RESEARCH PAPER

Ingredients Tracking of Cosmetic Formulations in the Skin: A Confocal Raman Microscopy Investigation

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ABSTRACT

Purpose Confocal Raman microspectroscopy (CRM) was used to follow the absorption of retinol into the skin and to track the absorption of ingredients in topically applied formulations.

Method Three surfactants, PEG20C12, PEG20C18:1 (hydrophilic) and PEG6C18:1 (lipophilic), were used in preparing three o/w emulsions and three surfactant solutions all containing retinol. Quantitative retinol penetration studies for 24 h were carried out using Franz diffusion cells. CRM was used to follow the skin penetration of retinol, oil and water and also to study a possible modification of the lipid skin barrier in the *stratum corneum* (*SC*) using the ratio of I_{2880}/I_{2850}.

Results The oily surfactant solution containing PEG6C18:1 and dodecane showed the highest retinol penetration rate. This appears to be related both to the short polar head group of the surfactant and to the effect of dodecane on skin lipids. All the surfactant solutions showed a higher penetration rate compared with the corresponding emulsions. CRM measurements showed that the ratios of I_{2880}/I_{2850} were significantly modified using surfactant solutions.

Conclusions Penetration behavior appeared to be dependent on the surfactant used in the formulation. CRM associated to the Franz cell method gives new insights on permeation of drug related to vehicle or ingredients.

KEY WORD confocal Raman microspectroscopy · delivery · formulation ingredient tracking · retinol · skin

ABBREVIATIONS

aq	aqueous
BHT	butylhydroxytoluene
Brij®98	polyoxyethylene (20) oleyl ether
CRM	confocal Raman microscopy
D	dermis
E	epidermis
FWHM	full width half maximum
HLB	hydrophilic-lipophilic balance parameter
HPLC	high performance liquid chromatography
Myritol®318	caprylic/capric triglyceride
PEG	polyethyleneglycol
POE	polyoxyethylene
R I_{2880}/I_{2850}	ratio of the peak intensities at 2880 and 2850 cm^{-1}
Rpm	revolutions per minute
SC	*stratum corneum*
SD	standard deviation
SEM	standard error of the mean

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INTRODUCTION

The transport of a pharmaceutical or a cosmetic active through the skin is the main goal of dermatological and cosmetical products. The delivery efficacy is always based on the penetration efficacy of actives from the formulation. The enhancement effect of vehicles on skin delivery can be achieved by two approaches (1): first the partition of the active from the formulation with the skin (i.e. *via* vehicles) and, second, the modification of the skin barrier by using chemical penetration enhancers, which might modify the lipid structure of the skin (termed *skin barrier disruption* or *skin lipids fluidization*). The transport of drugs or cosmetic actives that are topically applied can be studied *in vitro* by the diffusion Franz cell method. Therefore, for any new formulation it is possible to determine *in vitro* whether or not an active ingredient is able to penetrate the skin barrier. *In vitro* studies also offer an interesting alternative when optimizing the composition of a formulation in order to achieve the required drug uptake across the skin. However, dermatological and cosmetic products are rarely as simple as an aqueous solution and also contain many ingredients, such as surfactants, which influence the active ingredient transport across skin. Until now, studies that attempted to predict skin absorption from complex formulations have been few in number; such studies are complicated in nature (2–4). Recently, Grégoire *et al.* developed a model, to predict the mass of a chemical absorbed into and through skin from a cosmetic or a dermatological formulation obtaining good correlations when taking into consideration the volume fraction of dispersed phase or continuous phase (5). However, in their model, the penetration-enhancing effects of a formulation matrix were considered to be negligible. In the current study, it was decided to choose another approach to optimize active delivery to the skin: to investigate active absorption and ingredient penetration into the skin by the non-invasive method of confocal Raman microspectroscopy (CRM). CRM was used in several works to study the penetration of drugs or active compounds (6–9), to investigate the influence of skin penetration enhancers (10–12) but also to evaluate skin properties and skin hydration (13,14). Concerning the first point, several authors demonstrated the applicability of confocal Raman microscopy as a non-invasive optical approach to study the delivery and the metabolism of active substances *in vitro* and *in vivo*. In some studies, retinol was the model active tracked in the skin. Mélot *et al.* demonstrated, using CRM as analytical method, the enhanced retinol penetration in the presence of skin enhancers. Failloux *et al.* showed a slower release of retinol from microparticles in the epidermis by CRM and an increase of its storage in epidermis (9). In some studies, researchers evaluated in the same time the active penetra-

tion but also the effect of these compounds on skin structure. For example, Zhang *et al.* studied the influence of two solvents, DMSO and chloroform/methanol, on the conformations of keratin. These two solvents are commonly used in dermatological research for studies of permeation enhancement and for extracting lipids from *stratum corneum* (15). They were shown to induce large reversible alterations (*alpha*-helix to *beta*-sheet) in the secondary structure of keratin. Tfayli *et al.* used this method for investigating the penetration of metronidazole in transcutol through human skin *in vitro*. In a second step, they analyzed structural modifications induced by the metronidazole on the skin by studying the changes in the spectral signature of the skin constituents (7).

Confocal Raman microspectroscopy allows simultaneous consideration of both the ingredients and the active penetration and also permits evaluation of the effects of formulation ingredients on the SC lipid bilayers arrangement.

Skin perturbation of lipid bilayers may explain skin enhancement (16) and should be examined carefully when optimizing a formulation (8,17). CRM was used in this study to follow an active substance, retinol, delivered into the skin from simple surfactant emulsions or surfactant solutions. The surfactants were esters of polyethyleneglycol, which varied in the length of both their alkyl chain (hydrophobic part) and their polyethyleneglycol (PEG) chain. It is well known that surfactants affect the permeability characteristics of several biological membranes, including skin (18,19), and for this reason they can enhance the skin penetration of other compounds as a result of their interactions with intercellular lipids. A series of papers have shown that certain PEG alkyl ethers were effective enhancers for oral, rectal or skin delivery of insulin, heparin and ibuprofen (20–22). In this preliminary study, tracking of water and an oily component (dodecane) was first demonstrated, together with the active substance retinol, from three simple o/w emulsions applied to the skin, each of them was stabilized by a different surfactant: PEG6C18:1, PEG20C12 and PEG12C18:1. Second, a comparison was made between the penetration depth for each emulsion ingredient in the *SC* and those from surfactant solutions formulated with the same oil (dodecane), and the same surfactant as the corresponding emulsion, in order to understand the component relationships and their different penetration behaviors using CRM. The fluidizing action of surfactants on intercellular lipids was also assessed by CRM. The extent of alkyl chain order was obtained from the ratio $v_{asym}CH_2/v_{sym}CH_2$, which is a measurement of the relative population of trans and gauche conformers and of the degree of order of the alkyl chain (10,23,24).

For a better understanding, a classical *in vitro* Franz cell experiment was also carried out and classical mass balance

of the penetrations into the three main skin compartments (*SC*, epidermis, dermis) was measured, as was permeation through the skin up to the receptor fluid. This work used CRM results for the first time to explain differences in retinol penetration behavior observed from the Franz cell experiments.

MATERIALS AND METHODS

Materials

All-trans-retinol and dodecane were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Butylhydroxytoluene (BHT) and α-tocopherol acetate were a kind gift from Jan-Dekker (Saint Germain en Laye, France), and the three different polyethyleneglycol esters (PEG6 and PEG20) with the carbon chain lengths of C12 and C18:1 were synthesized by Gattefossé SAS (Saint Priest, France) with a minimum 70 wt % of monoesters. Oleth-20 was a gift from Croda (Trappes, France). Analytical grade methanol was purchased from Carlo Erba (Milan, Italy). Deionized water of 16 MΩ.cm^{-1} resistivity was used throughout the work. D(26)-n-dodecane was purchased from Cambridge Isotope Laboratories and deuterium oxide from Sigma-Aldrich.

