Department of Pharmacy[1], the First Affiliated Hospital of China Medical University; School of Pharmacy[2], China Medical University, Shenyang, P. R. China; Department of Basic Pharmaceutical Sciences[3], School of Pharmacy, Husson University, Bangor, USA

Microdialysis as a tool to determine the skin concentration of mometason furoate in rats

Qizhen Gao[1,2], Yanan Zhao[2], Jiankun Yu[2], Tianzhi Yang[3], Zhaolu Zhu[2], Pingtian Ding[2]

Received February 27, 2014, accepted June 13, 2014

Qizhen Gao, Department of Pharmacy, the First Affiliated Hospital of China Medical University, 155 Nanjing Street, Shenyang, P. R. China
shiyin@qq.com

Pharmazie 69: 787–791 (2014) *doi: 10.1691/ph.2014.4036*

The objective of this study was to investigate the feasibility of microdialysis as a tool to determine the skin concentration of mometason furoate (MF), a lipophilic and highly protein-bound compound. The relative recovery (RR) of mometasone furoate was determined by an *in vitro* no-net-flux method using three different perfusates (40% PEG400, 5% fat emulsion, and 20% fat emulsion) and four flow rates (0.5, 1, 2, and 4 $\mu L \cdot min^{-1}$). With the increasing of flow rate, the relative recovery was decreased from 48.8% to 3.1%. The *in vitro* recovery was increased to 23.71%, 42.76% and 56.21% when 40%PEG400, 5% fat emulsion or 20% fat emulsion was used as microdialysis perfusates, respectively. Fat emulsion (5%) was chosen as the perfusate to evaluate the *in vivo* recovery by a retrodialysis method, in which mometasone furoate concentration in different tissues was determined. The result showed that concentrations of mometasone furoate in the dermis was greater than that in the subcutaneous or muscle tissue. It was concluded that a recovery enhancer could be used in microdialysis technique, especially for determining skin concentrations of lipophilic and high protein-bounds.

1. Introduction

Treatment of skin disease requires the efficacy of drugs at the target site of action for patients. Therefore, determination of intradermal drug concentration is a critical issue in both formulation design process, and clinical trial phases. To date most of transdermal penetration data have been obtained from diffusion cells methods, tissue homogenate, suction blister or *in vivo* tape stripping method. These traditional methods in skin research are invasive and involve many animals in the study. In addition, there are other drawbacks associated with these traditional methods, such as many individual differences, and lack of true information from continuous measurements (Surber et al. 1993). Cutaneous microdialysis is a sampling technique developed from traditional microdialysis methods and used to study stratum corneum barrier function and pharmacokinetics of drugs in many sampling sites (Morgan et al. 2003). The method has undergone a significant development and been proved to be a safe, versatile and valuable tool in numerous researches. Meanwhile, it makes monitoring the concentration of drugs in skin possible.

However, due to the low molecule weight cut-off and the non-specific binding between drugs and probe membranes, the applicability of microdialysis has some limitations on sampling lipophilic, protein-bound and high-molecular drugs (Groth et al. 1997; Holmgaard et al. 2010). Microdialysis perfusates are usually aqueous solutions. The composition, pH, osmotic pressure, and ionic strength of microdialysis perfusates should be closer to the environment of sampling sites. Traditional perfusates are always accompanied by poor solubility for the aqueous perfusates, which results in low or no recovery in experiments (Holmgaard et al. 2012).

In this study, we chose mometasone furoate as a model drug, which is a highly potent synthetic chlorinated glucocorticoid with high oil/water partition coefficient (Log P = 3.9) (Wang et al. 2008). The highly lipophilic property of mometasone furoate causes the low recovery of the drug, so it could not be detected in *in vivo* experiments. We explored the feasibility of improving the microdialysis recovery by using recovery enhancer, such as PEG400 and fat emulsion, and chose a suitable recovery-enhancing perfusate to study the tissue distribution of mometasone furoate in rat model.

2. Investigations and results

2.1. In vitro solubility and relative recovery of mometasone furoate

The result showed that the equilibrium solubility of mometasone furoate in 40%PEG400, 5% fat emulsion or 20% fat emulsion was significantly increased compared to that in Ringer's solution (Table). It was indicated that either recovery or delivery in 5% fat emulsion was significantly higher than that in 40%PEG400 (Fig. 1, $p < 0.01$). Due to the larger distribution coefficient and lower membrane-binding rate of mometasone furoate in 20% fat emulsion, drug recovery was significantly higher than the drug delivery in 20% fat emulsion (Fig. 1, $p < 0.05$). However, the recovery of mometasone furoate was similar to the delivery of the drug (Fig. 1, $p > 0.05$) in 5% fat

