastic and rigid vesicles on the penetration of lidocaine across human skin. STP Pharma 2002; 12:257–262.

53. Bouwstra JA, De Graaff A, Groenik W, et al. Elastic vesicles: interaction with human skin and drug transport. Cell Mol Biol Lett 2002; 7:222–223.
54. Touitou E. Compositions for applying active substances to or through the skin. US patent 5540934. 1996.
55. Fang JY, Yu SY, Wu PC, et al. In vitro skin permeation of oestradiol from various proniosome formulations. Int J Pharm 2001; 215:91–99.
56. Vora B, Khopade AJ, Jain NK. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Release 1998; 54:149–165.
57. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. Drug Dev Res 2000; 50:406–412.
58. Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. J Control Release 2004; 94:365–379.
59. Touitou E, Godin B, Dayan N, et al. Intracellular delivery mediated by an ethosomal carrier. Biomaterials 2001; 22:3053–3059.
60. Horwitz E, Pisanty S, Czerninski R, et al. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 87:700–705.
61. Lodzki M, Godin B, Rakou L, et al. Cannabidiol-transdermal delivery and anti-inflammatory effect in murine model. J Control Release 2003; 93:377–387.

74 Polymers Effect on Chemical Partition Coefficient Between Powdered Human Stratum Corneum and Water

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INTRODUCTION

Macromolecules have gained interest as potential active entities and as modulators of percutaneous delivery systems. Some success in percutaneous delivery using enhancer is being reported, despite these compounds exceeding the "500 Dalton rule" (1). Another approach is to use macromolecules as transdermal transport facilitators, where the presence of macromolecules can interact at the skin surface that facilitates the passage of another chemical or drug (2). These macromolecules can interact at the skin surface, interphasing with cosmetic ingredients to alter the initial phase of percutaneous absorption, which is the initial partition into the skin.

Powdered human stratum corneum (PHSC), a product made from foot callus, can be cut into smaller pieces and ground with dry ice to form a powder. Uniformity in particle size is achieved with sieving. The callus is human stratum corneum (SC) derived and thus, should retain some physical and chemical characteristics of human SC (3,4). Two macromolecular polymers have been synthesized to hold cosmetic and drug compounds on the skin surface. The effect these two polymers have on the partition coefficient (PC) (SC/water) was investigated on the PHSC.

MATERIALS AND METHODS

Compounds

MacroDerm^{TM} L (lipophilic MW 2081) is a block polymer of propylene oxide with hydrocarbon end groups that is completely insoluble in water. MacroDerm H (hydrophilic MW 2565) is a block polymer of ethylene oxide with hydrocarbon end groups. This latter material swells in water and exhibits amphipathic characteristics, but does not dissolve. Both materials are soluble in most organic solvents, including ethanol (5).

[^{14}C]-Estradiol—a model compound—(DuPont NEN) had specific activity of 54.1 mCi/mmol, and radiochemical purity was 99%, as determined by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC).

Preparation of Powdered Human Stratum Corneum

Human foot callus was cut into fine pieces and ground in a Micro-mill Grinder (Bel-Air Products, Pequannode, New Jersey, U.S.) in the presence of dry ice to form powder. Particles of PHSC that pass through a 50-mesh sieve but not 80-mesh sieve were used. This particle size (180–300 μm) has previously been used in published studies (3,4).

Partition Coefficient Incubations

Into a specially designed glass centrifuge tube (6 cm length × 1 cm diameter; O.Z. Glass Company, Pinole, California, U.S.), with a glass fritte (40 μm) fitted in on the end, 10 mg of PHSC was mixed with 4 mL of [^{14}C]-estradiol in water. After incubating with estradiol for different lengths of time (0, 2, 6, 12, and 24 hours), the mixture was separated by centrifugation (15 minutes at 1500 × g). Water was passed through the glass fritte and collected into receiving

centrifuge tubes. PHSC was then washed twice with water, with vigorous mixing and further centrifugations. Each subsequent supernatant was collected separately. Water and remaining dry PHSC were mixed with the scintillation cocktail (Universol-ES, ICN, Costa Mesa, California, U.S.), and then assayed for radioactivity by liquid scintillation spectrophotometry. Five replicate tubes were prepared for each test sample. Three concentrations of estradiol were chosen (2.8, 0.28, and 0.028 μm/mL).