Full-thickness pig skin from the flanks (mean thickness ± SEM = 0.84±0.02 mm) (Laboratoire de Physiologie, Université Claude Bernard Lyon 1, France) was used in the skin absorption experiments. Animals were three months old. The skins of three donor animals were washed and excised, the subcutaneous fatty tissue was carefully removed, and the skin pieces were stored flat at −20°C until use. The skin is considered as dead, and consequently no metabolism of retinol is likely to occur during skin absorption. It is known that retinol can be converted metabolically, mainly to retinyl esters but also, to a lesser extent, to retinal or retinoic acid (25–27). The thickness of each skin piece was measured with a micrometer (Mitutoyo). TransEpidermal Water Loss (TEWL) was assessed using a Tewameter®TM300 from Courage and Khazaka, Cologne, Germany. These measurements were performed in triplicate on skin pieces just before starting the skin absorption studies. Skin samples with TEWL value higher than 15 g.m^{-2} h^{-1} were discarded (28).

Formulations

Retinol saturation concentration in dodecane containing 0.50 wt. % BHT was measured: 16.67±0.660 mg/g (±SD). Its water saturation concentration was obtained from the literature: 0.06 μM (i.e. 17.2 μg/g) at pH 7.3 (29).

A retinol solution in pure dodecane containing 0.50 wt % of BHT and 0.50 wt % (5 mg/g) of retinol was tested in the same way as the formulations described below.

All formulations contained 0.5 wt % of retinol. Three o/w emulsions and three surfactant solutions were prepared using three surfactants differing in their PEG chain or alkyl chain lengths: PEG6C18:1 (HLB: 11.3), PEG20C12 (HLB: 17.1) and PEG20C18:1 (HLB: 15.9). Their hydrophilic-lipophilic balance parameter (HLB) values were calculated in accordance with Griffin's method (30). The oil/aqueous phase ratio for emulsions was ~55:37 (wt:wt) for stability purposes.

The PEG6C18:1-based surfactant solution was directly prepared in dodecane because of the insolubility of this surfactant in water. Conversely, PEG20C12 and PEG20C18:1 surfactant solutions were prepared in water and contained, retinol as the dispersed phase, similarly to the emulsions. Each formulation was prepared twice: the first time for skin absorption studies using the Franz cell method and the second time with deuterated ingredients D(26)-dodecane and deuterium oxide, D$_2$O (also known as 'heavy water'), for CRM studies. The compositions of the formulations are shown in detail in Tables I and II. The batch size for each preparation was 50 g and 7 g for preparation with heavy water.

To prepare the emulsions, the oil and aqueous phases were mixed together with an UltraTurrax® device (Ika®T25 Germany) working at 9000 rpm for 2–5 min, then at 12000 rpm for 12 min and finally at 9000 rpm for 20 min at room temperature. Surfactant solutions were prepared by mixing the components together using an ultrasonic bath for 15 min. The stability of retinol in formulations was investigated for 48 h at 20°C. The retinol degradation was comprised between 8.3×10^{-4} mg.g^{-1}.h^{-1}. and 0.025 mg.g^{-1}.h^{-1}. All formulations were used for skin permeation studies immediately after preparation.

Physicochemical Characterizations

Emulsions droplet size distributions were measured by small angle light scattering using a MasterSizer® 2000 (Malvern Instruments Ltd., UK). The refractive indices used for the "optical model" were 1.332 for water and 1.460 for the emulsion droplets. The average size and polydispersity index of retinol droplets dispersed in surfactant solutions were measured by means of dynamic light scattering using a NanoZS® instrument (Malvern Instruments Ltd., UK). The droplet mean sizes are given in Tables I and II. The size distributions of the different formulations were monodisperse.

The viscosity of emulsions was measured at 20°C using a Couette rheometer TV-e 05 (Lamy, France) equipped with a mobile system MS-BV 100 rotating at 600 rpm. All

Table I Composition of Emulsions and Surfactant Solutions for Quantitative Measurements Using the Franz Cells Method Together with the Mean Diameters of Micelles and Emulsion Droplets Based on a Spherical Model ($n = 3 \pm$ SD) and Emulsion Viscosities

	Oily surfactant sol. PEG6C18:1	Emulsion PEG6C18:1 (o/w)	Aq. surfactant sol. PEG20C12	Emulsion PEG20C12 (o/w)	Aq. surfactant sol. PEG20C18:1	Emulsion PEG20C1:1
Retinol	0.52 wt.%	0.53 wt.%	0.47 wt.%	0.50 wt.%	0.49 wt.%	0.51 wt.%
PEG6C18:1	7.35 wt.%	7.09 wt.%	–	–	–	–
PEG20C12	–	–	7.07 wt.%	7.15 wt.%	–	–
PEG20C18:1	–	–	–	–	7.00 wt.%	7.06 wt.%
BHT	0.52 wt.%	0.50 wt.%	0.49 wt.%	0.59 wt.%	0.53 wt.%	0.50 wt.%
Dodecane	91.61 wt.%	54.95 wt.%	–	54.60 wt.%	–	54.80 wt.%
H_2O	–	36.93 wt.%	91.97 wt.%	37.17 wt.%	91.98 wt.%	37.13 wt.%
Mean diameter (nm±SD)	357.1±43.0	722±1	24.3±1.0	2075±1	17.2±0.1	2578±51
Size distribution	0.34±0.41 (PDI)	0.98±0.01 (Span)	0.80±0.03 (PDI)	0.72±0.03 (Span)	0.49±0.01 (PDI)	1.10±0.06 (Span)
Viscosity (mPa.s)	–	147	–	366	–	236

emulsions were in a "liquid" state with viscosities between 137 and 385 mPa.s.

Mean size and viscosity both may influence the skin penetration (4). To avoid considering these parameters, the objective was to use emulsions with the same size and viscosity. Results presented in Tables I and II show that the mean size varied from 17.2±0.1 nm to 357.1±43.0 nm for the surfactant solutions and from 585±9 nm to 2578±51 nm for emulsions and could be considered as identical for emulsions, and viscosities were not significantly different.

In Vitro Penetration Studies

The thawed skin was mounted in two-chamber glass diffusion cells. The effective penetration area was 2.54 cm^2, and the volume of the receiver chamber was 11 cm^3. The receiver solution was composed of a phosphate buffer at pH 7.4 containing 1.5% Brij®98 (Oleth-20) and 0.1% BHT. Brij®98 and BHT were dissolved in this buffer solution at 60°C; the solution was filtered after cooling. Retinol solubility in the receptor fluid was 45 mg ml^{-1}; its stability was the same as that measured in the formulations.

The study was carried out in occlusive conditions for 24 h in static Franz cells. Freshly prepared formulations were spread uniformly on skin surfaces in the donor compartments of diffusion cells at a retinol concentration of 1,900 µg/cm². With these quantities, an infinite dose and sink conditions were ensured as recommended by OECD guidelines (28). The experiment was repeated six times for each formulation ($n=6$). At the end of the study the receptor fluid was removed, filtered and analyzed by high performance liquid chromatography (HPLC). The formu-

Table II Composition of Emulsions and Surfactant Solutions Used for CRM Measurements Together with the Mean Diameters of Micelles and Emulsion Droplets Based on a Spherical Model ($n = 3 \pm$ SD) and Emulsion Viscosities

