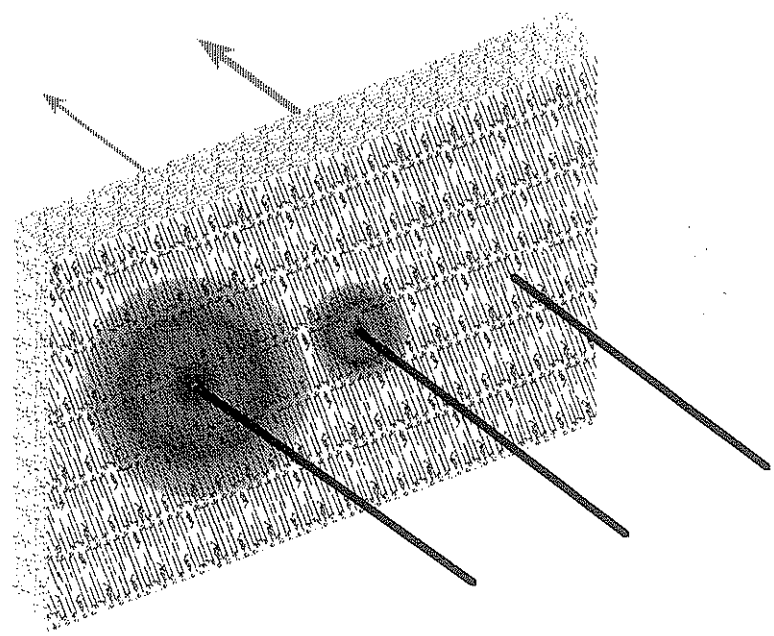
Prediction of Percutaneous Penetration

Volume 4b



Edited by K.R. Brain, V.J. James and K.A. Walters

Prediction of Percutaneous Penetration

Methods Measurements Modelling
Fourth International Conference

April 18-22, 1995 – La Grande Motte, France

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Proceedings of the Fourth International Prediction of Percutaneous Penetration Conference held in La Grande Motte, April 1995

Acknowledgements

The organisers are grateful to all of the organisations and individuals who have supported the Fourth Prediction of Percutaneous Penetration Conference.

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EFFECT OF LIPOSOMES ON PERCUTANEOUS PENETRATION USING THE TAPE STRIPPING TECHNIQUE.

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INTRODUCTION

While liposomes have been investigated for many years as parenteral drug carrier systems, they have only for approximately one decade been considered for topical drug delivery[1,2]. Despite numerous studies corroborating the advantages of liposomes as drug carriers in the skin and an increasing in the use of cosmetics with a liposome base, little is known about the molecular mechanism by which the penetration of liposomal lipophilic and hydrophilic drugs is promoted[3].

Most investigators would agree that no transport of lipids takes place across the whole skin[3]. Intact liposomes are mainly confined to the horny layer, do not penetrate deeper, but enhance penetration of hydrophilic and moreover lipophilic drugs[4]. Fluidisation of intercellular lipid domains in the stratum corneum (SC) has been suggested as a relevant hypothesis. Therefore the penetration enhancement of drug transport observed when drugs are encapsulated in vesicles may be due to SC structural modification.

The SC, despite being the main barrier by which substances enter the skin, also acts as a reservoir for topically applied substances[5]. A relationship between the reservoir effect of the horny layer and percutaneous absorption of molecules[6] has been established. A weak reservoir capacity would correspond to a weak penetration and, hence, a strong barrier. Inversely, a high reservoir capacity would provide a high penetration and, therefore, a weak barrier effect. Consequently, barrier and reservoir functions of the horny layer may reflect the same physiological phenomenum[5].

Thus, the study of the reservoir capacity of the SC by the tape stripping technique when sodium fluorescein encapsulated in vesicles is applied on the skin could provide a good predictive assessment of the total amount of hydrophilic drug that penetrates with liposomes. This could improve our understanding of the mechanism involved in transdermal transport of drugs when applied in vesicles to skin.

