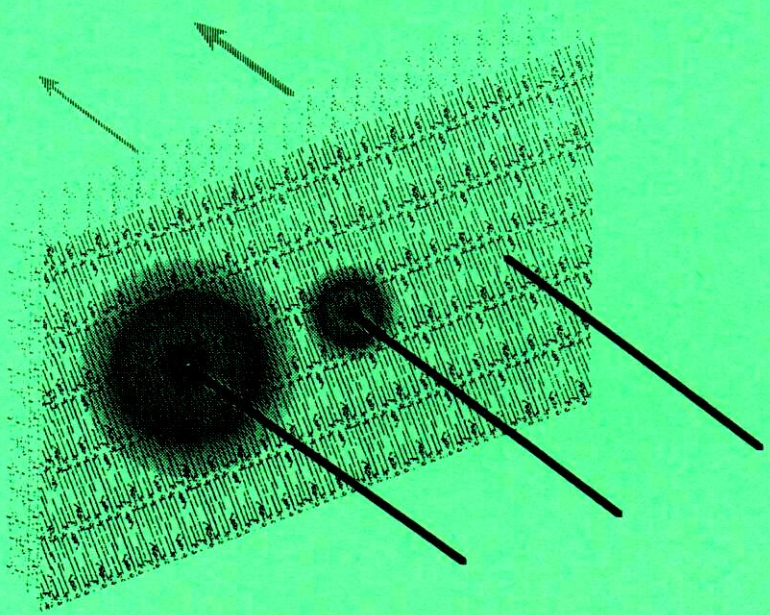
Perspectives in Percutaneous Penetration

Volume 7a



Edited by K.R. Brain and K.A. Walters

Abstracts of presentations at the Seventh International Perspectives in Percutaneous Penetration Conference held in La Grande Motte, April 2000

Acknowledgements

The organisers are grateful to all of the organisations who have supported the Seventh Perspectives in Percutaneous Penetration Conference.

3M Medica GmbH
An-eX
Avon
Bristol Myers Squibb
L'Oreal
OSI Pharmaceuticals
Uniqema
Unilever
Zeneca Central Toxicology Laboratory

IN VITRO SKIN DELIVERY OF 5-FLUOROURACIL FROM ULTRADEFORMABLE VESICLES **58**
GMM El Maghraby, AC Williams and BW Barry

PENETRATION ENHANCERS IN LIPOSOMES FOR SKIN DELIVERY OF OESTRADIOL: CAN THEY REPLACE SURFACTANTS? **59**
GMM El Maghraby, AC Williams and BW Barry

A COMPARATIVE STUDY OF TRANSDERMAL PERMEATION FROM 5-AMINOLEVULINIC ACID FORMULATIONS **60**
L Patel, AC Wiliams and BW Barry

COMBINED EFFECT OF OLEIC ACID AND PROPYLENE GLYCOL ON THE PERCUTANEOUS ABSORPTION OF TENOXICAM **61**
E Larrucea, A Arellano, S Santoyo and P Ygartua

IN VITRO PERCUTANEOUS ABSORPTION AND SKIN RETENTION OF PIROXICAM: EFFECT OF SKIN PRE-TREATMENT WITH LAURIC AND OLEIC ACIDS **62**
S Santoyo and P Ygartua

INFLUENCE OF PIROXICAM-HYDROXYPROPYL-β-CYCLODEXTRIN (PX-HP-β-CYD) COMPLEXES ON PERCUTANEOUS PENETRATION OF PIROXICAM **63**
A Doliwa, S Santoyo and P Ygartua

RETARDATION EFFECTS OF β-CYCLODEXTRIN AND POLYETHYLENE GLYCOLS ON PERCUTANEOUS ABSORPTION OF NICOTINE **64**
HR Moghimi and A Shakerinejad

THE INFLUENCE OF PROGESTOGENS ON THE ABSORPTION OF OESTRADIOL ACROSS MEMBRANES: AN EMPIRICAL ASSESSMENT **65**
M Saunders, WJ Pugh and J Hadgraft

POTENTIAL OF HALOPERIDOL PRODRUGS FOR TRANSDERMAL DELIVERY **66**
A Morris, KR Brain and CM Heard

DESIGN OF BIFUNCTIONAL MOIETIES WITH IMPROVED SKIN DELIVERY POTENTIAL COMBINING DEXAMETHASONE AND NSAIDS **67**
MH Nazemi, KR Brain and CM Heard

THE EFFECTS OF CYCLOPENTADECANONE, PENTADECALACTONE AND SEPA® ON PORCINE SKIN PERMEATION **68**
E Gauthier, S Krauser and C Samour

A NOVEL METHOD TO STUDY DRUG PERMEATION THROUGH THE HUMAN NAIL PLATE **69**
E Gauthier, S Krauser, C Samour and KA Walters

PHYSICO-CHEMICAL PARAMETERS AND SKIN PERMEATION ENHANCING EFFECT OF ω-AMINO ACID DERIVATIVES **70**
A Hrabálek, P Dolezal and K Palát

