

INTERNATIONAL CONFERENCE ON ENGINEERING

ICE UBI2011

28-30 Nov 2011

University of Beira Interior

Covilhã Portugal

ICEUBI2011

International Conference on Engineering UBI2011





Skin Delivery of Gallic Acid from Biofunctional Cotton Fabric.

Vanessa Martínez, Cristina Alonso, Alfons de la Maza, José L. Parra, Meritxell Martí and Luisa Coderch

Advanced Chemical Institute of Catalonia, (IQAC-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

Contact e-mail: meritxell.marti@iqac.csic.es

Scientific Area- CT24

Abstract

In these recent years, many studies about biofunctional textiles have been published. These textiles have incorporated different types of cosmetic or pharmaceutical active compounds. These compounds are able to move to the skin of the person who wears the biofunctional garment and they can act as a conventional cosmetic or pharmaceutical product.

The absorption and desorption of the antioxidant Gallic acid vehiculised in liposomes and mixed micelles applied onto a cotton fabric has been studied. A much greater absorption was found for the mixed micelles treatment related to liposomes. However, also a much higher desorption for the mixed micelles treated fabric could indicate a superficial absorption of mixed micelles easily lost with the aqueous washings.

The active compound penetration was followed by a specific *in vitro* percutaneous absorption methodology. Penetration of acid Gallic formulated as liposomes is much higher than when formulated as a mixed micelles. When Gallic acid is embedded into the biofunctional textile, it always promoted a reservoir effect; similar penetration was obtained for the textiles treated with Gallic acid in mixed micelles or liposomes in the skin compartments such as stratum corneum, epidermis and even the dermis. This methodology could serve to verify penetration in human skin of encapsulated substances which can exert a marked influence on specific doses of active agents to be released to the skin.

Key Words: biofunctional textile, antioxidant, Gallic acid, liposomes, mixed micelles, percutaneous absorption.

Introduction

Biofunctional textiles are the textiles with smart and new properties and added value especially related to comfort. Such textiles constitute the basis for the delivery system of cosmetic or pharmaceutical substances when the textile comes into contact with the skin. As most of the human body is covered with some sort of textile, the potential of biofunctional textiles is considerable. Textiles that have functional properties for the skin have been studied and patented in recent years (1, 2).

Encapsulation is one of the techniques used to apply substances onto textiles (3). Vesicles, in particular lipid vesicles (liposomes), have an impact on a variety of areas, which range from fundamental science to biotechnology. Vesicles serve as models for cell membranes and allow the study of the basic mechanisms of membrane function, such as fusion (4, 5). Furthermore, liposomes of controllable size are used as biocompatible and protective structures to encapsulate a wide spectrum of active principles that include cosmetics (6-10), compounds for gene therapy (11), for the treatment of allergies (12), as carriers of enzymes (13) and antibiotics (14), they are also used in textile industry (15-17).

There are different methodologies to prepare lipid vesicles; one of them is using detergent removal techniques: dilution, dialysis, gel exclusion chromatography, adsorption onto polymeric materials, temperature changes, or biochemical reactions (18). All these methods rely on the very high solubility of detergents compared to lipids. The equilibrium and transient structures encountered in lipid-surfactant mixtures and the kinetics of the dissolution and of the closure to vesicles are important issues, and have attracted considerable interest (19, 20).

In this research due to the mixed micelles constituted by a lipid and a surfactant agent are capable of transforming in liposomes when the surfactant is eliminated by simple dilution with water, a new application will be proved with the aim to study their application onto

textile fabric and later by dilution (water washing treatment) its potential ability to be structured as liposomes into the textile fabric.

Antioxidants are substances used as natural resources to regulate the processes related to the external aggressions, preventing the oxidative stress. One of the organism defence systems is the generation of endogenous antioxidants, but the organism also incorporates exogenous antioxidants with the diet. On the other hand, the skin is a zone increasingly used as topical route of application of compounds. There has been demonstrated that these exogenous antioxidants can diminished the effects of the free radicals with analogous defence mechanisms that the endogenous antioxidant ones.

In this sense, the textiles containing antioxidants might have a similar diffusion than the transdermal liberation patches used in the pharmaceutical field. Nowadays, the majority of the exogenous antioxidants used come from natural extracts as polyphenols from the grape or the tea (epicatechin, quercetin, resveratrol, etc.) (21), isoflavons from the soybean (22, 23) or phenols from the olive oil (hydroxytyrosol) (24, 25) among others. In this study, an antioxidant (Gallic Acid, GA) was used as active principle for its interest as chemical trustworthy tracer and for its eventual beneficial action when, incorporated in a biofunctional fabric, intended to be used in contact with the skin. Liposomes and mixed micelles made up with the same phospholipids but with the presence of Oramix as a surfactant, were used as vehicles to study their absorption and desorption properties when applied to cotton fabric. Skin delivery of Gallic acid embedded into the textile was determined at the different skin layers (stratum corneum, epidermis or dermis) by a specific *in vitro* percutaneous absorption methodology to determine the suitability of the vehicles for skin permeation (26).