	Oily surfactant sol. PEG6C18:1	Emulsion PEG6C18:1 (o/w)	Aq. surfactant sol. PEG20C12	Emulsion PEG20C12 (o/w)	Aq. surfactant sol. PEG20C18:1	Emulsion PEG20C18:1 (o/w)
Retinol	0.51 wt.%	0.50 wt.%	0.52 wt.%	0.38 wt.%	0.49 wt.%	0.49 wt.%
PEG6C18:1	7.13 wt.%	7.28 wt.%	–	–	–	–
PEG20C12	–	–	7.04 wt.%	7.2 wt.%	–	–
PEG20C18:1	–	–	–	–	7.23 wt.%	7.21 wt.%
BHT	0.49 wt.%	0.51 wt.%	0.73 wt.%	0.56 wt.%	0.67 wt.%	0.53 wt.%
n-dodecane	45.73 wt.%	27.50 wt.%	–	27.02 wt.%	–	27.37 wt.%
D(26)-n-dodecane	47.96 wt.%	27.75 wt.%	–	27.05 wt.%	–	27.78 wt.%
H_2O	–	9.85 wt.%	22.65 wt.%	9.34 wt.%	22.96 wt.%	9.19 wt.%
D_2O	–	27.04 wt.%	69.07 wt.%	28.46 wt.%	68.65 wt.%	27.45 wt.%
Mean diameter (nm ± SD)	232.9±18.6	585±9	41.0±3.0	1180±1	26.5±0.4	1915±4
Size distribution	0.08±0.03 (PDI)	131.49±21.22 (Span)	0.92±0.03 (PDI)	0.92±0.01 (Span)	0.78±0.01 (PDI)	0.86±0.04 (Span)
Viscosity (mPa.s)	–	137	–	385	–	253

lation in each donor compartment was collected in a vial. The remaining formulation was absorbed by two filter papers, which were also collected in a vial. The skin samples were then separated into *SC*, epidermis and dermis. The *SC* was separated by the cyanoacrylate skin surface biopsy method. It has been shown in previous work (31) that this method was the most effective for the removal of the entire *SC*. For this procedure a microscope slide (76 × 26 mm; Roth, Karlsruhe, Germany) was coated with cyanoacrylate resin (Loctite® Super Glue-3 from Henkel, France) on the *SC* for a defined polymerization time of 15 min. Removing the slide detached the *SC* (32). The viable epidermis was separated from the dermis by heat treatment in water at 60°C for 45 s. After separation, the epidermis and dermis were cut into pieces with a scalpel. Retinol was extracted, and samples were filtered and analyzed by HPLC. Methanol with 0.5 wt.% α-tocopherol acetate was used for the extraction of retinol *via* a double extraction under agitation. This extraction procedure showed a complete retinol extraction (>99%). On the third extraction no more retinol could be detected in the solvent. Skin samples and biopsy slides were immersed in extracting medium under agitation for 90 min in order to achieve full extraction. Using BHT and α-tocopherol acetate and undertaking all experiments in the dark avoided degradation of retinol under light (33–35).

HPLC Analysis of Retinol Content

The samples were analyzed for retinol content using liquid chromatography with a reverse phase column coupled with a UV detector. The HPLC system from Waters (St Quentin en Yvelines, France) was composed of a Waters 717 injector, a Waters 600 pump, a reverse phase column XTerra®MS C18 (3.9 mm×150 mm, 5 μm) and a Waters 2996 photo-diodearray UV detector working at 325 nm wavelength. Elution with methanol/water (85:15) solvent at 1 cm^3/min flow rate and 25°C gave a retention time of 8.8 min for retinol. Injection volume was 20 μl. The calibration curve for quantitative analysis was linear up to 85 $\mu g.ml^{-1}$ and the LOD and LOQ were respectively 15 $ng.ml^{-1}$ and 50 $ng.ml^{-1}$

HPLC Quantitative Data Analysis

The mean and standard error of the mean (SEM) of $n=6$ determinations were calculated. Statistical comparisons were made using the Student's *t*-test (two samples assuming different variances) with the level of significance at $p \leq 0.05$.

Confocal Raman Microscope Studies

The experimental device included a Raman spectrograph (LabRam HR800, Horiba Jobin Yvon) and a confocal microscope probe (BXFM Olympus). The objective used in this experiment was a long working distance 50× of magnitude lens (Mitutoyo) with a numerical aperture of 0.42, operating in air. For axial profiles the objective was displaced manually. The minimum graduation limit was 1 μm, and in this work axial profiles were measured in 2 μm steps. The excitation source was a green 514.5 nm argon ion laser (Spectra Physics), delivering about 10 mW CW at sample level. The spectrograph was equipped with an air-cooled CCD detector (Synapse, Horiba Jobin Yvon) and a 600 gr/mm grating, which allows the covering of the large spectral range from 200 to 3,900 cm^{-1} in three shot acquisitions with a spectral resolution of about 4 cm^{-1}, which is retained at deeper regions. The acquisition time was three times 5 s, which enabled rapid measurements to be made at the surface as well as in deeper layers. This configuration allowed an adequate signal/noise ratio without damaging the skin during the period of measurement. The surface of the skin was not covered by a window in order to avoid interferences by Raman features generated from the window material itself when in contact with skin. Moreover, the presence of a window on the skin may generate physiological and mechanical stress, which could affect skin penetration. This also limited refractive index effects induced by an additional interface depending on the material used.

The axial resolution, which was a critical achievement for the measurements, was determined by plotting the intensity of the Raman peak associated to the Si-Si vibrational mode of silicon at 520 cm^{-1} against the position of the laser focus. The axial resolution was inferred from the full width at half maximum (FWHM) of this response curve with about 5.6 μm with a confocal opening aperture of 200 μm.

The differences of the refractive index between the air and the skin, but also between the different skin layers, induces a deviation of the light beam, which gives an incorrect estimate of the depth value. This problem was studied and described by many groups (36–44), and a recent study by Xiao *et al.*, using multilayered systems constituted with polymers films with refractive indexes close to those of the human skin, showed that the error in the measurement led to an underestimation of the depth value of 15–20% (45). Therefore, all depth values deduced from Raman measurements should be theoretically corrected by applying a 15–20% factor. Several authors then developed mathematical models to represent the laser beam distortions to be able to calculate the depth resolution taking into account the refraction and diffraction effects (36–41,44). Tfayli *et al.* compared these theoretical estimations with experimental measurements on PET films and skin slices samples (44). They showed that, at low depth value (below 20 μm), the difference between the nominal depths

calculated from three models and the real one was small. However, the measurement error increased when probing deeper in the skin sample, the models giving an overestimation of the depth. The authors also proved that models gave acceptable estimation of the depth resolution. However, Tfayli *et al.* highlighted the difficulties encountered when working with such a complex tissue as skin, which presents heterogeneities and a layered structure, each layer having a different refractive index. To simplify the analysis, they did not take into account these differences and limited their work to *stratum corneum*. This correction was not done in the present work because the aim of the research was not to measure accurately the exact quantity of a formulation component at an exact depth in the skin but rather to provide relative information about formulation components and formulation type effects on the active substance distribution.

Data reproducibility was experimentally checked by recording ten successive spectra at the skin surface showing very slight spectral features and intensity variations of less than 3%.

Data Analysis

To remove the intrinsic skin fluorescence, a linear baseline was subtracted. As a result of the loss of light from an increase in scattering when probing deeper into the sample, the signal becomes weaker (17). To be able to compare the intensity of bands in the region, 2,600–3,900 cm^{-1} and 1,500–300 cm^{-1} spectra were equalized for the 2,940 cm^{-1} and 1,450 cm^{-1} band intensities, respectively. The band at 2,940 cm^{-1} corresponds to the protein CH_3 symmetric stretching band, and 1,450 cm^{-1} corresponds to the δCH_2 scissoring bands of proteins and lipids in the *SC*. The C–H bands (at 1,450 cm^{-1} and 2,940 cm^{-1}) protrude outside the protein chain and do not take part in strong intermolecular interactions; therefore, the C–H band is not modified by alterations in secondary protein structure and is used for equalization of the spectra (14,46).

The thickness of the *SC* cannot be measured directly by CRM. But it is known (1) that the water content rises from 13% in the *SC* to >50% in the viable epidermis. To measure the water content the ratio of the C–H (around 2,933 cm^{-1}) and O–H stretching bands (around 3,250 cm^{-1}) was calculated as described elsewhere (13).

The D(26)-dodecane and deuterium oxide contents were measured following a similar procedure using the characteristic band at 2,099 cm^{-1} with an integral area from 2,075 to 2,115 cm^{-1} for D(26)-dodecane and the band at 2,500 cm^{-1} with an integral area from 2,340 to 2,680 cm^{-1} for D_2O. The retinol content was measured by dividing the intensity of the characteristic band at 1,585 cm^{-1} by the intensity of the protein band at 1,450 cm^{-1}. The mean and standard error of the mean (SEM) of $n=3$ determinations were both calculated. Statistical comparisons were made using the Student's *t*-test (two samples assuming different variances) with the level of significance at $p \leq 0.1$.

The ratio I_{2880}/I_{2850} reflects the lateral packing of lipids. The 2,880 cm^{-1} band is sensitive to both intra-chain and inter-chain interactions. The integrated intensity and the peak height of the band decrease as intramolecular chain disorder (*trans-gauche* C–C bond isomerization) increases.