DIFFERENTIAL SCANNING CALORIMETRIC STUDY OF RAT STRATUM CORNEUM SHEETS PRETREATED WITH TRETINOIN **71**
A Ramírez Diéguez, MJ Fresno Contreras, MM Jiménez Soriano and E Sellés Flores

PERMEATION OF ETHYL ETHER OF DIETHYLENE GLYCOL THROUGH PALATAL MUCOSA **72**
V Koprda, G Touraille, L Bohacik, Z Kassai and F Falson-Rieg

RECONSTITUTION OF LIPOSOMES INSIDE THE INTERCELLULAR LIPID DOMAIN OF THE STRATUM CORNEUM BY DILUTION OF PHOSPHOLIPID/OCTYL GLUCOSIDE MICELLAR SOLUTIONS **73**
O López, M Cócera, P Walther, E Wehrli, L Coderch, JL Parra and A de la Maza

EFFECT OF WOOL LIPID LIPOSOMES ON SKIN BARRIER FUNCTION **74**
M de Pera, L Coderch, J Fonollosa, A de la Maza and JL Parra

IN VITRO INVESTIGATION OF PENETRATION INTO AND PERMEATION THROUGH HUMAN SKIN OF HEPARIN FROM AQUEOUS AND LIPOSOMAL FORMULATIONS **75**
G Betz, R Imboden and G Imanidis

IN VITRO SKIN PERMEATION STUDY OF TRETINOIN FROM STRATUM CORNEUM LIPID LIPOSOMES **76**
MM Jiménez Soriano, MJ Fresno Contreras and A Ramírez Diéguez

RECONSTITUTION OF LIPOSOMES INSIDE THE INTERCELLULAR LIPID DOMAIN OF THE STRATUM CORNEUM BY DILUTION OF PHOSPHOLIPID/OCTYL GLUCOSIDE MICELLAR SOLUTIONS

O López[1], M Cócera[1], P Walther[2], E Wehrli[2], L Coderch[1], JL Parra[1] and A de la Maza[1]
[1]Dpto. de Tensloactivos, I.I.Q.A.B., C.S.I.C., C/ Jordi Girona 18-26, 08034 Barcelona, Spain and [2]ETH-Zentrum, Lab. for Electron Microscopy, Universitiitsstrasse 16, CH-8092, Zürich, Switzerland

The use of liposomes in dermatology has become progressively popular in recent decades. However, a controversy exists on the mechanisms of the interaction of these vesicles with the skin. Thus, there is not consensus that lipid vesicles dermally applied really penetrate into skin and on the possible mechanisms of such penetration.

In previous works we have studied the effect of liposomes[1] and different solubilizing agents[2] on the stratum corneum tissue (SC), as well as the ability of SC lipids to form liposomes[3]. Taking into account that the structure of liposomes is similar to the layered structure of the SC intercellular lipids, it is reasonable to consider the possibility of application of liposomes as protective agents of this tissue. As a consequence, in this work we sought to evaluate the protective effect caused by PC liposomes on the SC structure against the action of the non-ionic surfactant octyl glucoside (OG). In addition, the ability of these vesicles to be reconstituted inside the SC tissue was examined. These studies were carried out in vitro using the technique of high-resolution low-temperature scanning electron microscopy.

Treatment of the SC tissue with OG led to breakage of the corneocyte envelopes and to the difusion of the protein material from the corneocyte into the intercellular domain. The interaction of this protein material with the intercellular lipids caused a certain alteration in the lipid structure that resulted in the formation of "rough structures"[4]. Previous incubation of SC with PC liposomes drastically reduced these alterations, this protection being associated with incorporation of about 10% of PC to the SC. In addition, in these samples, the presence of intact PC vesicles in the intercellular lipid domain was detected after OG treatment and subsequent washing. This, and the fact that the OG/PC molar ratio incorporated in the SC corresponded to that at which the micelle-lamella transition occurred on dilution demonstrated the ability of PC liposomes to be reconstituted inside the SC. Hence, the presence of PC vesicles inside the SC could be explained by the incorporation of an OG/PC micellar system and the subsequent reconstitution in PC vesicles by continuous dilution during washing.

1. L Coderch et al (1999), The effect of liposomes on skin barrier structure, ***Skin Pharmacol.Appl. Skin Physiol.,*** 12, 235
2. O López et al (1997), Study of the composition and structure of pig stratum corneum based on the action of different solubilizing agents, ***Colloids Surfaces***, 415, 123-124
3. O López et al (1996), Formation and characterization of liposomes from lipid/proteic material extracted from pig stratum corneum, ***J.Am.Oil Chem.Soc.,*** 73, 443
4. O.López et al (1998), Stratum corneum structural modifications by effect of different solubilizing agents: a study based on high-resolution low-temperature scanning electron microscopy. Perspectives in Percutaneous Penetration, Vol 6a, (Eds. KR Brain, VJ James & KA Walters), STS publishing, Cardiff, p111