MATERIALS AND METHODS

Fluorescein, sodium salt (NaFl) was purchased from SIGMA CHEMICAL Company (Missouri, USA). Boric acid, potassium chloride, sodium hydroxide, sodium chloride and hydrochloric acid fuming 37% were supplied by MERCK (Darmstadt, Germany). All chemicals were of the purest grade available.

Borate Buffer (pH = 9.0) wich was prepared from boric acid and potassium chloride solution, adding the specified volume of the sodium hydroxide solution[7] and was used as a buffer solution in this study.

Liposomes

Small unilamellar vesicles of an approximate size of 100 nm, performed with 5% of soya lecitin (containing 93-97% Phosphatidilcholine), water solution of NaCl 0.9%, ethanol and Dl-α-tocopherol (as antioxidant) were used.

13 ml of liposome solution containing 10 mg/ml of soya lecitin and 1 mg/ml of NaFl, were cleared of unencapsulated fluorescent probe by ultracentrifugation, at 55.000 rpm for 24 hours, separating the pellet from the supernatant. The NaFl content in the pellet fraction was spectrofuorimetrically determined obtaining an encapsulation efficiency of 15.89% ± 0.49 (n=36). The pellet was therefore resuspended in 2ml water solution of NaCl 0.45% in order to obtain approximately 1 mg/ml of sodium fluorescein just before application to the skin.

Penetration studies

Penetration behaviour was investigated "in vivo" according to the stripping method[6]. The study was carried out with equal concentrations (1mg/ml) of NaFl in all formulations: water solution, liposomes, liposomes free of unencapsulated probe and the supernatant.

Topical application assays and SC strippings were performed in a conditioned room at 20°C with 60% relative humidity. Previously the test subject was permitted to become acclimatized for 30 minutes in these conditions.

The volume applied with an EXMIRE microsyringe (Fuji, JAPAN) was 10μl and the applied dose of fluorescent probe was 2.5 $\mu g/cm^2$. Preparations were applied onto areas of 4 cm^2, of the central area of the forearm of 3 Caucasian volunteers aged 30 to 40. Each assay was experimentally repeated 3 to 5

times. The application area was delimited by an adhesive cell. A DOW CORNING Medical Adhesive (Brussels, BELGIUM) was placed around the area in order to prevent lateral diffusion.

After 30 minutes of contact, fifteen successive tape strips of the SC were carried out with Scotch Magic™ 810 adhesive tape under defined conditions (pressure: 4 times with a 1 Kg roller; rapid stripping off; same investigator). The extraction of the NaFl content in the different samples was done 4 times with borate buffer pH 9,0 (Vortex and Ultrasounds). The liquid extracted from the 1st, 2nd and 3rd stripping was separately analyzed and the rest of strips were joined in groups of three (4 to 6, 7 to 9, 10 to 12, 13 to 15), in order to obtain a good detection level.

NaFl fluorescence found in these samples was measured by spectrofluorimetry (SHIMADZU RF-540, CRF-1 program) at 20 °C (Termocirculator Heto-Birkerod) : λ excitation 493 nm and λ emission 513 nm. The NaFl quantities were determined with the help of calibration curves.

We have always substracted the fluorescence values of the control strips from those of the corresponding assays to avoid any interference of the auto-fluorescence of the biological material itself by spectrofluorimetry.

RESULTS AND DISCUSSION

As stated above, most investigators agree that the enhancement of drug transport observed when vesicles are applied may be due to a mechanism of SC structural modification rather than to a vehiculizing mechanism in which the liposomes would penetrate deeper in the skin.

In order to shedlight on these hypotheses we designed a study to determine the amount of NaFl that penetrates using a small unilamellar vesicle formulation with, in one case, an encapsulation efficiency of approximately 15% and, in another case, the same liposomes with theoretically 100% of encapsulation obtained separating the pellet from the supernatant as described in the experimental part. These two experiments were also compared with the study of NaFl penetration in water and in the supernatant obtained from separation of the pellet from the liposome solution.

The different results of these four experiments would enable us to assess the possible modification of the barrier effect of SC and the importance of the vehiculizing mechanism which would be reflected in the differences obtained using liposomes with several encapsulation efficiencies.