Table 1: Solubility of mometasone furoate in different perfusates

Perfusates	Solubility ($\mu g \cdot mL^{-1}$)				
	2 h	4 h	8 h	10 h	12 h
Ringer's solution	0.026	0.056	0.063	0.068	0.077
40% PEG400	25.79	37.02	35.52	39.8	40.02
5% fat emulsion	27.85	33.35	36.55	41.25	39.64
20% fat emulsion	31.12	54.73	74.75	78.72	76.53

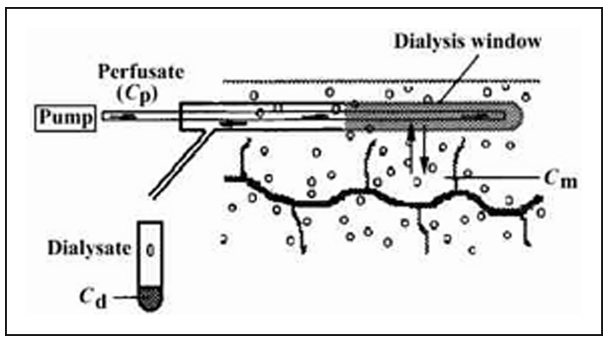

Fig. 1: Schematic diagram of microdialysis.

emulsion. Therefore, 5% fat emulsion was selected as the perfusate to evaluate the drug recovery in the following *in vivo* experiment.

It was observed that the *in vitro* mometasone furoate recovery was decreased with the increasing of flow rate (Fig. 2). This was mainly because the drug across the probe membrane is always maintained at a dynamic diffusion state. Increasing the flow rate made the equilibrium more difficult to achieve, which resulted in the lower recovery. When the flow rate was changed from 0.5 to 1.0 $\mu L \cdot min^{-1}$, the decline of recovery was not significant (Fig. 2, $p > 0.05$). It was reported previously that drug binding to microdialysis tube devices was relatively weakened due to higher flow rate infusion and shorter corresponding duration (Araujo et al. 2008). A flow rate of 1.0 μL•min-1 was chosen in this study in order to collect sufficient amounts of the dialysates for further analysis.

2.2. In vivo relative recovery and tissue distribution studies of mometasone furoate

In vivo microdialysis experiments were carried out in a rat model. The results showed that the relative recoveries of

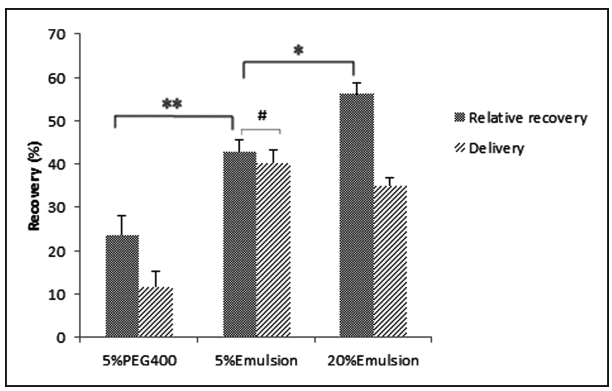

Fig. 2: *In vitro* relative recovery and delivery of mometasone furoate using different microdialysis perfusates with the flow rate of 1.0 μL•min^{-1} (n = 3). ** Very significant difference, * Significant difference, # No significant difference.

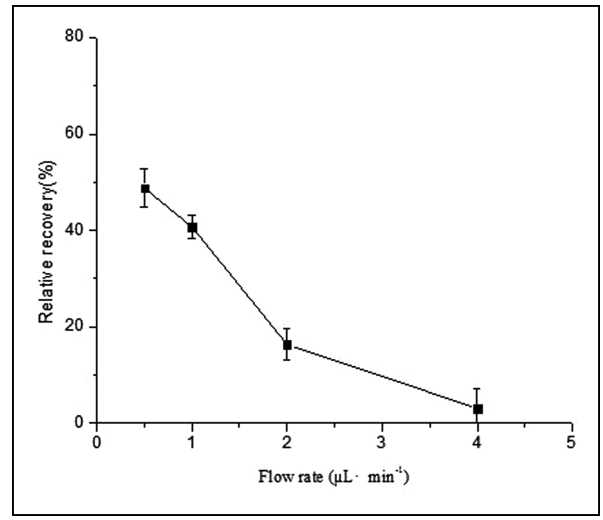

Fig. 3: The relationship between microdialysis flow rate and relative recovery (n = 3). The 5% fat emulsion was used as the perfusate and the probe was immersed in a 0.4 μg•mL^{-1} MF solution.