INDEX

2

2-butoxyethanol....118, 119
2-ethoxyethanol....117
2-ethylhexyl-4-methoxycinnamate....130
2-ethylhexylsalicylate....130

3

3,3',4,4'-tetrachlorobiphenyl....114, 115

5

5-ALA....60
5-aminolevulinic acid....60
5-fluorouracil....58

a

Aiache....33
Aillaud....31, 32, 142
Alewaeters....108
Allen....4
Allender....47, 113, 139
allergy....147
alpha hydroxy acid....10, 87, 96
Ananth....9
André....109
angiotensin ii....106
Anissimov....19
antibacterial....7
anti-flash creams....124
anti-inflammatory....7
antimicrobial....83, 102
Appa....96
applied toxicology....13
Arellano....61
Arock....34
ascorbic acid....42
Asgedom....139
Ashley....123, 124
aspirin....67
ATR....44
atracurium....34
ATR-FTIR....45
avobenzone....131

b

Baldo....136
Barel....108, 130
Barkovic....96
barrier....49
barrier cream....121
barrier function....53
barrier integrity....50
Barry....55, 58, 59, 60, 111
Bayer....53
Beck....27
Becket....107
Belfihadj....102
Benech-Kieffer....54, 136
Benfeldt....30
Benford....29
Benson....26, 91
benzoic acid....54
benzophenone-3....129
beta-hydroxy acids....10
betamethasone 17-valerate....134
betamethasone dipropionate....25, 94, 95, 100
Betz....75
Beyssac....33
Bica....23, 24, 25
Bieli....128, 130
Biesse....31, 32
bioavailability....54, 136, 137
bioequivalence....134
biomodule....87
biophysical analysis....123
biophysical characteristics....143
bisphenol a....116
Blankschtein....40
Boeninger....29
Bohacik....72
Boix....92
Bonnabry....33
Bonner....111
Bonnet....51
Boogaard....29
Bouwstra....16, 35, 38, 80
Brain..15, 22, 27, 47, 57, 66, 67, 83, 111, 113, 139
Brondeau....29
Brusq....143
Bucks....6
Bunge....20, 21
bupivacaine....85, 88
butoxyethanol....118
butyl salicylate....138
butylmethoxydibenzoylmethane....26

c

caffeine....31, 54, 141
calcium....35
Calpena....84, 85, 86
Cameron....48
Campos....23, 25
Cantor....12
Carli....89
carvone....81
Casiraghi....45
Cedeño Arias....93
ceramides....35, 36, 52
Chang....9
Chatelain....128, 131
Chaulet....103
CHG....103
Chilcott....49, 112, 121, 122, 123, 124
chiral....55
chlorhexidine....102, 103
cholesterol....35, 122
cholesterol sulfate....35
Chromameter....108, 134, 137
ciclosporin a....90
Cilurzo....45
cineole....60
Clarys....108, 130
clobetasol propionate....133, 134
clotrimazol....44
clotrimazole....91
cobra....33
Coceani....89
Cócera....73
Coderch....73, 74
COLIPA....11, 28
comparative in vitro dermal absorption study....120
confocal....8, 9
confocal laser microscope....41
Corish....29
corneocytes....47

corticosteroid....134
Cottin....54, 136
Courtellemont....31, 32
Cross....19, 26, 147
Cullander....41
cultures....53
current density....111
cyanophenol....20
cyclodextrin....63, 64
cyclopentadecanone....68
cypermethrin....127
cytochrome p_{450}....115

d

Daddona....5
databases....15
Davey....91
Davis....4
de Graaff....80
de Heer....29
de la Maza....73, 74
de Paepe....146
de Pera....74
de Richter....51
DEET....125, 128
Degobert....102, 103
Delgado-Charro....109
delivery....58, 59
Demaret....86
dermal exposure variables....114
dermatitis....101, 146
Dettmar....83
dexamethasone....67
diastereoisomers....56
Diaz....103
diclofenac....51, 67, 84
diethylamine salicylate....138
diethyltoluamide....125
digital image analysis....134
disposition....114
dithranol....44
Djordjevic....98
dodine....126
Dolezal....70
Doliwa....63
Domenech....85, 92
Dong....40
Doucet....53
doxepin....92
doxycycline....139
DPPG....103
Dreher....54
Dressler....14, 27, 46
Drexler....29, 119
drug-in-adhesive....12
DSC....36, 65, 71
Dubbelaar....16, 35
Dunn....7
Dunne....124
Dürsch....119
Dusseau....102

e

ECETOC....28
ECVAM....28
efficacy....128, 137
el Maghraby....58, 59
electrodes....110
electroosmotic flow....107
Emanuel....49
EMLA....86
enhanced....91
enhancer....57, 59, 60, 81, 97
enhancing....70
epi-dihydrojasmonate....57
episkin....54
erythromycin....104
Escribano....84, 85, 86
Esdaile....11, 126, 127
ester hydrolysis....66
estradiol....31, 68
ethoxyethanol....117
ethyloleate....81
E-trans[tm]....5
eusolex....31
eutectic....86
ex vivo....34