The fluorescent probe was selected for its solubility, specificity, selectivity and atoxicity[8] (widely accepted for cosmetical formulation and ophthalmic use).

Calibration curves with different amounts of NaFl in alkaline borate buffer solution were obtained with a λ excitation at 493 nm and λ emission at 513 nm. The linear zone of fluorescent response used in this study is shown in Fig 1.

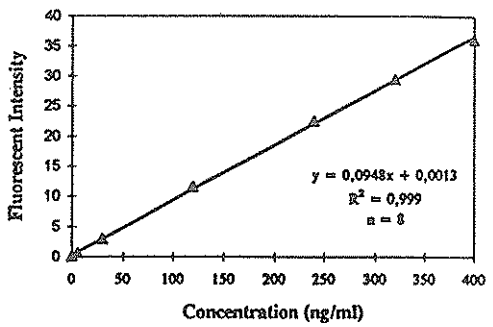

Fig 1. Calibration curve for Sodium Fluorescein

Ten μl of the water and liposome formulations were spread on the surface of the tape and extracted with the methodology described in the experimental part, in order to assess the extraction capacity of the borate buffer and the possible interferences of the adhesive components in the fuorescence results.

The espectrofluorimetrical detection of the extractive liquid showed that the total % recovered exceeded 95% in all cases (Table I). It can therefore be concluded that there is a good recovery of NaFl with this methodology with borate buffer and that there is no interference of the tape components or of the liposome components in the NaFl detection.

Table I. Total NaFl recovered after extraction (mean ± SD)

FORMULATION	% RECOVERED	n
Water	101.02 ± 1.04	21
Liposome	101.03 ± 4.0	24

The potential interference due to the autofluorescence of the biological material was also controlled. This interference of the SC was very low; however, we always substracted, were necessary, the fluorescence values of the control strips from those of the corresponding assays.

The amount of NaFl recovered in every strip or group of strips expressed in percentage with respect to the total applied dose, for the different formulations was determined. The experiments were performed 5 times for water and liposome formulations and 3 times

Table II. Recovery % of the applied dose in the differents strips (mean ± SD)

Number of Strip	Water (n=5)	Liposome (n=5)	Supernatant (n=3)	Pellet (n=3)
1st	32.66 ± 3.37	20.12 ± 2.03	26.89 ± 2.56	22.42 ± 5.48
2nd	14.88 ± 4.00	11.94 ± 4.37	19.32 ± 1.44	12.26 ± 6.66
3rd	7.78 ± 1.46	8.65 ± 1.89	12.05 ± 0.95	7.99 ± 0.21
4th-6th	12.43 ± 0.69	14.60 ± 4.73	12.01 ± 2.30	12.98 ± 3.22
7th-9th	5.99 ± 0.47	8.68 ± 2.58	5.39 ± 1.47	7.96 ± 1.71
10th-12th	4.08 ± 0.40	5.34 ± 1.73	4.50 ± 1.66	4.87 ± 1.34
13th-15th	3.17 ± 0.96	3.76 ± 1.16	2.31 ± 0.86	4.02 ± 1.44
Total %	81.01 ± 6.30	73.08 ± 10.26	82.48 ± 4.16	72.51 ± 3.13
Stratum Corneum %	48.34 ± 3.67	52.96 ± 10.42	55.59 ± 6.55	50.09 ± 4.53

for supernatant and pellet formulations. The mean and standard deviations were calculated (Table II).

Bearing in mind that the NaFl content in the first strip accounts for the non-penetrating content, the higher amount of NaFl content obtained in this first strip when water and supernatant are applied with respect to liposome and pellet should be noted. These results support the weakness of the SC barrier effect when liposomes are applied.

The total percentages of NaFl analyzed by adding all strips are also lower in the case of liposome and pellet penetration. This result reflects a higher penetration in deeper layers of skin, wich will also corroborate the already mentioned weak barrier effect.

Moreover, the total amount of NaFl in the SC (from 2nd to 15th strip) was also calculated to determine the reservoir capacity of this layer. The lowest amount of NaFl in SC when water is applied is in accord with the low penetration and strong barrier effect of this layer discussed above. However, the similar mean values obtained and the standard deviation values in the amount of NaFl in the SC do not allow us to reach more reliable conclusions.