In vitro percutaneous absorption is an interdisciplinary subject that is relevant to a number of widely divergent fields (27). The main areas of interest are the development of transdermal devices, dermatological formulations and safety assessment of cosmetics topically applied. There exists suitable evidence that *in vitro* data may be predictive for *in vivo* percutaneous absorption in both animals and humans (27). Furthermore, the usefulness of the pig skin as an experimental model for skin permeation in humans is widely appreciated because their permeability properties are remarkable similar (28).

Experimental Part

Materials

The standard fabric used was plain cotton fabric (CO), (Bleached Desized Cotton Print Cloth, Style 400 ISO 105-F02).

Liposomes were prepared using commercial lipids (phospholipids) Emulmetik 900 (Lucas Meyer GmbH, France) and mixed micelles using the same lipids and the surfactant Oramix CG 110 (Sppic Italy Srl, Italy). An antioxidant active principle Gallic Acid (GA)(Sigma-Aldrich) was used. All chemicals used were of analytical grade. For high-performance liquid chromatography with UV detector (HPLC-UV) analysis, methanol, (HPLC grade) and distilled water were used. Methanol (Carlo Erba, France) was used for the GA extractions from textiles.

Liposome preparation

Liposome (4 wt% of lipid, 2wt% GA) was prepared using a thin-film method reported elsewhere (10).

Mixed micelles preparation

Mixed micelles (30 wt% of surfactant, 4 wt% of lipid, 2wt% GA) were prepared mixing all the ingredients; solubilisation was performed by gently shaking until absolutely clear solutions were obtained. All actions took place at room temperature.

Vesicles size and zeta potential

Liposome and mixed micelle sizes were measured using Malvern Zetasizer Nano ZS90.

Textile application procedure

Liposomes or mixed micelles were applied onto cotton fabrics in triplicate by bath exhaustion, bath ratio 1/5, at 60°C for 60 min with manual stirring every 10 minutes. To quantify the amount of liposomes absorbed into the fabrics, the samples were weighted before and after 24h application under standard ambient conditions (20±2°C and 65±5% relative humidity, ISO 554-1976).

Desorption procedure

The treated cotton fabrics were washed in three different water baths at room temperature, the samples were weighted before and after 24h washing with deionised water (1/5 bath ratio) during 5 min with magnetic stirring, under standard ambient conditions (20±2°C and 65±5% relative humidity, ISO 554-1976). Samples were also weighted before and after washing at standard ambient conditions in a second wash with deionised water (1/10 bath ratio) during 5 min with magnetic stirring, and the third wash, with deionised water (1/25 bath ratio) during 5 min with magnetic stirring.

In vitro percutaneous absorption experiments (Franz diffusion cells)

For these studies pig skin was used from the unboiled back of Landrace large white pigs weighing 30-40 kg. The pig skin was provided by the Clínic Hospital of Barcelona, Spain. After excision, the skin was dermatomed to a thickness of about 500 ± 50 µm with a Dermatome GA630 (Aesculap, Germany). Skin discs of a 2.5 cm inner diameter were prepared and fitted into the Franz-type diffusion cells.

The skin absorption studies were initiated by applying 10 µL of liposomes or mixed micelles (about 70 µg/cm²) or by applying the fabrics treated with the same liposomes or mixed micelles (about 150-250 µg/cm²) onto the skin surface. Between the textile and the skin, 20µL of distilled water was added to ensure a close contact. A control skin disc (without product application on the skin surface) was used to rule out possible interferences in the analysis of the GA by HPLC-UV. According to the OECD methodology used (27); the skin penetration studies were performed after 24 h of close contact between the textile and the skin.

In order to increase the contact pressure between textile fabric and skin, skin permeation experiments were also carried out by placing a steel cylinder on the textile-skin substrate at a constant pressure in accordance with standard conditions (125 g/cm²) (ISO 105-E04, 1996)). A thin Teflon disc full of holes served as a rigid base to prevent an eventual flexing effect on textile or on skin because of the cylinder pressure. The real conditions of “corset” use can be simulated with the device illustrated in Figure 1.

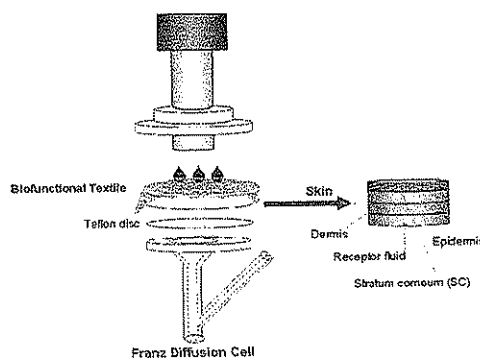


Figure 1. Diagram of *in vitro* percutaneous absorption experiments.