f

Fabre....51
Falson....102, 103, 109
Falson-Rieg....72
Farinha....23, 24, 25
Fartasch....119
Fasano....50, 120
fatty acids....122
FDA guidance....25, 134
FDA guideline....24
fentanyl....144
Fernanda Bahia....99, 100
Fernandez....129
Ferreira....99
finite dose....19
Finkey....96
Finnin....17
fluocinonide....134, 135
fluorescence....8, 9, 39
fluorescent probes....38
follicular pathway....1
Fonollosa....74
Förster....36
Fouchard....53
free fatty acids....35
Freris....17
Fresno Contreras....71, 76, 77, 78, 79
Frokjaer....81
FTIR....44, 65
FTIR-ATR....44
function....49

g

Gabard....128, 130, 131
Garcia....53, 92
Gauthier....68, 69
Gelis....82
Ghidoni....52
Giese....39
glucose....112
Goddard....83
Göen....29, 119
González....9, 84
Gooris....16, 35
Grabarz....27
Grams....38
Grassi....89
Green....27
Griffin....29
Groth....95, 138

h

Hachem....146
Hadgraft....65
Hagemeister....133

Hahn.....10
Haigh.....33, 134, 135
hair dye.....14, 27
hair follicle.....3
hair regrowth.....97
hairless rat.....30
haloperidol.....66
handwash.....83
Hanh.....44
Hayden.....26
Heard.....22, 47, 57, 66, 67, 83, 113, 139
Henrichs.....9
heparin.....75
hexyl nicotinate.....108
hibitane.....103
histamine.....10, 34, 110
Hoeck.....81
homosalate.....131
Honeywell-Nguyen.....80
Hotchkiss.....29, 121
Howes.....27, 118
Hrabálek.....70
human.....28
Humbert.....34, 42
Hung.....105, 106
Hutton.....122
hyaluronic acid.....71, 76, 77, 78
hydration.....45, 53, 111
hydrocortisone.....99

i

ibuprofen.....31, 32, 67
ICP-AES.....132
Imanidis.....75, 135, 137
Imboden.....75
in vitro14, 19, 23, 24, 25, 28, 31, 32, 33, 46, 50, 62, 75, 76, 77, 78, 79, 92, 94
in vivo.....30, 95
inflammation.....10
infrared imaging microspectroscopy.....43
infrared spectroscopy.....44
insect repellent.....125, 128
interlaboratory.....14, 27, 31
interspecies.....27
in-use conditions.....83
in-vivo imaging.....8
iontophoresis.....106, 108, 109, 110, 111
iontophoretic device.....105
irritation.....13
Ischenko.....101

j

Jahn.....90
Janssens.....146
jasmonates.....57
Jenner.....121, 122, 123, 124
Jett.....4
Jiménez Soriano.....71, 76, 77, 78, 79
Jørgensen.....94
Jornet.....85
Junginger.....38, 80

k

Kantelip.....34, 42
Kassai.....72
Kasting.....3
Kaushik.....4
Kaye.....4
Keister.....144
keratinocytes.....52
Kesel.....87
ketoconazole.....24, 44, 99
ketoprofen.....67, 68
Kezic.....29
Kietzmann.....116
Kitchiner.....27, 46
Kitson.....2
Klede.....119
Kokkat.....145
Koprda.....72
Kotlyar.....101
Krause.....88
Krauser.....68, 69
Kreilgaard.....81
Kusum Devi.....145

l

Lademann.....1, 133
Lagarde.....104
Lambrecht.....130
Langer.....40
Lapillonne.....142
Larese.....29
Larrucea.....61
Laugier.....32
lauric.....62, 81
Lauroba.....84
laurocapram.....70
Lboutounne.....102
Leclaire.....54, 136
Leclerc.....136
Leveque.....42
l-glutamic acid.....111
lidocaine.....88, 105, 110
lidocaine-prilocaine.....86
Liebschutz.....142
lignocaine.....85
Limasset.....29
limonene.....59
Lin.....106
lindane.....127
Linscheid.....133
Liorzou.....143
lipid profile.....37
liposomal.....97
liposomes.....58, 59, 73, 74, 75, 76, 79, 96
local anaesthetics.....85, 88
Lockley.....118
Long.....141
López.....73
Lüdi.....128, 130, 131
Lui.....105, 106
Luzy.....126, 127

m

Mackay.....55
Macroflux[tm].....5
Madden.....48
Madsen.....81
Maibach.....13
Maillols.....129
Makki.....42
malathion.....127
Manning.....50
mannitol.....106
Marques.....136
Marti-Mestres.....129
Martin.....86
Marty.....31, 32, 53, 127, 143
Mary.....34, 42
mass spectrometry.....42
Maurer.....41
Mavon.....82, 104, 132

