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Assessment of the recovery of three lipophilic psoralens by microdialysis: an in vitro study

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Abstract

The microdialysis method is extensively developed in neuropharmacological experiments. It is a non-invasive technique with promising applications in biological research. The aim of this work was to establish and to optimize a process to microdialyse chemicals. Three lipophilic molecules used in dermatology were tested: 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP) and trimethylpsoralen (TMP). These psoralens have the same basic chemical structure (furocoumarin). The different radical substitutions are responsible for their different lipophilicities. In this work, different microdialysis parameters were studied: the relative recovery and the equilibrium time in relation to the perfusion rate and to the drug lipophilicity. The equipment was composed of a CMA/100® microinjection pump, CMA/20® microdialysis probes (membrane length of 10 mm, cut off 20 and 100 kDa) and a CMA/140® microcollector. The perfusate was a phosphate buffer 0.06 M (pH 7.4). The psoralen dialysates were assessed by high performance liquid chromatography. The relative recovery of psoralens increased with the decreasing perfusion rates and with the drug lipophilicity. The equilibrium time decreased with the increasing perfusion rates. TMP was not detected under these experimental conditions. This work defines the optimal parameters for in vivo studies and shows the limit of this technique for the investigation of lipophilic drugs. © 1998 Elsevier Science B.V.

Keywords: Microdialysis; Recovery; Psoralens; Lipophilicity; Percutaneous absorption

1. Introduction

Up until now, the pharmacological and pharmacokinetic study of skin in vivo required sampling the extracellular fluid, which is the action

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site of drugs on all surface-bound receptors. The skin examinations have been restricted by lack of appropriate methods and by their invasive character (i.e. punch biopsy). Unlike the suction blistering technique (Kiistala and Mustakallio, 1967), in vivo microdialysis causes minimal tissue damage and no physical alteration to the skin of the tested subjects (Petersen, 1995).

One of the advantages of microdialysis sampling is that, once a molecule crosses the dialysis membrane, no enzymatic degradation or protein binding can occur, and that tissue can be directly sampled without significant tissue fluid loss (i.e. suction blister). In addition the molecules are finally isolated in the buffer solution and consequently no extraction is needed.

Microdialysis has been used for more than 20 years in experimental brain research in animals, and more recently in peripheral tissues. Its basic principle is to mimic the function of a capillary blood vessel by perfusing a thin semipermeable dialysis tube (Fig. 1) implanted in the tissue. A constant flow through the probe creates a concentration gradient along the membrane so the compounds diffuse through it from the interstitial fluid to the perfusate or from the perfusate to the interstitial fluid. The outlet perfusate is collected in microtubes. The principal diffusion parameter is expressed as the relative recovery (RR) which is the ratio of two concentrations: one from the dialysate to that of the medium surrounding the probe.

5-Methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP) and trimethylpsoralen (TMP) (Fig. 2) are usually used to treat skin diseases such as psoriasis and vitiligo. The aim of this study was to perform an in vitro calibration, in respect of 5-MOP, 8-MOP and TMP. The calibration parameters were: the perfusion rate, the RR, the equilibrium time and the detection limits (Groth, 1996). This technique was also used to study the effects of the lipophilicity on the RR.

2. Materials and methods

2.1. Microdialysis system

The microdialysis system consisted of a CMA/100® syringe pump (Phymep, Paris, France), which delivered Sorensen phosphate buffer (0.06 M, pH 7.4) and a CMA/140® microfraction collector. The microdialysis probes (CMA/20®) had a 20 kDa cut off with a polycarbonate membrane, or a 100 kDa cut off with a polyethylenesulfone membrane (10 mm length).

2.2. In vitro experiments

Three probes were placed in a vial containing a Sorensen phosphate buffer solution of 1 μg/ml of each psoralen. Samples were analyzed at flow rates of 1, 2, 3, 5 and 7.5 μl/min. They were collected at these flow rates every 30 min for a period of 5 h. Each experiment was repeated four times at the perfusion speed of 3 μl/min. An unpaired nonparametric Mann–Whitney two sample test was used to study the effect of molecule lipophilicity and the influence of the membrane.