After the exposure time, the receptor fluid was collected and brought to 5 ml in a volumetric flask. In the case of formulations, the skin surface was washed with a specific solution (500µl SLES (Sodium Lauryl Ether Sulphate) (0.5%) and twice 500µl distilled water) and dried with cotton swabs. In the case of textiles, the fabrics were removed from the skin surface and collected together with the top of the cell. In both cases, after eliminating the GA excess from the skin surface, the stratum corneum of the skin was removed using adhesive tapes (D-Squame, Cuderm Corporation, Dallas, TX, USA) applied under controlled pressure (80 g/cm²

for 5 sec). The first tape was individually separated, the second to the fourth, and the fifth to the eighth tapes were pooled together. The epidermis was separated from the dermis after heating the skin at 80°C for five seconds.

GA was extracted from the different samples (surface excess, cotton or skin layers) using methanol:water (50:50), 30 min at room temperature by ultrasound bath. The receptor fluids were directly analyzed. After filtration on a Millex filter (0.22 μm , Millipore, Bedford, MA, USA), the solutions containing GA were assessed by HPLC-UV.

Gallic Acid analytical detection

GA from the different extraction bath samples was determined by HPLC equipped with an UV-Vis detector previously described (10). The column used was a LiChrocart 125-4/Lichrosorb RP-18 (5 μm) (Darmstadt, Germany). The mobile phase was 80% water / 80% methanol at 1 mL/min flow rate. The GA retention time was about 3.3 min. The area below the peak was used to calculate the concentration of GA using external standards that showed linearity over the concentration range of 0.25 to 100 $\mu\text{g/ml}$. The intra-day and inter-day variations of the method were less than 5%.

Statistics

Standard deviations were calculated for all mean values. Analysis of variance (ANOVA) with a one-way layout was used for group comparisons.

Results and discussion

The precise amount of active agents present in the biofunctional textile has been established before its use as a textile drug delivery system. The amount of vehicles (liposomes or mixed micelles with GA incorporated into textiles was determined by weighting of the fabrics before and after the exhaustion treatment. The amounts of formulation absorbed and desorbed in cotton are detailed in Table 1 and Figure 2.

Table 1. Product percentage absorbed onto cotton fabric after treatment, after first water washing, and after total water washings. (%owf: % over weight of fiber)

Fabric	Type of Treatment	Treatment (% owf)	After 10ml wash (% owf)	After total wash (% owf)
CO	Mixed Micelles	35.43 \pm 2.73	12.72 \pm 0.44	2.42 \pm 0.06
CO	Liposomes	10.99 \pm 0.39	7.32 \pm 2.43	5.58 \pm 0.39

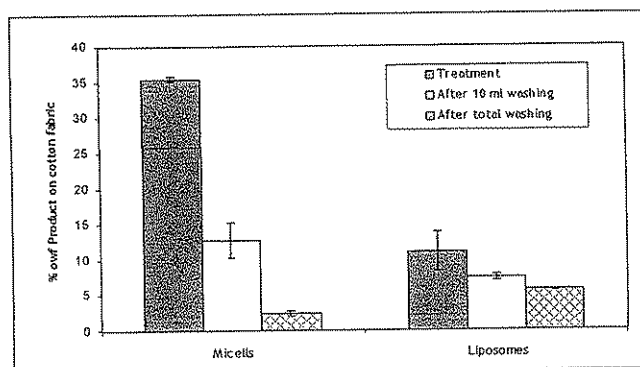


Figure 2. Product percentage absorbed onto cotton fabric after exhaustion treatment, and remaining after first water washing, and after total water washings.

A much greater absorption was found for the mixed micelles treatment related to liposomes. The high amount of surfactant present in the mixed micelles formulation absent in the liposome formulation could be the reason. However, there is a much higher desorption for the mixed micelles treated fabric due to the high solubility of the surfactant present, leading to

less amount of absorbed material in the textile after washing related to the liposome treated fabric.

Based on the different sizes between micelles and liposomes (around 10nm and 500nm respectively) higher penetration of the mixed micelles formulation onto the cotton fabric was expected. This could be supported by the high amount of product absorbed with micelles related to liposomes. However, the micelle-vesicle transition due to surfactant dilution in the washing baths which would account to an increase of the vehicle size, were expected to increase retention of the formulation inside the fibre after washing. Indeed, the opposite effect was found. Much desorption of mixed micelles were observed which could indicate a superficial absorption of mixed micelles easily lost with the aqueous washings.

Particle size was determined to elucidate the vehicle size and their changes with elution on the initial baths and washing baths. Mean particle size and a polydispersity index were obtained by dynamic light scattering measurements with Malvern Zetasizer Nano ZS90 (Table 2).

Table 2. Size (Z-Average) and Polydispersity Index of different baths with mixed micelles and liposomes.