Mayoral....84
McAllister....4
McCafferty....110, 141
McCarley....20
MCHM 20-001....69
McNaughton....47
Mehta....107
Melchior....94
melting point....55
Mendelsohn....43
Menné....30
menthol....55
Messager....54
metformin....109
methoxsalen....44
methyl dihydrojasmonate....57
methyl nicotinate....108
methyllaurate....81
microautoradiography....46
microdialysis....30, 34, 42, 119
microemulsions....89
microneedles....4
Mikler....31, 32, 142
Milic....125
Miljkovic....125
Milutinovic....125
Minghetti....45
minoxidil....97
Minter....46
MIP....113
Miquel....104, 132
Mitragotri....40
model....3, 17, 19, 21, 31, 32, 51, 54
Möder....116
modulation....103, 122
Moghimi....64
moisturising....98, 146
molecularly imprinted polymers....113
Molzahn....133
monoclonal antibody....93
Montanari....45
Montenegro....93
Monti....52
Moore....43
Morales....93
Morgan....17
Morris....66
mosaic model....16
Moss....110
Motta....52
Mouysset....132
MTT....82
Mueller....137
multiple emulsion systems....100
Muñoz....93
Muret....34, 42

n

n,n-diethyl-m-toluamide....128
n,n-dimethyldodecylamine....47
nail plate....69
nanocapsules....102, 136
nanoparticles....1
Nazemi....67
NDELA....15
NDOMA....15
neomenthol....55
Neubert....36, 44, 88, 90
Neudecker....39
new-born-rat model....1
nicotinate....137
nicotine....64, 145
Niemiec....96, 97
nitroglycerin....23
Nitsche....3
n-methyl-pyrrolidone....81
non invasive....39, 44
non-invasive monitoring....113
nonylphenol....116
NORAN....41
NSAIDs....67

o

Obach....85, 86
Oberthür....87
occlusion....147
Ocheltree....144
octocrylene....26
octyl glucoside....73
octyl methoxycinnamate....128, 131
octyl salicylate....15, 26, 128, 131
octyldimethyl-paba....26
octylmethoxycinnamate....26
OECD....28
OECD guidelines....11
oestradiol....59, 65
oleic....62
oleic acid....36, 40, 59, 61, 81
Oliveira....99
Ouvrard-Baraton....31, 32
oxybenzone....26, 128, 129, 130, 131

p

PAH....17
Paillous....82
Palát....70
palatal mucosa....72
paraben....19
parabens....147
Parra....73, 74
PAS....44
patch adhesion....143
Patel....60
Patouillet....54
Payan....29
Payne....18
Payonk....96
PCBs....114
PCMX....83
PDMS....65
peel patch tester....143
Peiffer....51
Pena Ferreira....100
Pendlington....27, 46
penetration....2
pentachlorophenol....115
pentadecalactone....68
percolation....2
perfused bovine udder....116
Perkins....29
pesticides....127
Petersen....138
phenylbenzimidazole suffonic acid....31
phenylenediamine....27, 46
physicochemical properties....142
pig ear....31, 32
pig skin....68
pilocarpine....110
Pirot....102, 103, 109
piroxicam....62, 63
polycyclic aromatic hydrocarbons....17
polyethylene glycols....64
poly-ε-caprolacton....102

Ponec....16, 35, 37
powdered solids....20
PPD....27, 46
Prausnitz....4
predict....22
prediction....15, 118, 144
preterm infants....141
prilocaine....85, 88
progesterone....33
progestogens....65
propylene glycol....61, 81
PSPDC....105, 106
Pugh....65
pulsed short-circuit pulsed direct current system105
Python....27, 33

q

Qiao....114, 115
quality control....25
quinidine....56
quinine....56
Qvist....81

r

rabbit skin....101
Rajadhyaksha....9
raman....36
Ramírez Diéguez....71, 76, 77, 78, 79
Rashid....111
rat....28
Raudenkolb....36
Raufast....132
reconstructed epidermis....51
Reddy....21
Reed....17
reference compounds....28
Reig....92
rejection criteria....48
release....26
Rengifo....93
repaired-rat-skin model....1
reproducibility....14
reservoir....104
retardation....64
retinoid....6
retinol....10, 96, 136
Rettig....36
reverse electroporation....112
Rice....121
risk assessment....29, 117
Riviere....114, 115
Roberts....19, 26, 147
Rodríguez....93
Rogiers....146
Roper....48, 120
Roseeuw....146
Roux....104
Rovira....85, 86
Rowland....112
Royer....34

s

Sala....52
salicylamide....138
salicylic acid....138
Samour....68, 69
sandwich....16
Santos....99
Santoyo....61, 62, 63
SAR....17
Sartorelli....29
saturation....135
Saunders....65
Sauvaire....51
Schaefer....1, 133
Schmelz....119
Schröder....27
Schüle....39
Schwarb....135
selective entrapment....113
self-organizing system....2
Sellés Flores....71
Semino....126
semi-solids....24, 25
sensory irritation....10
SEPA....68
Serup....30
Shah....133
Shakerinejad....64
Sharma....27, 46
Sialve....104
silicone rubber....20
Simonnet....136
Simonsen....138
Simpson....48
skin barrier....8, 74
skin penetration....1
Smith....134, 135
Smyth....107
snake skin....33
So....40
sodium salicylate....105
solar irradiation....82
Soler....92
solubility....12
solubility parameter....22, 66
Sournac....143
spatial distribution....21
standardization....29
Steiling....27
stereoselectivity....56
Sterry....133
Stevenson....29
Stoddart....22
stripping....136
strontium....10
Stubbs....123
Subramanyam....9
substance p....34
sucrose....107
Sugimoto....27
sulphur mustard....121, 122, 123
sun protection factor....131
sunscreen....31, 128, 129, 132
supac-ss....24, 25
supersaturated....135
Surber....134, 135, 137
surfactants....59
swine model....114