2.3. Quantitative assessment of psoralens

The concentration of 5-MOP, 8-MOP and TMP in the dialysate was determined by high performance liquid chromatography (Stolk, 1980), with a fluorimetric detection (λ emission 314 nm, λ excitation 490 nm). The mobile phase was of

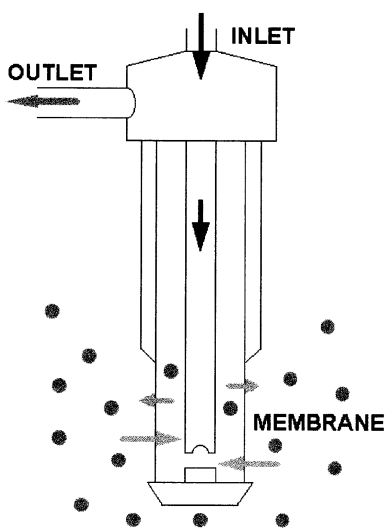
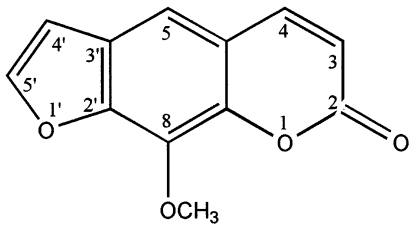


Fig. 1. Microdialysis probe.



5-Methoxypsoralen

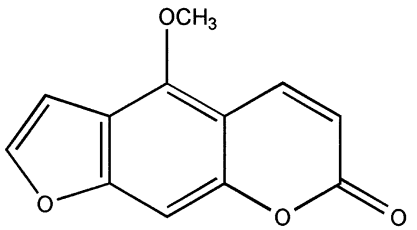
molecular weight: 216 g/mol

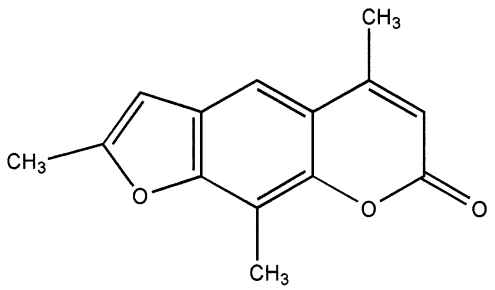
logKo/w = 2.00 ± 0.01 (mean ± s.d.)

8-Methoxypsoralen

molecular weight: 216 g/mol

logKo/w = 1.93 ± 0.01 (mean ± s.d.)





Trimethylpsoralen

molecular weight: 228 g/mol

logKo/w = 3.14 ± 0.01 (mean ± s.d.)

Fig. 2. Formula for the three psoralens used in PUVA therapy.

methanol–water (60/40 v/v) and the column was a LiChrospher® 100 RP-18 (5 μm) in Lichro-CART® 125-4 (Merck, Darmstadt, Germany).

3. Results

The in vitro RR from 1 μg/ml solutions of 5-MOP, 8-MOP and TMP are represented in Fig. 3. TMP was not recovered in the microdialysates, whatever the optimal conditions adopted. There was a decrease in the 8-MOP RR (from 40.9 to 11%) and in the 5-MOP RR (from 21.3 to 5.2%) with increasing perfusion rates (from 1 to 7.5 μl/min) when using the classical membranes with a cut off at 20 kDa (Table 1). For the two molecules, the equilibrium time increased (from 0.5 to 3 h), while the perfusion rate decreased (Table 1). At the optimal perfusion rate of 3 μl/min, the 5-MOP RR 21.3 $\pm$ 1.1% (mean $\pm$ S.D.) was 32% less than the 8-MOP RR 31.2 $\pm$ 1% when 20 kDa membranes were used ($p < 0.0005$) (Fig. 4a). The 5-MOP RR 23.6 $\pm$ 0.4% was 17% less than the 8-MOP RR 28.4 $\pm$ 0.6% when 100 kDa membranes were used ($p < 0.0005$) (Fig. 4b). When the effect of the chemical nature of the membranes was tested, there was no significant differences of RR: the 8-MOP RR and the 5-MOP RR were slightly influenced by the 20 or 100 kDa membranes ($p = 0.031$ for 8-MOP and $p = 0.026$ for 5-MOP).