To calculate this ratio, the intensities at 2,850 cm^{-1} and 2,880 cm^{-1} were measured. Each skin sample was measured along the *SC* at 0, 4, 6 and 8 µm, and the mean was taken as the ratio I_{2880}/I_{2850} for the skin sample.

RESULTS

Raman Characteristic Peak Identification

The Raman spectra of D(26)-dodecane and retinol were recorded in order to verify that their characteristic peaks do not interfere (Fig. 1a). The characteristic peak of D(26)-dodecane at 2,099 cm^{-1} and the one for *trans*-retinol at 1594 cm^{-1} can readily be observed in Raman spectra. Moreover, they could easily be observed in skin spectra, which is illustrated in Fig. 1b, showing a skin sample treated with an emulsion containing retinol, D(26)-dodecane and heavy water, stabilized by a surfactant.

In the same manner, a peak for heavy water (~2,500 cm^{-1}) arising from its use in some formulations can be observed independently of endogenous water.

Retinol Penetration

The retinol content was measured after 24 h in the donor compartment (depot), *SC*, epidermis, dermis and receptor fluid. The results are given in Table III as a percentage of applied retinol per cm^2. The first result indicated that no retinol was recovered in the receptor fluid. Therefore, only the retinol distribution within skin layers will be compared.

For a better understanding, a comparison will be made, first, of the influence of the different formulation types (emulsion and surfactant solution) and, second, of the influence of the different surfactants.

Comparison of Vehicles

The retinol distribution according to the vehicle is illustrated in Figs. 2 and 3.

In Fig. 2, the amounts of retinol that penetrated into the whole skin, the lipophilic *SC*, and the hydrophilic part of the skin after 24 h are given as percentages of the applied
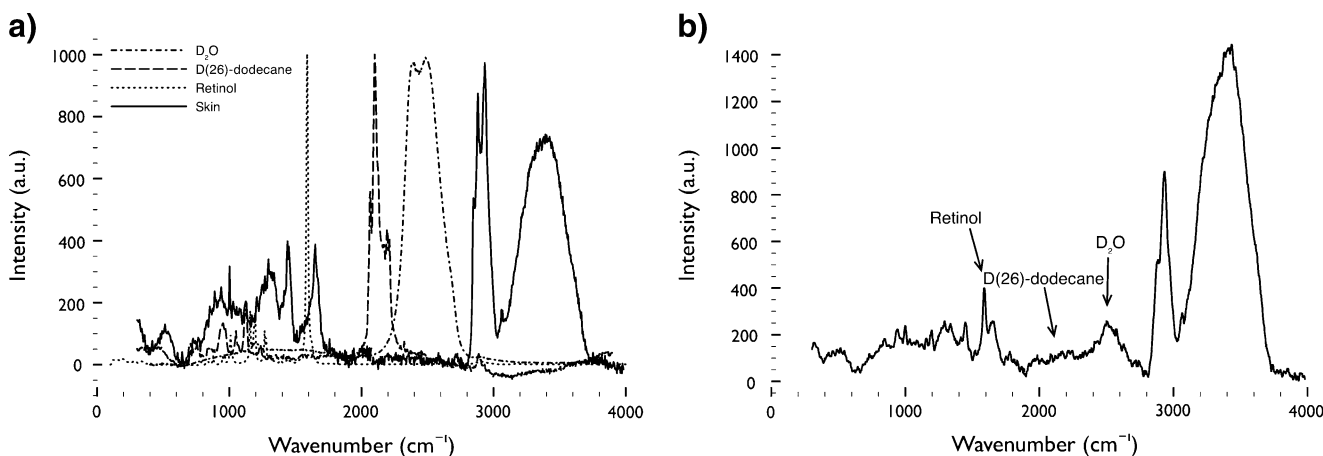
Fig. 1 a) Raman spectra of an untreated skin, retinol (1,594 cm^{-1}), D(26)-dodecane (2,099 cm^{-1}) and heavy water (2500 cm^{-1}); **b)** Raman spectra of a skin treated with an emulsion containing retinol, D(26)-dodecane and heavy water.

dose. In Fig. 3, the penetration of retinol, is shown, as determined using CRM from the use of formulations based on different vehicles.

Whole Skin. Considering complete formulations, it is clear from Fig. 2 that surfactant solutions promoted significantly greater retinol absorption in skin when compared with the emulsions.

The distribution of retinol in skin layers is different according to the vehicle used. The control dodecane solution loaded with retinol was compared only with the PEG6C18:1 surfactant solution because of the oily nature of its vehicle.

Stratum Corneum. As previously mentioned, all emulsions (stabilized by PEG6 or PEG20) were of the oil-in-water type.

The three surfactant solutions were different: the PEG6C18:1 surfactant solution was formulated in dodecane, while the other two surfactants were formulated in water. Using the surfactant solutions, the penetration of retinol in the *SC* was $0.30\pm0.05\%/cm^2/24$ h for the PEG6 (Fig. 2 left), $0.30\pm 0.03\%/cm^2/24$ h for the PEG20C18:1 (Fig. 2 middle), and $0.34\pm0.06\%/cm^2/24$ h for the PEG20C12 (Fig. 2 right). These values were significantly three times higher than for the corresponding emulsions ($p\leq0.05$) ($0.11\pm0.01\%/cm^2/24$ h for PEG6C18:1, $0.09\pm0.01\%/cm^2/24$ h for PEG20C18:1 and $0.11\pm0.01\%/cm^2/24$ h for PEG20C12). Retinol from the control dodecane solution penetrated poorly into the *SC*, ($0.05\pm0.01\%/cm^2/24$ h) being twice as low as for the PEG6C18:1 ($0.11\pm0.01\%/cm^2/24$ h) emulsion and as much as six times lower than for the oily PEG6C18:1 surfactant solution ($0.30\pm0.05\%/cm^2/24$ h).

Table III Quantitative Penetration Results for Retinol After 24 h as a Percentage of the Applied Quantity per cm^2 in Different Skin Compartments. All Results are the Mean of Six Experiments (PEG6C18:1 Surfactant Solution $n=12$), and the Error is the SEM

| Depot	SC	Epidermis (E)	Dermis (D)	Receptor fluid	E+D	Skin	Total
Retinol solution in dodecane; % of applied retinol per cm^2 (%/cm^2/24 h)							
96.68±0.34	0.05±0.01	0.13±0.01	0.19±0.03	0.01±0.01	0.32±0.04	0.37±0.04	97.07±0.30
Emulsion (o/w) PEG6C18:1; % of applied retinol per cm^2 (%/cm^2/24 h)							
103.42±0.50	0.11±0.01	0.15±0.01	0.25±0.03	0.00±0.00	0.40±0.05	0.51±0.03	103.94±0.51
Oily surfactant solution PEG6C18:1; % of applied retinol per cm^2 (%/cm^2/24 h)							
95.77±2.42	0.30±0.05	0.22±0.02	0.23±0.01	0.05±0.03	0.45±0.00	0.75±0.06	96.57±2.36
Emulsion (o/w) PEG20C12; % of applied retinol per cm^2 (%/cm^2/24 h)							
102.93±1.54	0.11±0.01	0.07±0.01	0.12±0.01	0.00±0.00	0.19±0.03	0.29±0.03	103.23±1.53
Aqueous surfactant solution PEG20C12; % of applied retinol per cm^2 (%/cm^2/24 h)							
107.69±2.24	0.34±0.06	0.06±0.01	0.09±0.01	0.01±0.01	0.15±0.02	0.49±0.07	108.19±2.27
Emulsion (o/w) PEG20C18:1 % of applied retinol per cm^2 (%/cm^2/24 h)							
94.15±2.96	0.09±0.01	0.05±0.00	0.13±0.01	0.01±0.01	0.18±0.04	0.28±0.02	94.43±2.97
Aqueous surfactant solution PEG20C18:1; % of applied retinol per cm^2 (%/cm^2/24 h)							
105.05±0.80	0.30±0.03	0.06±0.00	0.05±0.01	0.01±0.01	0.11±0.01	0.41±0.04	105.47±0.75
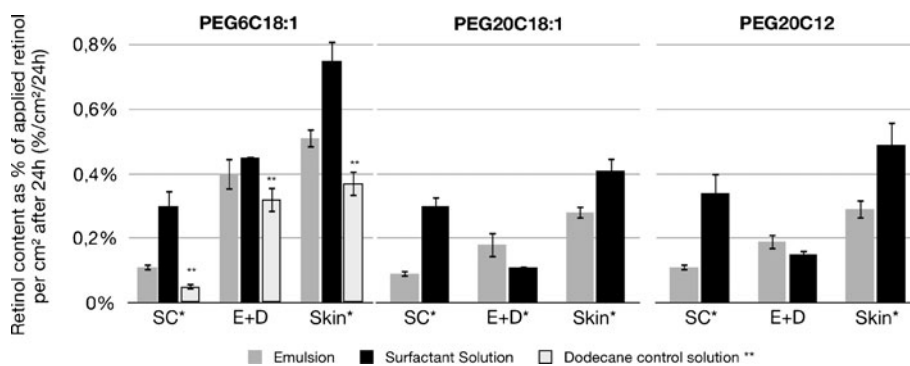
Fig. 2 Retinol content given as a percentage of applied retinol per cm^2, measured via HPLC, in different skin compartments (*stratum corneum* (SC), epidermis + dermis (E+D) and whole skin) after 24 h of exposure time with a surfactant solution, Dodecane control solution (left); from emulsions with surfactants PEG6C18:1 (left), PEG20C18:1 (middle) and PEG20C12 (right). The error bars represent the SEM ($n = 6$ and $n = 12$ for PEG6C18:1 surfactant solution). Columns marked with a * represent a significant difference ($p \leq 0.05$) between the surfactant solution and the emulsion. ** indicates that the retinol content from the surfactant-free dodecane control solution is significantly different from the PEG6 surfactant solution in the SC, E+D and skin and from the PEG6 emulsion in the SC and skin.