Treatment	Analysed Bath	Size (Z-Average) Diameter (nm)	Pdl
Mixed Micelles	Initial Bath	6.94±0.82	0.98±0.04
	Bath after exhaustion treatment	102.18±30.95	0.98±0.03
	Bath after 1sr water washing (10 ml)	206.66±176.46	0.34±0.12
	Bath after 3rd water washing (50 ml)	210.97±38.20	0.31±0.02
Liposomes	Initial Bath	525.60±26.06	0.68±0.03
	Bath after exhaustion treatment	375.25±64.56	0.51±0.12
	Bath after 1sr water washing (10 ml)	474.33±32.22	0.68±0.20
	Bath after 3rd water washing (50 ml)	623.53±18.75	0.52±0.03

As it could be expected, in the initial bath of mixed micelles particles of around 7nm were found and in the initial bath of liposomes much bigger particles of about 500nm were detected. The particles elution from the initial bath to the last water washing baths does not have much effect with liposome size which is maintain around 500nm, however a big increase in size from 7 to 200nm was found for mixed micelles which indicate liposome formation due to surfactant dialysis. This increment in size does not help the formulation to remain in the textile, on the contrary it favours its desorption.

Having in mind the percentage of GA present in each formulation, the theoretical amount of GA in the textiles was calculated, without considering possible preferential affinities of a particular component by the fibre (Table 3 and Figure 3)

Table 3. Gallic Acid percentage absorbed onto cotton fabric after treatment, and remaining after first water washing, and after total water washings.

Fabric	Type of Treatment	Treatment (% owf)	After 10ml wash (% owf)	After total wash (% owf)
CO	Mixed Micelles	1.98±0.15	0.71±0.02	0.14±0.00
CO	Liposomes	3.66±0.13	2.44±0.24	1.86±0.13

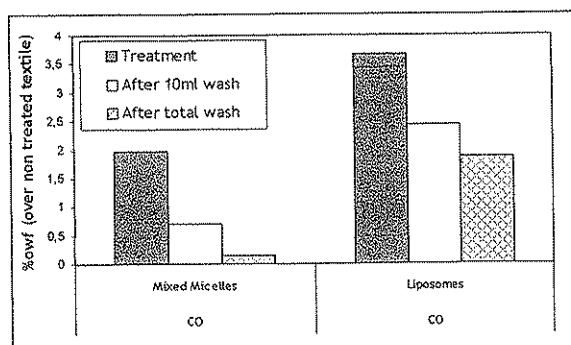


Figure 3. Gallic Acid percentage absorbed onto cotton fabric after exhaustion treatment, and remaining after first water washing, and after total water washings.

The comparison between Figure 2 and Figure 3 indicates that the higher amount of formulation absorbed in the mixed micelle treated textile is mainly due to the presence of surfactant Oramix. Therefore, if all components of formulations have the same affinity for the fibre, a lower amount of GA will be present in the cotton fibre both after the absorption process but moreover after the desorption one.

To study the active principle penetration into the skin, an *in vitro* methodology based on percutaneous absorption has been applied to demonstrate the delivery of an encapsulated principle from the textile to the different skin layers (stratum corneum, epidermis or dermis). Percutaneous absorption of the two formulations; 2% GA, 4% of PC liposomes and 2% GA, 4% of PC and 30% of Oramix CG mixed micelles were evaluated as well as the cotton textiles impregnated with the same liposomes or mixed micelles. The two formulations were formed in the presence of 2% of GA as described in the experimental part. The cotton textile, were treated with the same formulations already described. The two formulations and the cotton textiles previously treated with the formulations were put in contact with the skin disks. In the case of textiles between the textile and the skin a little amount of distilled water was added to ensure a close contact. Besides, in order to increase the contact pressure between textile fabric and skin, skin permeation experiments were carried out by applying a steel cylinder on the textile-skin substrate at a constant pressure according to standard conditions (125 g/cm²) (26) (See experimental part).

The aim of this assay was to demonstrate the tracer delivery in to the different layers of the skin. GA encapsulated in mixed micelles and PC liposomes which were embedded or not in cosmeo-textiles were applied to skin to study their percutaneous absorption. After three experiments with each sample, GA was analysed from each extract of washing sample, fabric, stratum corneum, rest of epidermis, dermis and receptor fluid. Results are listed in Table 4.

Table 4. *In vitro* percutaneous absorption of GA (Gallic Acid) from Mixed Micelles and Liposomes and their biofunctional textiles (CO: cotton) (SC: stratum corneum, R. Fluid: receptor fluid)

Mixed Micelles Compartments	GA in Mixed Micelles		GA in mix. Micelles on Cotton	
	%	µg/cm ²	%	µg/cm ²
Total applied	-	70.34	-	242.61
Wash/Fabric	69.30±4.92	48.80±3.46	86.91±6.30	210.85±15.68
SC	4.62±0.62	3.25±0.44	5.21±1.02	12.64±2.48
Epidermis	1.60±0.60	0.87±0.06	1.83±0.35	4.45±0.86
Dermis	1.57±0.13	1.10±0.09	0.87±0.54	2.12±1.30
R. Fluid	12.58±0.35	8.85±0.25	4.70±0.44	16.10±1.03
Recovery	92.37±6.49	63.67±4.50	99.51±4.82	241.47±11.69