4. Discussion

It is admitted that the quantity of a compound recovered by microdialysis is only a fraction of the quantity present in the tissue. The results are expressed by the RR. This RR is dependent on several parameters: perfusion rate, membrane nature, temperature, physicochemical properties of

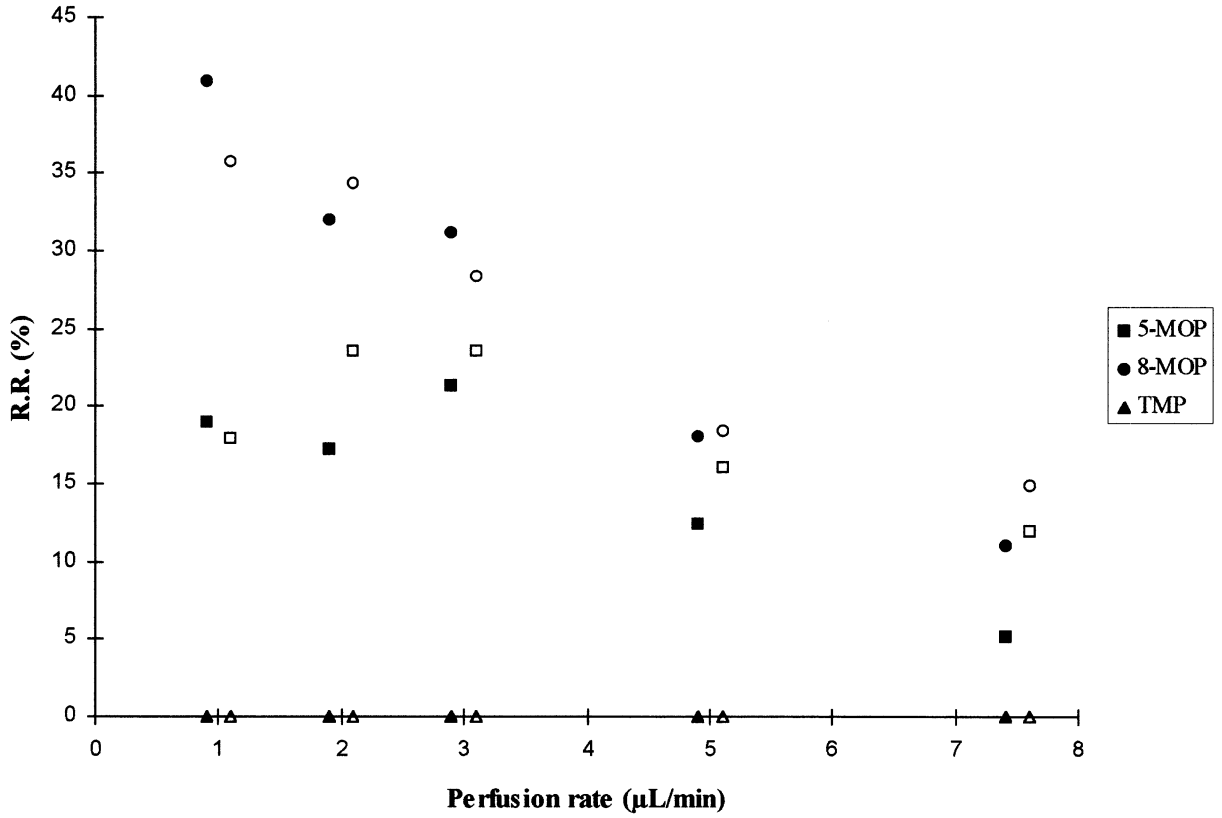


Fig. 3. Evolution of the psoralen RR in relation to the chemical nature of the membrane (20 kDa membranes, open) (100 kDa membranes, filled) and the perfusion rates. According to the two tailed nonparametric test of Mann–Whitney, with a significance probability $P < 0.005$, there is significant differences between the RR of the three psoralens, and these RR were not significantly changed when the effect of the chemical nature of the membrane was tested.

the compound studied, etc. Thus, experiments need to be performed in vitro in order to determine the exact conditions for collecting the maximal fractions of the molecules.