In order to clearly follow the penetration profile of NaFl in the different strips, cumulative percentages were calculated and plotted as a function of the strip numbers.

A regression analysis was performed in order to confirm a mathematical relationship between the NaFl cumulative values and the number of strips,. By the non linear regression technique[9, 10] a modified hyperbolic function $y = 100/(a + b*x^c)$ was found to be the best, y being the cumulate % of applied dose, x the number of strip and a, b and c the equation estimated coefficients. Parameters a, b, c and the determination equation coefficient R^2 can be seen in Table III. In all cases R^2 are higher than 0.9994, reaching the value 1 in the case of liposome formulation. Plots of these curves can be seen in Figure 2.

Table III: Mathematical equation parameters and Determination coefficients according different formulations.

FORMULATION	a	b	c	R^2
Liposome	1.05	3.92	0.93	1.0000
Water	1.09	1.96	0.93	0.9996
Pellet	1.11	3.35	0.90	0.9998
Supernatant	1.17	2.54	1.37	0.9994

This figure shows the similar behaviour of the liposome and pellet formulations as well as the water and supernatant formulation with a similar higher enhancement of liposome formulations with respect to the others.

From the curves obtained it seems that NaFl included in liposome and pellet formulations penetrates the SC more readily than FlNa both in water and in supernatant. A similar conclusion can

be deduced if we consider the number of strips needed to obtain in all cases a cumulative 50% of applied dose.

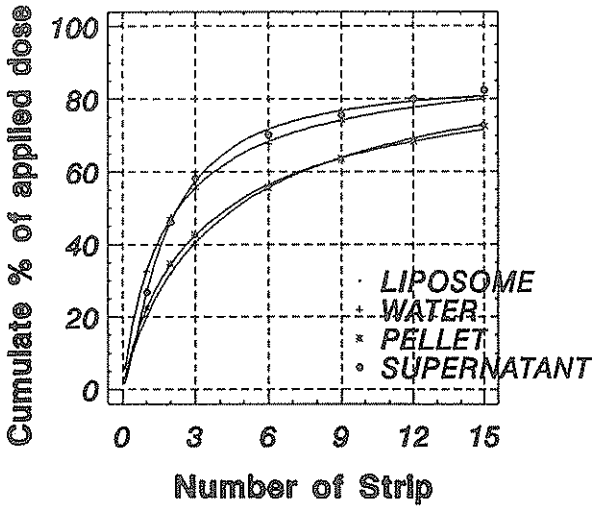

Fig 2. Penetration profile of Sodium Fluorescein into SC after 30 minutes using four vehicles.

Similar values were obtained when liposomes with varying amounts of encapsulated probe were applied. This findings lend further support to the hypothesis that the liposome penetration mechanism is not related to the amount of encapsulated probe, which would have indicated a vehiculizing mechanism of the vesicles. The lipophylic components of liposomes may co-diffuse into the SC and thus produce a change in the skin barrier properties by possibly making the SC environment more acceptable to the fluorescent probe.

CONCLUSION

Tape stripping associated with spectrofluorimetry is applied to the study of "in vivo" penetration behaviour of the hidrophilic probe in the SC in accordance with the vehicle structure, in this case liposomes.

Our results indicate the penetration enhancement of liposome formulations for delivery of hydrophilic substances. The low non-penetrating content and the high reservoir capacity of the SC correspond to a high penetration and therefore a weak barrier effect.[4]

The penetration profile of sodium fluorescein in the different strips were found to fitted as an hyperbolic function with appropiate correlation

From these curves it can be deduced that liposome formulation allows a deeper penetration followed by pellet formulation, being water and supernatant formulations the most superficial.

Moreover, the similar values obtained when the same liposomes with a varying encapsulation content were applied would support the penetration mechanism wich account for the fact that the vesicle enhancement is mainly due to SC structural modification.

ACKNOWLEDGEMENTS

The authors thanks to Mr. J. Caelles for his valuable informatical suport and Mr. G. Knorring for his expert technical assistance.

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