mometasone furoate in different tissues were significantly reduced compared to the data obtained from *in vitro* studies the recoveries of probes in different tissues were significantly reduced compared to *in vitro* (Fig. 3). There are two major reasons contributing to the reduction. First, mometasone furoate binding with tissue proteins was restricted by biological membranes, which made the detection of the drug incomplete. Second, due to the different resistance of drug diffusion in tissue medium, increased curvature in sampling sites and smaller diffusion space, the diffusion of drug through the dialysis membrane was declined and the recovery was decreased *in vivo* (Wang et al. 2008). In addition, several other factors need to be taken into consideration, such as the metabolism, other active reactions that the analytes may undergo, eluting effect caused by local perfusion, convection factor and perfusate loss across highly permeable probes caused by the altered osmotic pressure in the area around the probe (Nelson et al. 1998).

The pharmacokinetic profiles of mometasone furoate in different rat tissues after 10 h continuous administration are displayed in Fig. 4. The mometasone furoate concentrations in dermis were much higher than those in subcutaneous tissue or muscle, with a maximum concentration of 0.28 μg•mL-1 and were maintained at a steady state until 10 h. This indicated that the drug might provide a major local therapeutic effect, especially at dermis site.

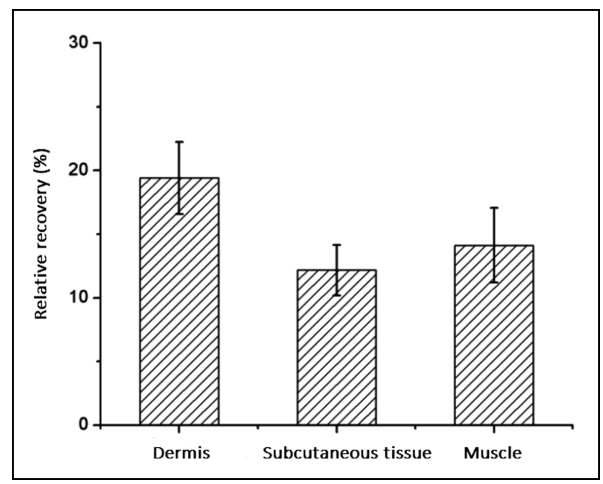

Fig. 4: Relative recovery of mometasone furoate in dermis, subcutaneous and muscle tissue in rats (n = 3). The probes were perfused with 5% fat emulsion, at the flow rate of 1 μL•min^{-1}.

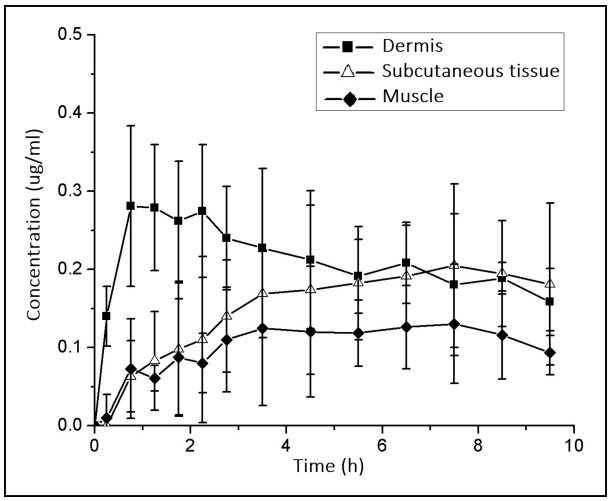

Fig. 5: Concentrations of mometasone furoate in dermis, subcutaneous and muscle tissue in rat skin with the dose of about 0.2 mg/cm^2. (n = 3).

Furthermore, the drug could penetrate into the deeper tissues over time, after being rapidly absorbed by blood capillaries in the dermis.

3. Discussion

Compared to the conventional sampling techniques, microdialysis has the following advantages: (1) determine the concentration of drug in the target tissues directly; (2) reduce the experimental error and tissue damage; (3) use self-control to avoid the individual differences. In our study, the observed maximal concentration of drug in dermis by microdialysis ($C_{max} = 0.2802\ \mu g \cdot mL^{-1}$) was significantly lower than the measured maximal concentration in skin by tissue homogenate sampling method ($C_{max} = 0.6217\ \mu g \cdot mL^{-1}$). This was mainly because the latter concentration included free drug, protein binding drug as well as some drug in subcutaneous tissue. The drug concentration measured by microdialysis technique only reflected the existing free drug format; therefore there is a better correlation between pharmacokinetics and pharmacodynamic data when microdialysis technique is applied.