The optimal perfusion rate must be a compromise between a speedy equilibrium setting on the probes and obtaining a sufficient RR. In our study, the optimal experimental conditions were obtained with a perfusion rate of 3 μl/min, after an equilibrium time of 30 min associated with a sufficient RR of 31.2 ± 1 for the 8-MOP, and $21.3 \pm 1.1\%$ for the 5-MOP (Table 1). This relation between flow rate and RR is in accordance with other studies: Ault et al. (1992) found this tendency for 5-fluorouracil. Le Quellec et al. (1995) results show an RR inversely related to the perfusion rate for a non-indolic melatonin analog (S20098).

The highest lipophilic compound (TMP), could not be detected by microdialysis under our experimental conditions. Studies conducted by a number of authors have shown similar problems such as drug-material binding and drug solubility which were provoked by the lipophilic nature of the compound, as was demonstrated by Groth (1996) with calcipotriol. Drug-membrane binding could be avoided by adding 0.2–0.5% of albumin to prevent adhesion to the membrane material, tubes and vials of sticky compounds (Petersen, 1995). However, estradiol (solubility in water 1/10 000) was shown to be dialysable only in small amounts in vitro, even with the addition of albumin to the perfusion medium (Müller et al., 1995). Currently, the major limitations of the microdialysis technique are: the low recovery for molecules

Table 1
In vitro RR for 1 μg/ml 5-MOP, 8-MOP and TMP solutions

Perfusion rate (μl/min)	T_{eq} (min)	RR (%) (mean ± S.D.) 8-MOP		5-MOP		TMP	
		20 kDa	100 kDa	20kDa	100 kDa	20 kDa	100 kDa
1	180	40.9 ± 1.8	35.8 ± 2	19 ± 1.9	18 ± 3	<1	<1
2	150	32 ± 0.9	35.7 ± 1.4	17.3 ± 1.2	23.5 ± 1.5	<1	<1
3*	120	31.2 ± 1	28.4 ± 0.6	21.3 ± 1.1	23.6 ± 0.4	<1	<1
5	60	18.1 ± 1	18.4 ± 0.4	12.5 ± 0.6	15.8 ± 0.3	<1	<1
7.5	30	11 ± 0.5	14.8 ± 1.1	5.2 ± 0.3	12 ± 1	<1	<1

Samples were collected every 30 min, for 5 h, at flow rates of 1, 2, 3, 5 and 7.5 μl/min ($n = 3$ or $n^* = 10$).
There is an inverse correlation between the perfusion rate and the RR.

having high atomic weight (> 20 kDa), high lipophilicity and/or high protein binding.

As has been suggested by Groth (1996), further investigation is needed to obtain higher recoveries. The studies could include alternative perfusion media, new tubing and membranes of different materials and/or with larger cut off values. In this work, the use of two types of membranes did not show any significant difference for the psoralen RR. Using a polycarbonate membrane (20 kDa) instead of a polyethylenesulfone membrane (100 kDa) did not significatively increase the psoralen RR: at the optimal perfusion speed of 3 μl/min, the 8-MOP R.R. varied from $31.2 \pm 1\%$ to $28.4 \pm 0.6\%$ ($p = 0.031$) and the 5-MOP RR from $21.3 \pm 1.1\%$ to $23.6 \pm °.4\%$ ($p = 0.026$). It is difficult to draw any conclusions from such results to the extent that there are two parameters which need to be taken into account: the material composition and the membrane cut off. It seems, however, that the chemistry of both the membrane and the studied compound constitutes the main factor influencing the recovery, even more than the physical structure of the membrane (cut off, preparation). The polyethylene sulfone membrane, viewed under a scanning electron microscope, showed circular cavities which seemed to be prepared by submitting a thin polymeric film to a large flux of high energy particles and then to chemical attack, in contrast to the polycarbonate membrane which appeared to be prepared by an inversion phase procedure where the polymer was dissolved in an appropriate solvent and then precipitated by cooling or by adding a non-solvent. Despite the differences in structure of the two types of membranes, the behaviour of the three psoralens by microdialysis was the same. This phenomenon could be due mainly to chemical interactions between the membranes and the psoralens. Buttler et al. (1996) tested different types of membranes for the microdialysis of alcohols and carbohydrates. Despite the weak molecular weight of the alcohols, the RR of these compounds with a 100 kDa polysulfone membrane was low (5% for the glycerol) These authors suggested an interaction between the hydroxy groups of the analytes and the chemical groups on the membrane surface. They concluded that the polycarbonate membrane was acceptable for all their experiments, whereas polyamide was more suitable for carbohydrates than for alcohols. Considering our results and these authors experiments, it is important to emphasis that for each compound studied by in vitro microdialysis, the effect of the membrane needs to be taken into account, especially for lipophilic compounds.