Liposomes Compartments	GA in Liposomes		GA in Liposomes on Cotton	
	%	µg/cm ²	%	µg/cm ²
Total applied	-	67.45	-	147.95
Wash/Fabric	69.83±5.48	47.10±3.69	88.73±9.72	131.28±14.38
SC	7.68±0.90	5.18±0.60	5.41±1.42	8.60±2.26
Epidermis	2.72±0.07	1.89±0.15	1.75±0.87	2.78±1.93
Dermis	4.29±0.27	2.89±0.18	0.72±0.41	1.15±0.66
R. Fluid	23.10±1.83	15.58±1.24	0.00±0.00	0.00±0.00
Recovery	107.62±4.26	72.65±2.82	96.61±7.91	143.81±4.25

Results in Table 4 and Figure 4 indicate that penetration of GA formulated as liposomes is much higher than when formulated as mixed micelles. All skin compartments have a higher amount of GA when vehiculized as liposome related to mixed micelles, when formulations alone are applied. When GA is embedded into the biofunctional textile, it always promoted a reservoir effect, a lower GA skin penetration was obtained than when it was applied directly onto the skin, being in this case much lower for the liposome applied cotton textile.

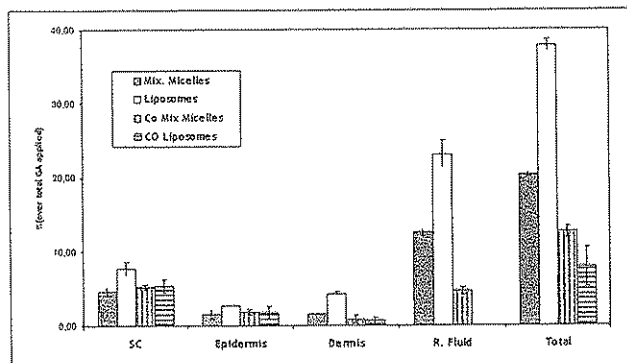


Figure 4. *In vitro* percutaneous absorption of Gallic Acid (GA) from mixed micelles, liposomes and their biofunctional textiles (CO: cotton). (SC: stratum corneum, E: epidermis, D: dermis, FR: receptor fluid)

The higher penetration of the PC liposomes related to mixed micelles can not be related to the different size since mixed micelles are much smaller. Perhaps the bilayer structure of the vesicles has more affinity to the similar lipid bilayer structures present in the stratum corneum and rest of the skin. Besides mixed micelles have been reported to be less deformable in nature and also have less skin permeation ability across the skin in comparison with elastic liposomes (29).

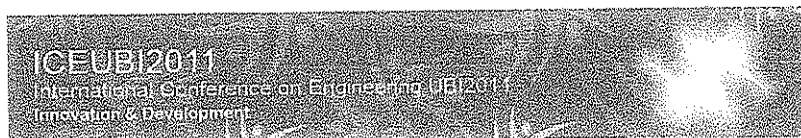
Similar penetration was obtained for the textiles treated with GA in mixed micelles or liposomes in the skin compartments, stratum corneum, epidermis and even in the dermis. Possibly, the textiles with the different vehicles embedded play a similar reservoir effect. However, a large difference was obtained in the receptor fluid for the differently treated fabrics, while liposome treated do not allow GA liberation into the receptor fluid, an important desorption ($\approx 5\%$) was found for the mixed micelle treated fabric. This could be related to the higher release properties of the GA when vehiculized with mixed micelles contrary to the lower desorption when vehiculized as liposomes.

Conclusions

The precise amount of active agents present in the biofunctional textile was established before its use as a textile drug delivery system. A much greater absorption was found for the mixed micelles treatment related to liposomes. However, there is a much higher desorption for the mixed micelles treated fabric, leading to less amount of absorbed material in the textile after washing related to the liposome treated fabric which could indicate a superficial absorption of mixed micelles easily lost with the aqueous washings. A big increase in size from 7 to 200nm was found for mixed micelles which indicate liposome formation due to surfactant dialysis. This increment in size does not help the formulation to remain in the textile, on the contrary it favours its desorption.

Percutaneous absorption of the two formulations; 2% GA, 4% of PC liposomes and 2% GA, 4% of PC and 30% of Oramix CG mixed micelles were evaluated as well as the cotton textiles impregnated with the same liposomes or mixed micelles. Results indicate that penetration of GA formulated as liposomes is much higher than when formulated as a mixed micelles. Perhaps the bilayer structure of the vesicles has more affinity to the similar lipid bilayer structures present in the stratum corneum and rest of the skin.