t

tape stripping....30, 37, 49, 128, 131, 132, 133
TCB....114
temperature....130
tenoxicam....61
terpenes....55
testosterone....28, 68
TEWL....31, 45, 49, 53, 75, 108, 123
Theaux....41
theophylline....113
Therkildsen....94
Thouret....82
TiO_2....1, 132

titanium dioxide.....132
TMF12.....7
Toledo-Crow.....8
topical delivery.....3
Torres.....93
Toscano.....23, 24, 25
Touraille.....72
transcutaneous.....119
transcutol.....72, 86
transdermal.....141, 142, 145
transdermal drug delivery.....4, 5, 26
transdermal drug delivery system.....12, 23
transfersomes.....58, 59
transkarbams.....70
tretinoin.....6, 71, 76, 77, 78, 79
triclosan.....83
trifluorosal.....7
tritiated water.....50
tritiated water permeability.....48
two-photon.....40

u

ultradeformable vesicles.....58, 59
US-EPA.....28
uv filters.....130, 131, 132

v

v Pelchrzim.....133
van de Sandt.....28, 29
van Hemmen.....29
Vanpee.....146
vasoconstriction.....134
vesicles.....80
Viayna.....84
Vicendo.....82
Vijaya.....145
Vincent.....31, 32
vitamin e acetate.....98
vitamins.....87
volpo.....18
Vuleta.....98, 125

w

Wahren.....116
Walters.....15, 27, 69
Walther.....73
Wang.....96, 97
Warnholtz.....26
Wartewig.....36, 44
Watkinson.....27, 111
Weerheim.....37
Wehrli.....73
Weigmann.....1, 133
Weiner.....97
Wick.....12
Wiliams.....60
Wilkins.....57
Wilkinson.....117
Williams.....29, 55, 58, 59, 117, 118, 124
Winckle.....47, 83
Wohlrab.....88, 90
Woolfson.....110

x

x-ray.....36

y

Ygartua.....61, 62, 63
Yu.....40

z

Zanini.....54
Zastrow.....53
Zhang.....105, 106
Zhao.....105
zinc oxide.....101

–

ω-amino acid.....70

RECONSTITUTION OF LIPOSOMES INSIDE THE INTERCELLULAR LIPID DOMAIN OF THE STRATUM CORNEUM BY DILUTION OF PHOSPHOLIPID/OCTYL GLUCOSIDE MICELLAR SOLUTIONS

O. López[1], M. Cócera[1], P. Walther[2], E. Wehrli[2], L. Coderch[1], J.L.Parra[1], A. de la Maza[1], [1]I.I.Q.A.B.-C.S.I.C. Barcelona, Spain, [2]ETH-Zentrum, Zürich, Switzerland

INTRODUCTION

A number of studies have been devoted about the mechanisms of the interaction of liposomes with skin. In this sense, various authors recently have claimed the advantages of topical application of phospholipid liposomes [1,2], however, there is not consensus in that lipid vesicles dermally applied really penetrate into the skin and which would be the cause of such possible penetration. Thus, whereas some authors claimed that liposomes are not able to penetrate deeper than SC [3,4], other reported various effects of these vesicles on the epidermis [5,6].

OBJETIVE: Use of high-resolution low-temperature scanning electron microscopy technique (HRLTSEM) to evaluate the ability of PC liposomes to protect pig SC against the action of the surfactant octyl glucoside and to be reconstituted inside of this tissue.

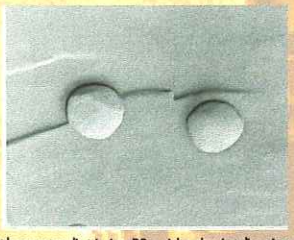
METHODS

PC liposomes :Preparation following a method previously reported [7].
SC native: Separation and isolation based on a method previously described [10,11].
SC treated with OG: Treatment of SC native with 20 mM OG by sonication at 25ºC for 15 min and incubation at the same temperature for 18 h in N_2 atmosphere [12].
SC incubated with liposomes: Incubation with PC liposomes 15 mM at 25ºC for 18 h, separation from the liposome solution, treatment with 20 mM OG and washing with buffer during 30 min.