Similar results could be observed using CRM (Fig. 3). It must be mentioned that no conclusions concerning the quantity of retinol in the epidermis can be made from CRM results, as the measurements were only made up to 30 μm and not for the whole skin, as a consequence of technical constraints. The retinol was tracked from 0 μm to 30 μm under the surface, which corresponded to a screening of the whole *SC* and the beginning of the epidermis. Significantly more retinol could be observed in the *SC* with a surfactant solution as compared to an emulsion (Table III, Figs. 2 and 3). A significant difference ($p \leq 0.1$) between emulsions and surfactant solutions could be observed from the retinol penetration patterns. It is clear from Fig. 3 that for surfactant solutions, absorbed retinol quantities, and with that the intensity of the characteristic retinol peak at 1,594 cm^{-1}, decreased significantly from 4 μm to 30 μm, while a constant retinol distribution over the same range was observed for emulsions independently of the surfactant used.

Epidermis and Dermis. It is interesting to notice that the amount of retinol was superior to that recovered in *stratum corneum* for emulsions contrary to surfactant solutions.

With the surfactant solutions (PEG20C12 and PEG20C18:1), the retinol content dropped dramatically between the *SC* and the hydrophilic parts of the skin (Figs. 2 and 3). However, there was no significant difference between amounts of retinol found in the epidermis and dermis using the different formulations except for the surfactant PEG20C18:1. Emulsions formulated with PEG20C18:1 showed a significant higher penetration of retinol in these layers than for the corresponding surfactant solutions.

Retinol from the control dodecane solution penetrated into the hydrophilic parts of the skin one third less than for the corresponding PEG6C18:1 surfactant solution.

Comparison of Surfactants

Skin absorption results related to the surfactants are reported in Fig. 4.

Whole Skin. The results in Fig. 4 show that the highest penetration of retinol using PEG6C18:1 formulations is independent of the vehicle. There were no significant differences between the other two surfactants.

Stratum Corneum. It can be seen that the retinol amount in the *SC* is similar for the three emulsions used (about 0.1%/cm^2/24 h). Looking at the data for the surfactant solutions, retinol accumulation was found to be greater than for emulsions (0.32%/cm^2/24 h), but there were no differences between the three surfactants.

Only the control dodecane solution displayed a significant lower retinol content (0.05 ± 0.01%/cm^2/24 h) in the *SC*.

Epidermis and Dermis. As illustrated in Fig. 4, the use of both the PEG6C18:1 surfactant solution and the corresponding emulsion showed a significantly higher retinol content in these layers than for the use of the PEG20 surfactant solution and emulsion.

The retinol content of 0.32 ± 0.04%/cm^2/24 h from the control dodecane solution was lower than for the oily PEG6C18:1 surfactant solution but was still higher than for the aqueous PEG20 surfactant solution.

Ingredient Penetration (D$_2$O and D(26)-Dodecane) by CRM

The use of CRM allows the water phase of the formulation to be tracked by replacing normal water with deuterium oxide, so-called 'heavy water', D$_2$O.
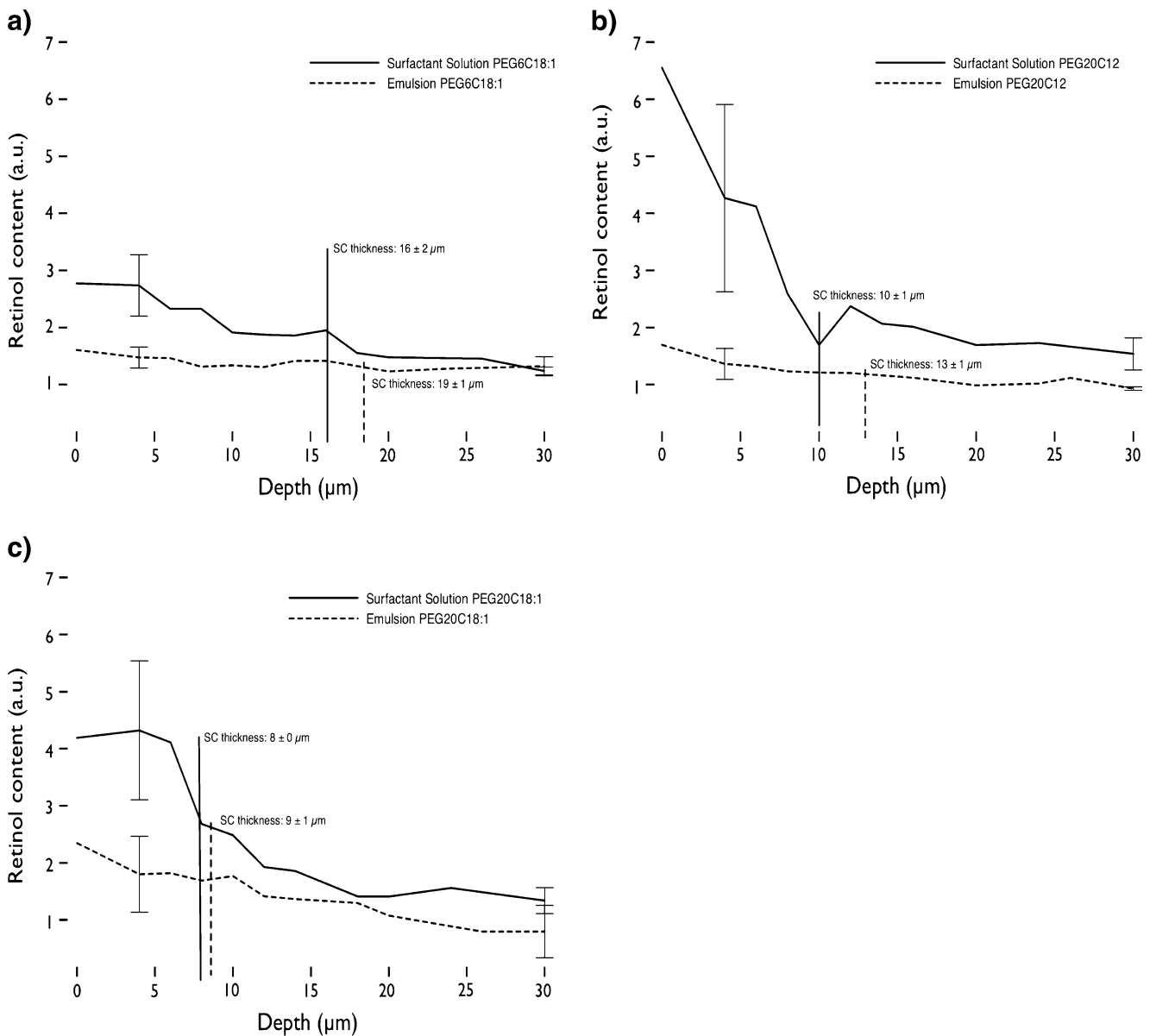
Fig. 3 Relative concentration profiles of retinol in the *SC* after 24 h exposure time for an emulsion and an oily surfactant solution using PEG6C18:1 (**a**), PEG20C18:1 (**b**) and PEG20C12 (**c**) in the *SC* as determined by Raman spectroscopy. For better visibility the SEM ($n = 3$) bars are given for 4 and 30 μm. For surfactant solutions, the decrease from 4 to 30 μm is significant with $p \leq 0.1$. *SC* thicknesses for skin samples treated with surfactant solution were measured using $16 \pm 2 \mu$m (PEG6C18:1), $8 \pm 0.7 \mu$m (PEG20C18:1) and $10 \pm 1 \mu$m (PEG20C12). For skins treated with emulsions, *SC* thicknesses were $19 \pm 1 \mu$m (PEG6C18:1), $9 \pm 1 \mu$m (PEG20C18:1) and $13 \pm 1 \mu$m (PEG20C12).