When GA is embedded into the biofunctional textile, it always promoted a reservoir effect, a lower GA skin penetration was obtained than when it was applied directly onto the skin, being in this case much lower for the liposome applied cotton textile. Similar penetration was obtained for the textiles treated with GA in mixed micelles or liposomes in the skin compartments, stratum corneum, epidermis and even in the dermis, which indicates a similar reservoir effect. However, while liposome treated do not allow GA liberation into the receptor fluid, an important desorption ($\approx 5\%$) was found for the mixed micelle treated fabric. This could be related to the higher release properties of the GA when vehiculized with mixed micelles contrary to the lower desorption when vehiculized as liposomes. This methodology



could serve to verify penetration in human skin of encapsulated substances which can exert a marked influence on specific doses of active agents to be released to the skin.

Acknowledgements

The authors wish to thank the Spanish National Project (Ministerio de Educación y Ciencia) CTQ-PPQ2009-13967-C03-01 for the financial support.

References

- (1) Wachter, R.; Weuthen, M.; Panzer, C.; Paff, E.: "Liposomes are used as textile finishes which not only improve elasticity and hand but can also be transferred to skin contact" (2005) Patent n° EP1510619-A2, DE10339358-A1, US2005058700-A1.
- (2) Guarducci, M.: "Product having particular functional properties for the skin and process for the preparation thereof" (2006) Patent n° WO/2006/106546.
- (3) Nelson, G.: "Application of microencapsulation in textiles" *International Journal of Pharmaceutics*, Vol. 242 (2002), ISSN 0378-5173, pp. 55-62.
- (4) D.D. Lasic and Y. Barenholz. (eds.). *Handbook of Nonmedical Applications of Liposomes*, Vol. 1-4. CRC Press, Boca Raton, 1996.
- (5) D. Lichtenberg. Liposomes as a model for solubilization and reconstitution of membranes. In *Handbook of Nonmedical Applications of Liposomes—Models for Biological Phenomena*, Vol. 2. 199-218. D. D. Lasic and Y. Barenholz, editors. CRC Press, Boca Raton, 1995.
- (6) Teschke, O.; de Souza, E.F. "Liposome structure imaging by atomic force microscopy: verification of improved liposome stability during absorption of multiple aggregated vesicles" *Langmuir*, Vol. 18 (2002), ISSN 0743-7463, pp. 6513-6520.
- (7) Lian, T.; Ho, R.J.Y. "Trends and developments in liposome drug delivery systems" *Journal Pharmaceutical Sciences*, Vol. 90 (2001), ISSN 0022-3549, pp. 667-680.
- (8) Betz, G.; Aeppli, A.; Menshutina, N.; Leuenberger, H. "In vivo comparison of various liposome formulations for cosmetic application" *International Journal Pharmaceutics*, Vol. 296 (2005), ISSN 0378-5173, pp. 44-54.
- (9) Aggarwal, A.; Dayal, A.; Kumar, N. "Microencapsulation processes and application in textile processing" *Colourage*, Vol. 45 n°8 (1998), ISSN 0010-1826, pp.15-24.
- (10) Ramón, E.; Alonso, C.; Coderch, L.; de la Maza, A.; López, O.; Notario, J.; Parra, J.L. "Liposomes as alternative vehicles for sun filter formulations" *Drug Delivery*, Vol. 12 (2005), ISSN 1071-7544, pp. 83-88.
- (11) Chaszczewska-Markowska, M.; Stebelska, K.; Sikorski, A.; Madej, J.; Opolski, A.; Ugorski, M. "Liposomal formulation of 5-fluorocytosine in gene therapy with cytosine deaminase for colorectal cancer" *Cancer Letters*, Vol. 262 n°2 (2008), ISSN 0304-3835, pp. 164-172.
- (12) Ichikawa, K.; Urakami, T.; Yonezawa, S.; Miyanchi, H.; Shimizu, K.; Asai, T.; Oku, N. "Enhanced desensitization efficacy by liposomal conjugation of a specific antigen" *Int. J. Pharmaceutics*, Vol. 336 n°2 (2007), ISSN 0378-5173 pp. 391-395.
- (13) Dong, K.; Damaglai, N.; Smiles, K.; Yarosh, D. "The 8-oxo-guanine repair enzyme OGG1 encapsulated in liposomes reduces MMP-1 secretion and increases collagen production by dermal fibroblasts in a paracrine system" *J. Am. Acad. Dermatol.* Vol. 58 n°2 (2008), ISSN 0190-9622, AB110.
- (14) Pasquardini, L.; Lunello, L.; Vanzetti, L.; Anderle, M.; Pederzoli, C. "Immobilization of cationic rifampicin-loaded liposomes on polystyrene for drug-delivery applications" *Colloids Surfaces B*, Vol. 62 n°2 (2008), ISSN 0927-7765, pp. 265-272.
- (15) Martí, M.; de la Maza, A.; Parra, J.L.; Coderch, L. "Dyeing Wool at Low Temperatures: New Method Using Liposomes" *Textile Research Journal*, Vol. 71 n°8 (2001), ISSN 0040-5175, pp. 678-682.