Delipidization of PHSC

PHSC was delipidized by incubation in chloroform:methanol (2:1) for 16 hours, then centrifuged and air-dried.

Polymer Incubation

Three concentrations of each polymer were used (1, 5, and 10% w/v in ethanol). There was a 30-minute preincubation period (with agitation) with the polymer solutions, and PHSC was washed twice with 50% ethanol and finally once with water. The resulting dry PHSC was then incubated for two hours with the same three concentrations of estradiol as previously described.

Calculation of PHSC/Water Partition Coefficient

The following equation was used to calculate PC:

$$\mathrm{PC}_{\mathrm{PHSC/Water}} = \frac{\mathrm{dpm\,PHSC/mg\,PHSC}}{(\mathrm{dpmi} - \mathrm{dpm\,PHSC/mg\,water})}$$

where dpmi represents the initial concentration of estradiol in water (in desintegration per minute units). Statistical significance was determined using sigma stat.

RESULTS AND DISCUSSION

The PC values obtained after different incubation times, different estradiol concentrations, and in delipidized PHSC are shown in Table 1. There was no statistically significant effect ($p > 0.05$) on PC values when the concentration of estradiol was increased 100-fold (from 0.028 to 2.8 μm/mL), when the incubation period was increased from 0 to 24 hours, or when PHSC was delipidized prior to incubation with estradiol. In general, the log PC values obtained for estradiol ranged from 1.6 to 1.9, indicating an intermediate affinity of estradiol for PHSC compared with water. The data indicate a rapid partitioning of estradiol into human skin with no apparent lag time in partitioning for this chemical, which is probably because of the lipophilicity of the compound (log $P_{o/w} = 2.70$). Therefore, a two-hour incubation period was chosen for further studies with estradiol. The log PC values obtained (1.6–1.9) agreed with other similar work; for example, Surber et al. (6) reported a log $\mathrm{PC}_{\mathrm{PHSC/water}}$ value for estradiol of 2.1 and Hui et al. calculated the value to be 1.2. Therefore, the new glass vials developed for these assays appear consistent with older methods. Surber et al., (6) also showed the process of delipidization of the SC that had little or no effect on the PC of estradiol. This result is consistent with the data reported here.

Table 1 Effect of Time, Estradiol Concentration, and PHSC Delipidation on the PHSC/Water Partition Coefficient

	Log $\mathrm{PC}_{\mathrm{PHSC/water}}$ (mean ± SD, $n = 5$)			
Time (hr)	Delipidized PHSC [2.8 μm/mL][a]	Normal PHSC [2.8 μm/mL][a]	Normal PHSC [0.28 μm/mL][a]	Normal PHSC [0.028 μm/mL][a]
0	1.22 ± 0.17	1.57 ± 0.17	1.81 ± 0.30	1.20 ± 0.32
2	1.67 ± 0.22	1.62 ± 0.14	1.68 ± 0.11	1.71 ± 0.13
6	1.89 ± 0.09	1.68 ± 0.14	1.78 ± 0.07	1.79 ± 0.05
12	1.75 ± 0.15	1.71 ± 0.26	1.70 ± 0.33	1.70 ± 0.23
24	1.88 ± 0.05	2.16 ± 0.55	1.79 ± 0.04	1.79 ± 0.17

[a]Concentration of estradiol used in each incubation.