Fig. 5a represents the water content as a function of skin depth penetration. This water profile is similar to that for endogenous water. The water from the formulations penetrated into the skin and increased slowly in the *SC*, then increased more rapidly at the border between the lipophilic *SC* and the hydrophilic epidermis, reaching values of around 8–9 µm for samples treated with PEG20C18:1, 13–14 µm for samples treated with PEG20C12, and 16–18 µm for samples treated with PEG6. The increases in the three penetration profiles are significant in the range 4 to 30 µm. For a clearer illustration of this, only the SEMs for 4 µm and 30 µm are given. However, no significant differences in the water content between the five formulations could be measured (Fig. 5a), nor could any correlation between retinol and water penetration be established. As mentioned in Materials and Methods, these depth values do not correspond to the real depths reached by the retinol under the skin but only to the displacement of the objective, which should be corrected to take into account laser beam diffraction and refraction. However, the data could be used for comparative purposes between the formulations.
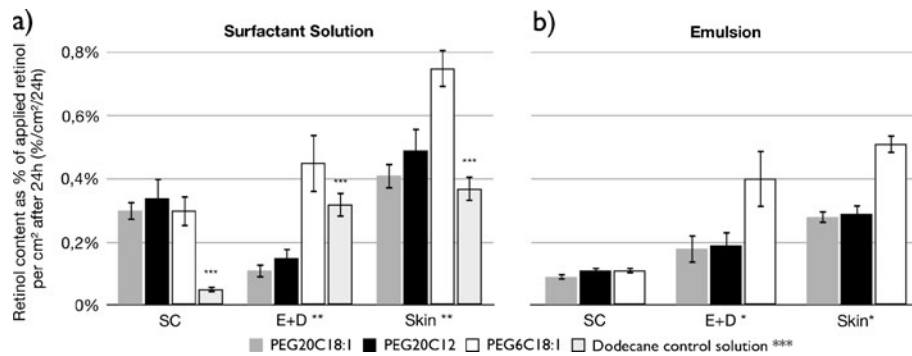
Fig. 4 Retinol content given as a percentage of applied retinol per cm^2, measured via HPLC, in different skin compartments *stratum corneum* (*SC*), epidermis + dermis (E+D) and whole skin after 24 h of exposure time with three different surfactant solutions using PEG20C18:1, PEG20C12 and PEG6C18:1, also including the dodecane control solution, (**a**), and an emulsion stabilized by the same surfactants, (**b**). The error bars represent the SEM ($n = 6$ and $n = 12$ for PEG6C18:1 surfactant solution). Columns marked with * represent a significant difference ($p \leq 0.05$) between the PEG6C18:1 and the PEG20 solutions. ** indicates a significant difference between all surfactants, the retinol content difference between the *SC* and E+D of each surfactant being significant. *** indicates that the retinol content from the surfactant free dodecane control solution is significantly different from the PEG6C18:1 surfactant solution in the *SC*, E+D and skin.

The dodecane also penetrated into the *SC* and the epidermis (Fig. 5b), except for the PEG20C18:1 surfactant solution, but between the four formulations, no significant differences in the quantities of D(26)-dodecane that penetrated were noticed.

Lateral Interaction (Ratio of I$_{2880}$/I$_{2850}$)

Each ratio I$_{2880}$/I$_{2850}$ value was determined in triplicate, and the values are given in Table IV.

Pure dodecane (R I$_{2880}$/I$_{2850}$ = 1.192 ± 0.042) without any surfactant influences the ratio I$_{2880}$/I$_{2850}$ significantly when compared with untreated skin (R I$_{2880}$/I$_{2850}$ = 1.615 ± 0.029) and with skin treated with water. This ratio decreased significantly in value, showing the strong effect

of dodecane on lipid fluidization. The oily surfactant solution of PEG6C18:1 also fluidized the lipid layer to a marked extent as indicated by a value of the I$_{2880}$/I$_{2850}$ ratio = 1.413 ± 0.033. This is a somewhat smaller decrease than for dodecane but still significant when compared with skin treated with water and with untreated skin.

Water itself appears to have no marked influence on the ratio of I$_{2880}$/I$_{2850}$ when compared to untreated skin nor do the emulsions. For the aqueous surfactant solutions, only the influence of the PEG20C12 gives a significant different value when compared with the water-treated skin and the untreated skin (Fig. 6).

No significant differences were observed for the ratio values between the three emulsions. In contrast, one of the
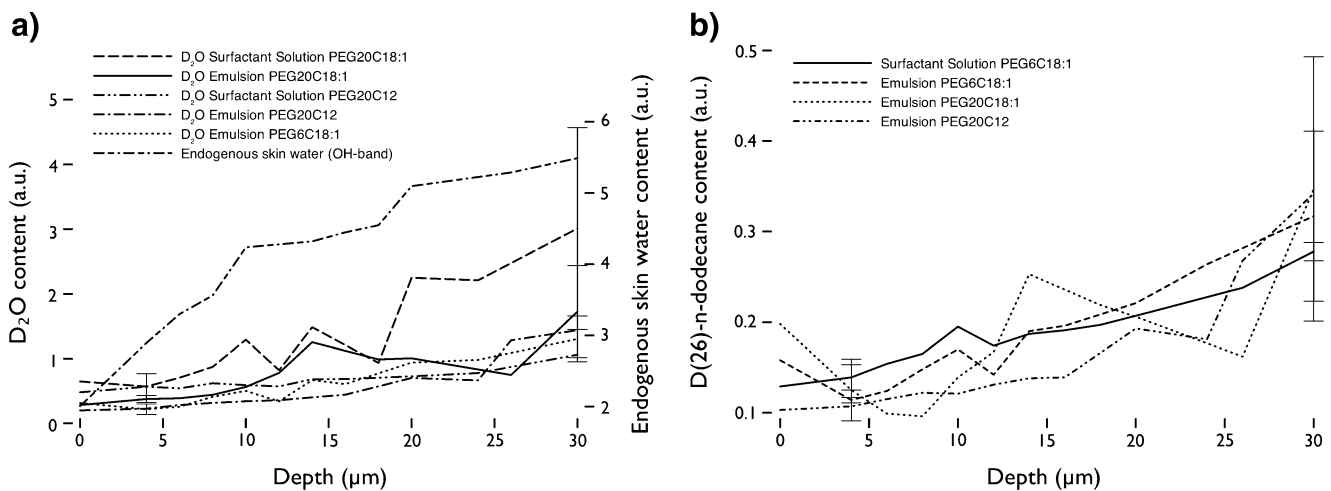

Fig. 5 Relative concentration profiles in the *SC* after 24 h exposure time of D$_2$O (**a**) for the three emulsions and for the two aqueous surfactant solutions using PEG20C18:1 and PEG20C12 together with the endogenous skin water for one skin sample and a comparison of the penetration patterns of D$_2$O and of D(26)-dodecane; (**b**) for the three emulsions and for the oily surfactant solution with PEG6C18:1 as determined by Raman spectroscopy. For clarity, the SEM ($n = 3$) bars are given for 4 and 30 μm. For all formulations the increase in dodecane (except the PEG20C18:1) and D$_2$O from 4 to 30 μm is significant with $p \leq 0.1$.