- (16) Martí, M.; Coderch, L.; de la Maza, A.; Parra, J.L. "Liposomes of phosphatidylcholine: a biological natural surfactante as a dispersing agent" *Coloration Technology*, Vol. 123, (2007), ISSN 1472-3581, pp. 237-241.
- (17) Montazer, M.; Validi, M.; Toliyat, T. "Influence of temperature on stability of multilamellar liposomes in wool dyeing" *Journal Liposome Research*, Vol. 16 n°1 (2008), ISSN 0898-2104, pp. 81-89.
- (18) Ollivon, M.; Lesieur, S.; Grabielle-Madelmont, C.; Paternostre, M. "Vesicle reconstitution from lipid-detergent mixed micelles" *Biochimica et Biophysica Acta*, Vol. 1508 (2000), ISSN 0006-3002, pp. 34-50.
- (19) Kragh-Hansen, U.; le Maire M.; Møller J.V. "The mechanism of detergent solubilization of liposomes and protein-containing membranes" *Biophysical Journal*, Vol. 75 (1998), ISSN 0006-3495, pp. 2932-2946.
- (20) Lasch J. "Interaction of detergents with lipid vesicles" *Biochim. Biophys. Acta*, Vol. 1241 (1995), ISSN 0006-3002, pp. 269-292.
- (21) Jayaprakasha G.K.; Tamil Selvi; Sakariah K.K. "Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts" *Food Research International*, Vol. 36 (2003), ISSN 0963-9969, pp. 117-122.
- (22) Mota, M.; Gargavu, S.; Popa, S.; Schiopu, S.; Panduru, N. M.; Mota, E. "Soya, the medicine food product" *Romanian Journal Internal Medicine*, Vol. 45 (2007), ISSN 1220-4749, pp. 113-121.
- (23) Valsecchi, A.E.; Franchi, S.; Panerai, A.E., Sacerdote, P.; Trovato, A.E.; Colleoni, M. "Genistein, a natural phytoestrogen from soy, relieves neuropathic pain following chronic constriction sciatic nerve injury in mice: anti-inflammatory and antioxidant activity" *Journal of neurochemistry*, Vol. 107 (2008), ISSN 0022-3042, 107 pp. 230-240.
- (24) Fernandez-Bolanos, J.G.; Lopez, O.; Fernandez-Bolanos, J.; Rodriguez-Gutierrez, G. "Hydroxytyrosol and derivatives: Isolation, synthesis, and biological properties" *Current organic chemistry*, Vol. 12 (2008), ISSN 1385-2728, pp. 442-463.
- (25) De Leonardis, A.; Aretini, A.; Alfano, G.; Macciola, V.; Ranalli, G. "Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea Europaea* L.) and evaluation of its antioxidant properties and bioactivity" *European Food Research and Technology*, Vol. 226 (2008), ISSN 1438-2377, pp. 653-659.
- (26) Rubio, L.; Alonso, C.; Coderch, L.; Parra, J.L.; Martí, M.; Cebrián, J.; Navarro, J.A.; Lis, M.; Valdeperas, J. "Skin delivery of caffeine contained in biofunctional textiles" *Textile Research Journal*, Vol. 80 n°12 (2010), ISSN 0040-5175, pp. 1214-1221.
- (27) A.C. Williams, "Transdermal and Topical Drug Delivery: from theory to clinical practice" *Pharmaceutical Press*, London, UK, 2003.
- (28) Simon, G.A.; Maibach, H.I. "The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations--an overview" *Skin Pharmacology and Applied Skin Physiology*, Vol. 13 (2000), ISSN 1422-2868, pp. 229-234.
- (29) Mishra, D.; Garg, M.; Dubey, V.; Jain, S.; Jain, N.K. "Elastic liposomes mediated transdermal delivery of an anti-hypertensive agent: Propanolol hydrochloride", *J. Pharmaceutical Sciences*, Vol. 96 (2007), ISSN 0022-3549, pp.145-155.



SKIN DELIVERY OF GALLIC ACID FROM BIOFUNCTIONAL COTTON FABRIC

Vanessa Martínez, Cristina Alonso, Alfons de la Maza, Meritxell Martí, and Luisa Coderch
Advanced Chemical Institute of Catalonia (IQAC-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain

Abstract

In these recent years, many studies about biofunctional textiles have been published. These textiles have incorporated different types of cosmetic or pharmaceutical active compounds. These compounds are able to move to the skin of the person who wears the biofunctional garment and they can act as a conventional cosmetic or pharmaceutical product. The absorption and desorption of the antioxidant gallic acid (GA) vehiculated in liposomes and mixed micelles applied onto a cotton fabric has been studied. A much greater absorption was found for the mixed micelles treatment related to liposomes. However, also a much higher desorption for the mixed micelles treated fabric could indicate a superficial absorption of mixed micelles easily lost with the aqueous washings. The active compound penetration was followed by a specific *in vitro* percutaneous absorption methodology. Penetration of GA formulated as liposomes is higher than when formulated as a mixed micelles. When Gallic acid is embedded into the biofunctional textile, liposomes promoted a reservoir effect more than mix. micelles. This methodology could serve to verify penetration in human skin of encapsulated substances which can exert a marked influence on specific doses of active agents to be released to the skin.