Microdialysis gave satisfactory results for slightly lipophilic compounds such as 5-MOP or 8-MOP (Fig. 3). Despite the small lipophilicity differences between the 5-MOP ($\log P = 2$) and 8-MOP ($\log P = 1.93$) (Saïd et al., 1996), the 8-MOP RR was twice that of the 5-MOP value, whatever the perfusion rate used. For all the experiments, there was an inverse correlation between the RR and drug lipophilicity. These results

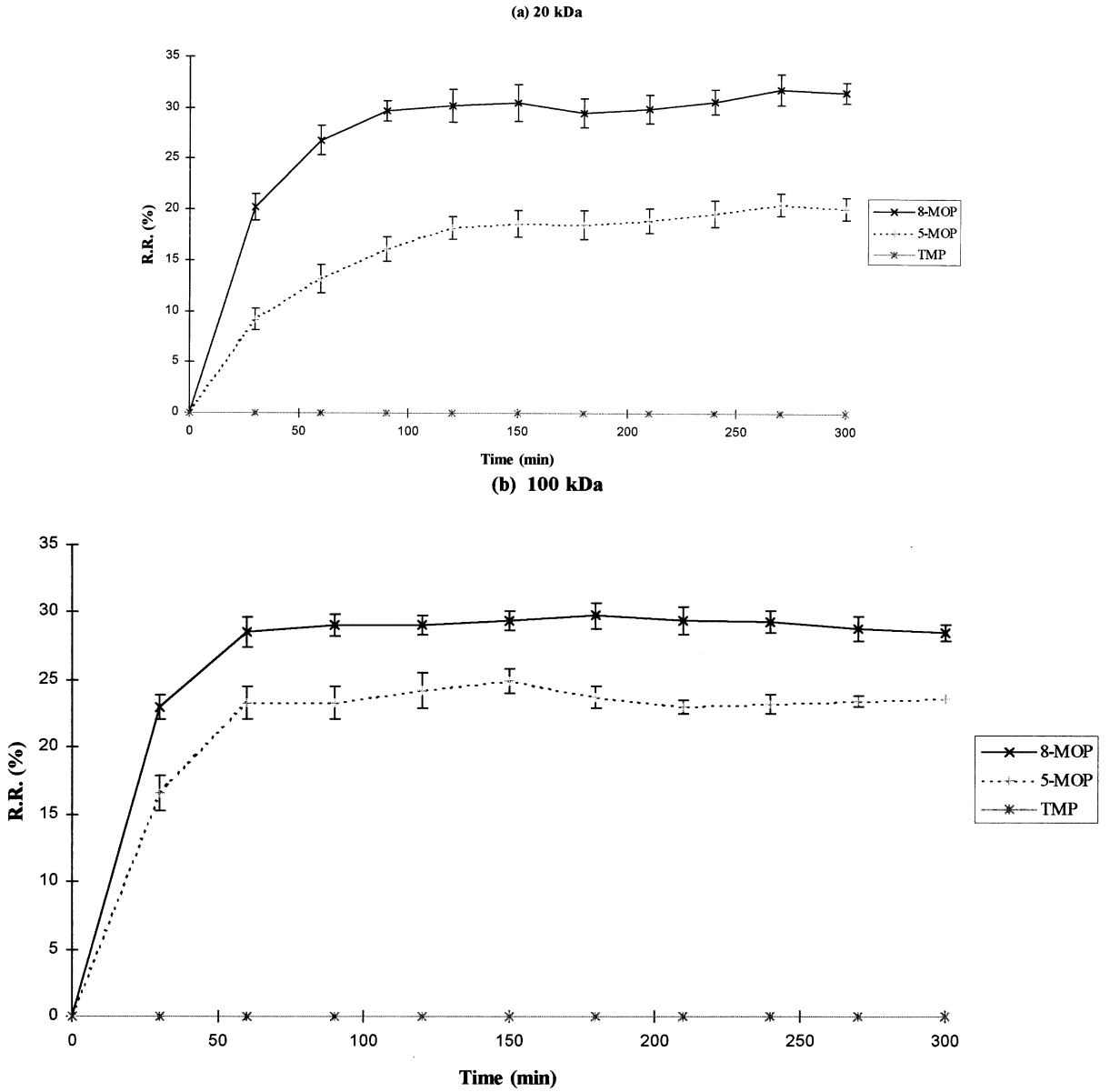


Fig. 4. RR of psoralens at 3 μl/min. A 10 mm membrane probe was used with a cut off of 20 kDa (a) or 100 kDa (b). Each value is represented by the mean $\pm$ S.D. ($n = 10$).

are in agreement with those of Groth (1996) concerning glucose ($\log P << 1$, RR $= 74.65 \pm 0.99\%$), sodium fusidate ($\log P = 2.68$, RR $= 40.99 \pm 5.57\%$) and βmethasone dipropionate ($\log P = 3.24$, RR $= 36.4 \pm 4.05\%$). These compounds do not belong to the same chemical family and a comparison of the results could be critical. This was not the case for psoralens which have the same basic chemical structure (8-MOP: $\log P = 1.93$, RR $= 31.2 \pm 1\%$; 5-MOP: $\log P = 2.00$, RR $= 21.3 \pm 1.1\%$; TMP: $\log P = 3.15$, RR $= 0\%$). This difference in the behaviour of psoralens, despite their similar characteristics, was in agreement with previous in vitro experiments

performed in our laboratory, concerning percutaneous absorption: the 8-MOP, the least lipophilic, was penetrated the most, and the TMP, the most lipophilic molecule, was penetrated the least (Makki et al., 1996).

Our in vitro results seem to be promising for future in vivo dermatological investigations. They demonstrate that microdialysis provides a powerful tool for kinetic analysis of psoralens with a low to medium degree of lipophilicity.