Table IV Ratios of I_{2880}/I_{2850} for Skin Samples After 24 h Treatment. All Parameters were Determined in Triplicate. For Clarity, the Significances are Discussed in the Text

Substance used for treating skin	Ratio I_{2880}/I_{2850}
Dodecane	1.192 ± 0.042
Water	1.562 ± 0.012
Emulsion (o/w)	
PEG6C18:1	1.503 ± 0.041
PEG20C12	1.509 ± 0.057
PEG20C18:1	1.44 ± 0.103
Surfactant solution	
PEG6C18:1 (oily)	1.413 ± 0.033
PEG20C12 (aqueous)	1.779 ± 0.037
PEG20C18:1 (aqueous)	1.546 ± 0.023
Untreated skin (control)	1.615 ± 0.029

I_{2880}/I_{2850} values for the surfactant solutions was significantly different (Figs. 6 and 7).

DISCUSSION

This work aimed at studying the penetration behavior of the main components of retinol formulations in order to better explain the influence of ingredients on the retinol penetration. To achieve this goal, the formulations may be very simple in their composition to clarify the involved mechanism.
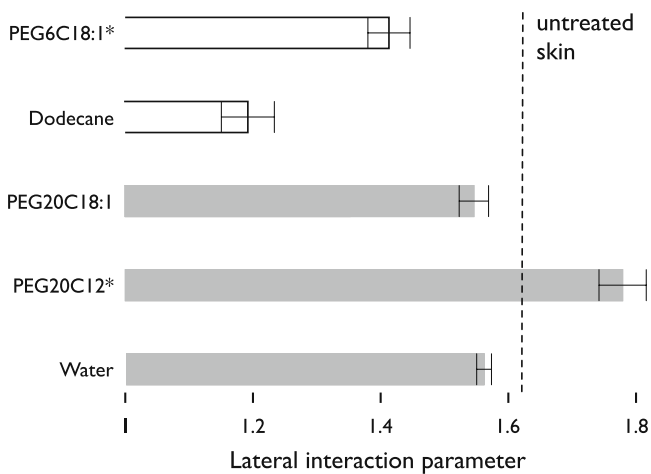

Fig. 6 Ratios of I_{2880}/I_{2850} for skin samples treated with dodecane, water and surfactant solutions; *white* indicating oily solutions and *grey* the aqueous solutions. The *error bars* represent the SEM ($n = 3$). Columns marked with * indicate a significant difference between the ratios of the treated samples as compared with the pure solvent ($p \leq 0.05$); "PEG6C18:1" compared to "dodecane" and "PEG20C12" to "water". All skin samples treated with the surfactant solutions are between themselves significantly different; the same is true for water-treated skin compared with dodecane-treated skin.

The formulations were based on retinol, water and dodecane as the main components. Three different surfactants were chosen because these were known to affect drug distributions in skin. The surfactants differed in their alkyl chain lengths and in their PEG numbers.

Dodecane was used as the oily phase because the band from trans-retinol at $1,594$ cm^{-1} and that of D(26)-dodecane at $2,099$ cm^{-1} can be observed simultaneously in Raman spectra (Fig. 1a and b) without interfering with the main bands from skin.

Retinol surfactant solutions in dodecane or in water were compared with their corresponding emulsions. A retinol solution in dodecane was used as control. PEG6C18:1 was not soluble in water, and consequently an oily surfactant solution was formulated in dodecane.

The model drug selected for this study was retinol, a widely used chemical for cosmetic as well as for pharmaceutical purposes. Retinol is highly lipophilic (logP = 7.62 (47)) and tends to accumulate in the epidermis (25,34). This drug is used in dermatological products as an ointment intended for skin repair. In cosmetic products, retinol is known to promote keratinocyte proliferation and to induce epidermal thickening in photo-damaged skin through its biologically active derivative, retinoic acid (27). The objective of this study was not to disclose a formulation that would provide a definite beneficial effect regarding the action of retinol, an action, which is mainly located in the basal cells of epidermis and dermis (26), but to use a substance that does not penetrate deeply into the skin so as to evaluate any possible enhancing effects caused by surfactants widely used in cosmetic formulations, and to permit the simultaneous tracking of all formulation ingredients by CRM.

Retinol did not penetrate into the receptor fluid from the different formulations (Table III). This result was expected because, in general, retinol penetrates the skin very poorly (48). In earlier skin absorption studies conducted on human or porcine skin, retinol was barely detectable in the receptor fluid (34,49).

In the present study skin, absorption of retinol was limited to between 0.28 and 0.80% of the applied dose. These results are in accordance with those of Jenning et al. (50), who applied a retinol-based nanoemulsion and an SLN (Solid Lipid Nanoparticles) suspension ten times less concentrated than in this study. They found a total retinol absorption into the skin between 0.5 and 0.8% of the applied dose, the exact value depending on the formulation. The total amount of retinol absorbed into the skin is, however, considerably different from one study to another for retinol dosage formulations varying between 0.1 and 0.5% of retinol. In this study, it was found that less than 1% of the applied dose accumulated in the skin, while Yourick and Bronaugh (51) found that 10% of the applied dose

accumulated in human skin after 24 h, and Frelichowska *et al.* found less than 0.1% accumulation in the skin from o/w emulsions (34). These results point out the importance of the vehicle on retinol penetration.

In this study, o/w emulsions were chosen as vehicles because the major part of topical formulations intended for pharmaceutical or cosmetic uses is based on o/w emulsions. For comparison purpose related to the surfactant effect on the retinol penetration surfactant solutions were also selected.

As previously observed in the literature, surfactants affect retinol penetration into the skin. PEG6C18:1 promoted retinol penetration whatever the formulation and especially in epidermis and dermis. Surfactants have been shown to affect the permeability characteristics of several biological membranes, including skin (19), and for this reason they can enhance the skin penetration of other compounds. Poly-ethoxylated non-ionic surfactants display penetration enhancement properties (18,20–22,52), but at the same time these surfactants can also decrease the permeation of lipophilic drugs into skin due to a decrease in thermodynamic activity when a drug of this type is solubilized in the micelles (52). The hydrophilic–lipophilic balance value (HLB) is not a good indicator of the enhancing effect of a surfactant, and, therefore, many studies have been focused on the influence of the alkyl chain length of the hydrophobic portion of non-ionic surfactants attached to a polar head group in the potency of surfactants as penetration enhancers (22,53,54) with a particular enhancing effect for the C12 alkyl chain being observed. A medium length alkyl chain surfactant (*e.g.* C12) may penetrate the lipid bilayers more easily because of its proper aqueous solubility and higher critical micellar concentration than a longer alkyl chain surfactant (*e.g.* C18). However, in this study, the C12 chain had almost the same effect as a C18:1 chain, but the C18 oleic chain, is known to exert a strong enhancer effect. Concerning longer alkyl chain lengths, it has been demonstrated earlier that for penetration purposes, enhancers containing unsaturated alkyl chains such as C18 appear to have near optimum penetration properties.

The effect of a polar head group of a surfactant is not as clear, in particular in the way in which they influence the activity of surfactants as enhancers. Recently, positive effects of polysorbates with short polar head groups on methanol penetration of skin have been reported by Cappel and Kreuter (52). They observed an enhancement effect for PEG4 sorbitan monolaurate and PEG5 sorbitan mono-oleate on methanol penetration. These researchers suggested that surfactants insoluble in water have a significant effect on the leaving tendency of the hydrophilic compound as a result of a higher thermodynamic activity and a modification of the vehicle/*SC* partition coefficient. Moreover, these short surfactants have been shown to affect the

barrier properties of skin to a greater extent than their hydrophilic counterparts. In this study, it was noticed that there was a stronger enhancement effect for a PEG6 polar head group compared to a PEG20 polar head group. Park *et al.* found a similar tendency when studying the influence of polyoxyethylene (POE) alkyl ethers on the permeation of ibuprofen through rat skin. The enhancers containing an ethylene oxide (EO)-chain length of 2-5, an HLB value of 7-9 and a C16-18 alkyl chain length appear to be very effective promoters for the skin permeation of such a drug.