Experimental Part I

LIPOSOMES PREPARATION

4 wt% lipid
2 wt% gallic acid

thin film method:

MIXED MICELLES PREPARATION

30wt% surfactant
4 wt% lipid
2 wt% gallic acid

mixing

PARTICLE SIZE

Malvern Zetasizer Nano ZS90

Experimental Part II

APPLICATION BATH EXHAUSTION

Bath ratio: 1/5
Temperature: 60°C
Time: 60 min
Stirring every 10 min

DESORPTION WASHING BATHS

Room Temperature
Time: 5 min with magnetic stirring
Water bath 1: 1/5
Water bath 2: 1/10
Water bath 3: 1/25

PRODUCT QUANTIFICATION

Samples were weighed before and after 24h application or washings.
Standard ambient conditions (20°C, 65% Relative Humidity)

Experimental Part III

PERCUTANEOUS ABSORPTION

From diffusion cell used to know the percutaneous absorption profile of a given compound coated on a skin disc.

Cotton with liposomes or mixed micelles

Receptor fluid

Epidermis

Stratum corneum (SC)

Separation of the different skin compartments followed by an extraction and quantification of gallic acid in each compartment

RESULTS: Absorption / Desorption

The amount of liposomes or mixed micelles with GA incorporated into textiles and maintained after washings was determined by weighting of the fabrica before and after the exhaustion and washing treatments.

Absorption /desorption formulations

Product percentage absorbed into cotton fabric: after exhaustion treatment, and remaining after first water washing, and after total water washings.

1) absorption for the mixed micelles in exhaustion treatment
2) Desorption for the mixed micelles in the washing treatment

High amount of surfactant present in mix. micelles is easily absorbed in the textiles and easily desorbed by washing.

Mixed micelles constituted by a lipid and a surfactant agent are capable of transforming in liposomes when the surfactant is eliminated by simple dilution with water => a new application will be proved with the aim to study their application onto textile fabric and later by dilution (water washing treatment) its potential ability to be structured as liposomes into the textile fabric.

Lipid structure size in different baths

Size of liposomes is maintained with dilution.
Size of mix. micelles is increased with dilution.

Increase in size of mixmicelles could favours its desorption

Having in mind the percentage of GA present in each formulation, the theoretical amount of GA in the textiles was calculated, without considering possible preferential affinities of a particular component by the fibre.

Gallic acid absorption / desorption

Gallic Acid percentage absorbed into cotton fabric after exhaustion treatment, and remaining after first water washing, and after total water washings.

Mixed micelles: 1) GA in the cotton fibre
Liposomes: 2) GA in the cotton fibres

Much more GA is absorbed and maintained in the textile treated with liposomes.

A: Initial bath B: Bath after exhaustion treatment C: Bath after 1st water washing D: Bath after 3rd water washing

RESULTS: *in vitro* percutaneous absorption

To study the active principle penetration into the skin, an *in vitro* methodology based on percutaneous absorption has been applied to demonstrate the delivery of an encapsulated principle from the textile to the different skin layers (stratum corneum, epidermis or dermis).

Formulations: Skin penetration GA in liposome >>> Skin penetration GA in mix. micelle
SC, Epidermis and R. Fluid: GA in liposome penetration > GA in mix. micelle penetration
Skin penetration GA in mix. micelle: Formulation + biofunctional cotton
Skin penetration GA in liposomes: Formulation +>>> biofunctional cotton
SC, Epidermis and Dermal: biofunctional cotton with lipos = biofunctional cotton with mix. Micelles
R. Fluid: liposomes <<<< mix. micelles

Maintenance of the substantial GA penetration of mix. Micelles when applied formulation or embedded into cotton and promotion of reservoir effect of liposomes when are absorbed into cotton biofunctional fabric.

CONCLUSIONS

Two formulations were prepared and applied onto cotton fabrics by bath exhaustion method: 2%GA, 4% lipid Liposomes and 2% GA, 4% lipid, 30% surfactant Mixed Micelles. Great absorption was found for mix. micelles, however high desorption was also found. This could indicate a superficial absorption of Mix. Micelles easily lost with aqueous washings. A big increase in size from 7 to 200 nm of diameter was found in mix. micelles which indicates liposome formation due to surfactant dialysis. This increment in size could favour its desorption from the cotton fabric. Percutaneous absorption results indicate that penetration of GA formulated as liposomes is higher than when is formulated as a mix. micelles. Perhaps bilayer liposome structure has more affinity to the same structures in the stratum corneum and rest of the skin. GA in biofunctional cotton do not modified its skin penetration in the case of mixed micelles, however it promotes a reservoir effect in the case of liposomes, where GA not achieve the receptor fluid. The *in vitro* methodology could serve to verify penetration in human skin of encapsulated substances applied onto biofunctional textiles.