TECHNIQUES

Thin layer chromatography flame ionization detection (TLC-FID) was used to analyze the amounts of PC and OG incorporated in the SC after incubation with liposomes or surfactant treatment respectively [11].
Dynamic light-scattering (DLS) technique was used to determined the hydrodynamic diameter (HD) of PC liposomes and OG/PC mixed micelles [8].
Freeze-fracture electron microscopy (FFEM) study of PC liposomes and reconstituted vesicles by dilution was done according to the procedure described by Egelhaaf et al. [13]. The replicas were examined in a Philips EM 301 electron microscopy at 80 kv.
High-Resolution Low-Temperature Scanning Electron Microscopy (HRLTSEM) Cylindrical pieces of each sample were fixed with 2% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer, cryoprotected with 30% (v/v) glycerol and mounted on a 3 mm aluminium holder. Thereafter, the SC samples were frozen by plunging into liquid propane and fractured in a Balzers BAF 300 freeze-etching device (Bal-Tec., Liechtenstein) at 10^{-7} mbar and a temperature of -110ºC. After etching for 2 minutes, the fracture plane was coated with 2 nm Pt/C and 5-7 nm C. Afterwards, the cold samples were immediately cryo-transferred on a Gatan cryo-holder into a Hitachi S-900, in lens field emission scanning electron microscope equipped with a highly sensitive annular YAG-detector for back scattered electrons [9]. Specimens were investigated at -110ºC. The beam current was $1-3x10^{-11}$ A as measured with a Faraday cage. The primary accelerating voltage was 10 kV. Images were obtained with the back scattered electron signal and recorded digitally with a Gatan Digiscan 688 connected to an Apple Quadra 950.

RESULTS AND DISCUSSION



FFEM micrograph corresponding to two PC vesicles showing diameters of about 180 nm, in agreement wit the DLS results

NATIVE STRATUM CORNEUM

This picture depicts corneocytes (black arrow) characterized by the particular pattern of keratine filling these structures and by the absence of cell organelles. In the intercellular space the fracture plane goes along the lamellae of the multilayered lipid organization resulting on the very smooth and relatively flat surface of the SC lipids (black arrowheads). Occasionally the bilayers were fractured straightacross, resulting in sharp edges (white arrows) indicating the multilamellar structure of the intercellular lipids.

STRATUM CORNEUM TREATED WITH OG

The corneocytes showed a more irregular shape than that observed in the native tissue (Fig 2). This perturbation was attributed to a partial breaking of the corneocyte envelope (white open arrow) that induced a release of the protein material into the intercellular lipid region. The presence of flat surfaces corresponding to the fracture along lipid lamellae (black arrowhead) and sharp steps corresponding to the fractures across the lipid lamellae (white arrow) indicates that the lamellar structure remained preserved after the OG treatment, despite the strong effect caused by OG on the protein structure. The main alteration of this lipid region was the formation of a wide area of new rough structure (white arrowhead). This structure, (not observed in the native SC, Fig 2), is very similar to those reported by Hofland et al, and Van' Hal et al. [14,15], who associated their formation to the disruption of the lipid lamellar structure. We attribute the formation of this "rough structure" to the interaction of the protein material liberated from the corneocytes (when the corneocyte envelope was damaged) and the lipids of the intercellular spaces, with the subsequent disorder in their lamellae structure.

SC INCUBATED WITH LIPOSOMES AND TREATED WITH OG

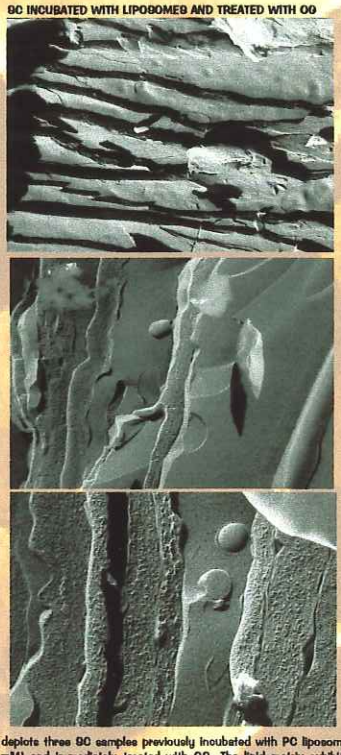
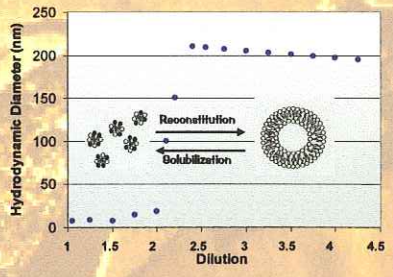


Figure 4 depicts three SC samples previously incubated with PC liposomes (PC conc 15 mM) and immediately treated with OG. The lipid matrix exhibited the structure of multiple layers (white arrows), indicating the preservation of these structures as occurred in the SC treated with OG without previous incubation (Fig 3). However, the rough structures observed in the SC treated with OG were not observed in any micrograph of this figure. Hence, the incubation with PC liposomes protected the SC against OG, being this protection associated to the incorporation of about 10% of PC into the SC. The most striking result was the observation in the intercellular lipid spaces of vesicular structures (curved arrow) of about 180 nm. (micrographs B and C). Given that these structures were not observed neither in the native SC (Fig. 2) or in the tissue treated with OG without liposome incubation (Fig 3), and taking into account their similarity in size and topology with PC vesicles (Fig 1), we could associate these structures to PC vesicles. However, bearing in mind the size of PC vesicles (180 nm) and the interlamellar distance of the ordered lipids (about 5-6 nm) the direct penetration of these vesicles into the SC appears to be difficult.