For one particular surfactant (PEG6C18:1), the oily surfactant solution significantly promoted retinol penetration in comparison with a PEG6C18:1 emulsion of the o/w type. PEG6C18:1 also promoted retinol penetration into the epidermis as shown by Fig. 4. The impact of surfactant on oil transport was reported by Mélot *et al.* (8), whose work showed similar enhancement effects. They found a significant effect for Triton X100 and oleic acid on the penetration of Myritol®318 (medium chain triglycerides). They reported oleic acid as a lipid fluidizer and Triton X100 as a lipid extractor but did not relate the observed enhancement effects directly to these two functions. Apart from the role of PEG6C18:1 on retinol penetration, the results of this study suggest a strong effect of the oil on the lipid barrier as shown by the R I_{2880}/I_{2850} modification.

The results reported in Table IV and illustrated in Fig. 6 show clearly the influence of pure dodecane on untreated skin (R $I_{2880}/I_{2850}=1.192\pm0.042$) compared with water-treated skin (R $I_{2880}/I_{2850}=1.562\pm0.012$). Pure dodecane induced the lowest I_{2880}/I_{2850} ratio, indicating the biggest fluidization of the lipids. It is noteworthy that this had no influence on the retinol quantity accumulated in the *SC* where the lipid barrier lies. All surfactant solutions showed a similar retinol quantity in the *SC* ($\sim$0.31%/cm^2/24 h). The retinol solution in dodecane associated with the most fluid lipid barrier resulted in the lowest retinol quantity in the *SC* (0.05%/cm^2/24 h). However, the modified I_{2880}/I_{2850} ratio correlated directly to the penetrated retinol amounts in the deeper skin layers. Dodecane showed the second highest retinol quantity in the dermis and epidermis after 24 h (0.32$\pm$0.04%/cm^2/24 h). The highest quantity (0.45$\pm$0.00%/cm^2/24 h) was obtained from PEG6C18:1 surfactant solution in dodecane, which gave a $_{2880}/I_{2850}$ ratio of 1.413$\pm$0.033. From pure dodecane solution, retinol penetration into epidermis and dermis was smaller than from the PEG6 surfactant solution because of two conflicting effects: while the dodecane affected the lipid barrier, at the same time the surfactant (PEG6C18:1) influenced the partition coefficient between retinol and skin. This effect of the surfactant was missing in the retinol solution prepared with pure dodecane; this may explain why using a skin with the lowest R I_{2880}/I_{2850} did not result in the highest penetration rate. The lipid fluidizer impact of

dodecane on a lipid barrier is well known and related to its skin irritation properties (55), which could explain the 'brutal' penetration enhancement of a dodecane surfactant solution when compared with an aqueous surfactant solution. This hypothesis is reinforced by R I_{2880}/I_{2850} calculated for the PEG6C18:1 o/w emulsion, which was not significantly different from the untreated skin or water-treated skin (Table IV and Fig. 7). In practice, in emulsions, dodecane is always in the disperse phase, and the observed differences cannot be assigned to a modification of the lipid barrier but are, more probably, linked to a surfactant effect. The influence of the surfactant on the retinol partition coefficient between the formulation and the skin might explain the higher penetration in epidermis and dermis using PEG6C18:1. To summarize, the particular behavior of PEG6C18:1 appears to be related to its oil solubility and its formulation in dodecane on the one hand, and to its short polar head group on the other.

The hydrophilic surfactants were formulated in aqueous or o/w vehicles. Surfactant solutions also promoted the penetration of retinol but mostly into the *SC*. Hydrophilic surfactants with long polar head groups did not have a similar enhancement effect to that observed with the shorter chain esters, as demonstrated earlier by Cappel *et al.* Additionally, no relationship between R I_{2880}/I_{2850} and the different retinol penetration behaviors was observed with these hydrophilic surfactants (Table IV, Figs. 6 and 7).

Only the surfactant solution of PEG20C12 had a significant influence on the lipid barrier with R $I_{2880}/I_{2850} = 1.779 \pm 0.037$ compared to untreated skin (R $I_{2880}/I_{2850} = 1.615 \pm 0.029$) or water-treated skin (R $I_{2880}/I_{2850} = 1.562 \pm 0.012$) (Fig. 6). Such an increase in the R I_{2880}/I_{2850} corresponds to a solidifying of the lipids. This
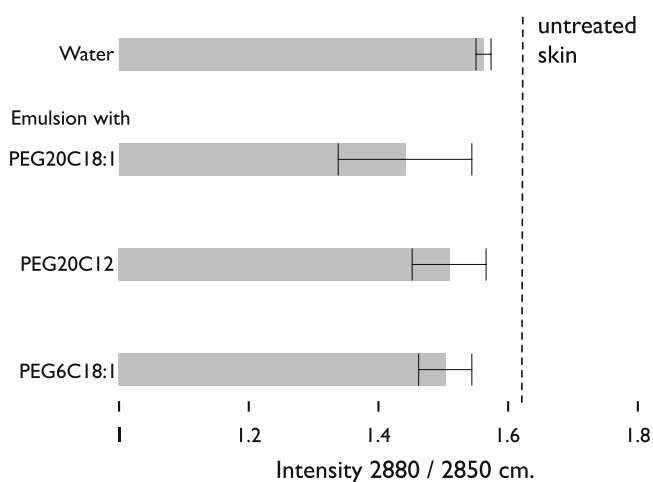
solidifying effect on the lipid barrier did not appear to have any influence on the retinol penetration into the epidermis and dermis.

The results appear not to be related specifically to the oil or to a change in the R I_{2880}/I_{2850}. They appear, instead, to result from a different partition coefficient between the formulation and the skin and, in particular, to the *SC*. In fact, it seems that this partition modification can have such a large effect that the quantity of retinol in the *SC* was very similar to that from an oily surfactant solution with a R I_{2880}/I_{2850} of 1.413 and from an aqueous surfactant solution with a R I_{2880}/I_{2850} of 1.779.

CONCLUSION

In this research project, the possibility of tracking the main components of two different formulation types, a surfactant solution and an o/w emulsion, has been demonstrated. The influence of these components on the penetration behavior of retinol, chosen as a model active substance, has also been studied. The work has shown the necessity for studying the impact of all formulation components for a complete understanding of the penetration behavior of a chosen active substance. The penetration behavior can be influenced by the partition existing between the formulation and the skin (quantified as the partition coefficient) and by modification of the lipid barrier. In this study, the combination of these two factors has been shown for each formulation type. Lipid organization was studied through the ratio of I_{2880}/I_{2850}, which decreases when lipid fluidization occurs. It has been shown that when the R I_{2880}/I_{2850} is low (less than 1.5 in this study), it is possible to link the decrease in the value of this parameter with an enhancement of retinol penetration into the hydrophilic parts of the skin. On the contrary, if the value of R I_{2880}/I_{2850} is high (>1.5 in this study), a change in value of this parameter indicates a change in lipid structure, which then appears to have less influence on penetration behavior. In this latter case, the suggested mechanism for explaining the change in penetration behavior is a change in the partition existing between the formulation and skin (quantified as the partition coefficient) rather than lipid structure influence. Results obtained with the oily soluble surfactant PEG6C18:1 confirmed the influence that a short polar head group has on penetration enhancement. This enhancement was also reinforced by the oil used to dissolve the surfactant, which showed itself a fluidization effect of the skin barrier.

These fundamental results were only possible through the use of CRM and very simple formulations. Using these techniques, the study provided some explanations about the impact of each emulsion component on penetration

Fig. 7 Ratios of I_{2880}/I_{2850} for skin samples treated with water and oil in water emulsions stabilized with PEG6C18:1, PEG20C12 and PEG20C18:1. The *error bars* represent the SEM ($n = 3$). No significant differences between the ratios of I_{2880}/I_{2850} ($p \leq 0.05$) could be observed.

behavior based on measured results and proof theoretical hypotheses.

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