References

Ault, J.M., Lunte, C.E., Meltzer, N.M., Riley, C.M., 1992. Microdialysis sampling for the investigation of dermal drug transport. Pharm. Res. 9, 1256–1261.

Buttler, T., Nilsson, C., Gorton, L., Marko-Varga, G., Laurell, T., 1996. Membrane characterization and performance of microdialysis probes intended for use as bioprocess sampling units. J. Chromatogr. A 725, 41–56.

Groth, L., 1996. Cutaneous microdialysis: methodology and validation. Acta Derm. Venereol. (Stockholm) 76, 197.

Kiistala, U., Mustakallio, K.K., 1967. Dermo-epidermal separation with suction. J. Invest. Dermatol. 48, 466–477.

Le Quellec, A., Dupin, S., Genissel, P., Saivin, S., Marchand, B., Houin, J.P.M., 1995. Microdialysis probes calibration: gradient and tissue dependent changes in no net flux and reverse dialysis methods. J. Pharmacol. Toxicol. Methods 33, 11–16.

Makki, S., Muret, P., Said, A., Bassignot, P., Humbert, P., Agache, P., Millet, J., 1996. Percutaneous absorption of three psoralens commonly used in therapy: effect of skin occlusion (in vitro study). Int. J. Pharmaceut. 133, 245–252.

Müller, M., Schmid, R., Wagner, O., Osten, B.V., Shayganfar, H., Heichler, H.G., 1995. In vivo characterization of transdermal drug transport by microdialysis. J. Control. Release 37, 49–57.

Petersen L.J.,1995. Skin microdialysis. In: Marks, R., Payne, A. (Eds.), Bioengineering and the Skin. CRC, Boca Raton, 641–649.

Said, A., Makki, S., Muret, P., Rouland, J.C., Toubin, G., Millet, J., 1996. Lipophilicity determination of psoralens used through solubility and partitioning: comparison of theoretical and experimental approaches. J. Pharm. Sci. 85, 387–392.

Stolk, L.M.L., 1980. Pharm. Weekbl. 2, 281–284.

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