In order to shed light on the mechanism of incorporation of PC vesicles inside the intercellular region of SC we determined the amounts of PC and OG incorporated into the SC during the two consecutive treatments using the TLC/FID technique. The total weight of PC and OG incorporated in 15 mg of tissue were 2.04 and 6.84 μmoles respectively, being the corresponding OG/PC molar ratio (Re) of 3.35. This molar ratio corresponded to a system mainly formed by OG/PC mixed micelles [16].
In a previous work Ollivon et al. [17] reported the formation of liposomes by continuous dilution of OG/PC mixed micellar solutions. We therefore hypothesize that the presence of PC liposomes inside the SC was due to the reconstitution of these vesicles in intercellular lipid spaces by dilution of the OG/PC micellar aggregates incorporated into the SC. In order to demonstrate this hypothesis a series of DLS experiments we performed to know the variation of the mean hydrodynamic diameter (HD) of the micellar solution of OG/PC (Re 3.35) during its continuous dilution with buffer. The curve obtained is plotted in Figure 5.



It can be seen that the HD of particles increased up to a maximum was achieved (from 9 to 190 nm), indicating the reconstitution of the vesicles by dilution of the mixed micelles. From these findings we assume that the presence of PC vesicles in the intercellular lipid region of the SC observed by HRLTSEM (Fig 4, micrographs B and C) is the result of the reconstitution of these structures inside the SC by means of a three steps process: First, formation in the SC surface of a OG/PC micellar system between the PC adsorbed during the incubation with liposomes and the OG added in the subsequent treatment. Second, incorporation of this micellar system into the intercellular lipid region during the surfactant treatment (incubation of 18 h after sonication). The fact that no PC was found in the treatment bath corroborates this process of incorporation. Third, reconstitution of PC vesicles inside the lipid spaces by dilution of this micellar system during the final washing of the SC. After reconstitution, the big size of the vesicles formed hinder their migration outside the tissue though the intercellular lipid spaces (distance of the ordered lipids of about 5-6 nm) by simple washing. This new strategy that favours the incorporation of PC vesicles inside the SC gives light on the controversy existing on the ability of PC liposomes to penetrate into this tissue and open up new avenues in the therapeutical applications of liposomes in skin.

References

1.Van den Bergh BAI, Salomons-de Vries I and Bouwstra JA, Int J Pharm, 167, 57-67 (1997).
2.Van Kuijk-Meuwissen EMJ, Junginger HE, and Bouwstra JA,. Biochim Biophys Acta, 137, 31-39 (1998).
3.Lasch J, Laub R and Wohlrab W, J Controll Release, 18, 55-58 (1991).
4.Korting HC, Stolz W, Schmid MH and Maierhofer G, Br J Dermatol, 132, 571-579 (1995)
5.Hofland HEJ, Bouwstra JA, Boddé HE and Spies F, Junginger HE Br J Dermatol, 132, 853-856 (1995).
6.Kirjavainen M, Urtti A, Jääskeläinen I, Marjukka Suhonen T, Paronen P, Valjakka-Koskela R, Kiesvaara J and Mönkkönen J, Biochim Biophys Acta, 1304, 179-189 (1996)
7.López O, Cócera M, Pons R, Azemar N, López-Iglesias C, Wehrli E, Parra JL and de la Maza A, Langmuir, 15, 4678-4681 (1999).
8.López O, de la Maza A, Coderch L, López-Iglesias C, Wehrli E and Parra JL, FEBS lett. 426, 314-318 (1998).
9.Walther P, Wehrli E, Hermann R and Müller M, J Microsc, 179, 229-237 (1995).
10.Wertz PW and Downing DT, J Lipid Res, 24, 753-758 (1983).
11.López O, De la Maza A, Coderch L and Parra JL, J Am Oil Chem Soc, 73, 443-448 (1996).
12.López O, De la Maza A, Coderch L and Parra JL Colloids Surfaces A, 123-124, 415-424 (1997).
13.Egelhaaf SU, Wehrli E, Müller M, Adrian M and Schurtenberger P, J Microsc 184, 214-228 (1996)
14.Hofland HEJ, Bouwstra JA, Boddé HE, Spies F, Nagelkerke JF, Cullander C and Junginger HE, J Liposome Res 5, 241-263 (1995).
15.Van' Hal DA, Jeremiasse E, Junginger HE, Spies F and Bouwstra JA, J Invest Dermatol, 106, 89-95 (1996).
16.De la Maza A and Parra JL, Eur J Biochem 226, 1029-1038 (1994).