Table 2 Effect of Two Polymers (L and H) on the Estradiol PC Between PHSC and Water

	$LogPC_{PHSC/water}$ (mean ± SD, $n = 5$)		
	Estradiol concentration (μm/mL)		
Polymer concentration	2.8	0.28	0.028
Polymer H (hydrophilic polymer)			
10%	2.31 ± 0.22[a]	2.36 ± 0.14[a]	2.13 ± 0.07[a]
5%	1.93 ± 0.10[b]	2.06 ± 0.21[b]	1.94 ± 0.06[b]
1%	1.71 ± 0.10	1.61 ± 0.19	1.59 ± 0.26
Polymer L (lipophilic polymer)			
10%	1.74 ± 0.10	1.65 ± 0.07	1.61 ± 0.14
5%	1.70 ± 0.20	1.62 ± 0.17	1.65 ± 0.09
1%	1.59 ± 0.19	1.57 ± 0.15	1.71 ± 0.07
Control (no polymer)	1.62 ± 0.14	1.68 ± 0.11	1.71 ± 0.13

[a]Statistically significantly different from control ($p < 0.01$).
[b]Statistically significantly different from control ($p < 0.05$).

The results in Table 2 show that polymer L had no effect on the estradiol PC between PHSC and water. This result was consistent for all three concentrations of estradiol. Polymer H showed a significant increase ($p < 0.01$) in log PC for estradiol concentrations of 2.8 and 0.28 mg/mL; this increase was dependent on the polymer concentration. At 10% polymer H, PC was increased by a factor of 2, and at 1% polymer, there was no statistical difference from control, untreated values. At the lowest estradiol concentration (0.028 μm/mL), the increase in PC was not as prominent, although still significant ($p < 0.05$) at 10% and 5% polymer. These results suggest that no polymer adhered to the PHSC during the 30-minute preincubation, or was completely removed by washing. The hydrophilic polymer H had a statistically significant effect ($p < 0.05$) on the estradiol PHSC/water PC, with increases of up to sixfold observed. The binding also exhibited a dose concentration-dependent response. This dose dependency is indicative of polymer binding to the SC and estradiol preferentially "binding" to the polymer rather than water. In addition, the dose dependency may be due to hydrophilic nature of polymer H, which can "swell" in the presence of water, hence absorbing additional water containing estradiol.

The data show a promising effect for a polymer in increasing the partitioning of a chemical of intermediate lipophilicity (be it a fragrance or other cosmetic ingredient) into the outer layers of the skin. Thus, a macromolecular chemical has the potential to alter the first step in percutaneous absorption, the partitioning of a drug or fragrance from its formulation into the skin. The data also show that PHSC may be a simple and cost effective method to screen macromolecules for potential improvement in percutaneous delivery. However, the consistency of this effect with other chemicals, of differing physicochemical properties, must first be established before firm conclusions can be drawn. These data derived from a model compound—estradiol—possibly to likely will be relevant to cosmetic/cosmeceutical ingredients of related physical/chemical properties. In addition, the newly designed glass tubes have proven to be a quick and reliable method for the determination of PHSC/water PCs.

REFERENCES

1. Bos JD, Meinardi MMHM. The 500 dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol 2000; 9:165–169.
2. Vanbever R, Prausnitz MR, Prat V. Macromolecules as novel transdermal transport enhancers for skin electroporation. Pharm Res 1997; 14:638–644.
3. Wester RC, Mobayen M, Maibach HI. In vivo absorption and binding to powdered human stratum corneum as methods to evaluate skin absorption of environmental chemical contaminants from ground and surface water. J Toxicol Environ Health 1987; 21:367–374.
4. Hui X, Wester RC, Magee PS, et al. Partitioning of chemicals from water into powdered human stratum corneum (callus) – a model study. In Vitro Toxicol 1995; 8(2):159–167.
5. Samour CM, Krauser SF. Cationic film-forming polymer compositions, and use thereof in topical agents delivery systems and methods of delivering agent to the skin. US patent 5 807 957. Sept 15, 1998.
6. Surber C, Wilhelm K-P, Hori M, et al. Optimization of topical therapy: partitioning of drugs into the stratum corneum. Pharm Res 1990; 7:1320–1324.