Microdialysis technique used in the skin pharmacokinetic study however has its own limitations. First, large molecular compounds can be intercepted by small pore of dialysis membrane. Second, bindings to the membrane and probe tube for lipophilic compounds, which hinder the microdialysis sampling. Recently, some alternatives have been proposed in the literature to overcome the limitations and allow the application of microdialysis for lipophilic compounds, including pH adjustment of the perfusate (Tre et al. 2012), adding some enhancers like glycerin, albumin (William et al. 2003), cyclodextrin (Khramov et al. 1999) and physiological lipid emulsions (Ward et al. 2003) into the perfusion fluid, which either prevent drug binding to the probe membrane or facilitate diffusion of the free analyte in perfusate.

In addition, quantitative analysis of high protein binding drugs and endogenous proteins by using of polymer dialysis membrane (with large pores) has been reported (Schnetz et al. 2001). The improved microdialysis technique can provide a broad development prospect for larger and more lipophilic molecules. In the study, we developed a new microdialysis method with long-chain fat emulsion as recovery enhancer for the determination of lipophilic substance. The solubility of mometasone furoate was indeed increased as compared to the Ringer's solution by solubilization effects of the fatty acid glycerides and amphiphilic lecithin (Chen et al. 2004), which consequently increased the concentration gradient across the membrane and improved the recovery. But it is a compromise between improving solubility and maintaining physiologically compatible conditions in order to prevent damages around skin tissues. We suggest that the new MD method may be a feasible approach to overcome the current recovery limitations to the use of microdialysis for lipophilic drug. Accurate determination of relative probe recovery is a major issue in conducting traditional microdialysis research to calculate the extracellular concentrations (Ward et al. 2003). So one of the key areas need to be addressed with this approach is the issue of probe calibration in the presence of recovery enhancers such as fat emulsion.

In our study, the recovery and delivery of mometasone furoate are not always similar in various perfusates. The phenomenon has been demonstrated for high protein binding and moderately or highly lipophilic drugs such as betamethasone dipropionate, calcipotriol, and sodium phenytoin (Groth et al. 1997) in many researches recently. The *in vivo* relative recovery could be decreased by increasing perfusate lipophilicity and has a linear relationship with the oil-water partition coefficient to a certain extent (Kurosaki et al. 1998). It is probably due to the poor solubility and low distribution coefficient of the drug in physiological solution, making it difficult to reach diffusive equilibrium. Meanwhile, the dissipation outwards of drug from the probe is higher than the diffusion dissipation from the surrounding solution to the membrane (Araujo et al. 2008), which leads to higher relative recovery than delivery as shown in our study. As a consequence, whether the recovery and delivery are similar or not, it should be validated before *in vivo* retrodialysis test.

4. Experimental

4.1. Materials

4.1.1. Chemicals and microdialysis system

Mometasone furoate (content, 99.67%) was purchased from Shanghai Xinhua Lianbang Pharmaceutical Company. Normal saline (NS) and mometasone furoate cream (0.1%) were provided by Shenyang Shengyuan Pharmaceutical Company. Fat emulsion containing purified soybean oil (0.1 g/ml), medium-chain triglycerides (0.1 g/ml), purified egg yolk lecithin (0.012 g/ml), and glycerin (0.025 g/ml) was obtained as a sterile 20% emulsion from Huarui Pharmaceutical Company. All other reagents and materials were of analytical grade. The microdialysis system consisted of a MD pump with a micro syringe (Kaikai Instrument Company, Shanghai) and LM-10 probes (BAS, America) with outside diameter of 0.32 mm, and membrane length of 10 mm. The probes of microdialysis system were prepared according to the manufacturer's instructions before using.

4.1.2. Animals

Wistar rats (male, 200 ± 20 g) were provided by the Shenyang Pharmaceutical University Animal Experiment Center.

4.2. Chromatographic system and condition

A high-performance liquid chromatograph (HPLC) method was used to measure mometasone furoate concentration. The HPLC system consisted of an L-7110 pump, and L-7420 photodiode-array UV/VIS spectrophotometric detector (Hitachi, Japan). The analytical column used was a Diamonsil-C8 column (200 mm × 4.6 mm, 5 μm particle size, Dikma Technologies, Beijing, China), connected with a C_8 pre-column (4 mm × 3.0 mm). The mobile phase consisted of methanol and water (75:25, v/v), and was run at a flow rate of 1.0 ml/min. UV detection wave was at 254 nm.

4.3. In vitro microdialysis studies

In vitro microdialysis experiments were carried out to investigate the effect of different perfusate and flow rate on the relative recovery by a no- net-flux method.

Under a given perfusion rate, the concentration of the drug cannot achieve absolute equilibrium between the extracellular fluid (C_m) and the perfusate (C_p) because of the continuous flux of perfusate (Araujo et al. 2008; Bungay et al 1990). The ratio between the concentration of dialysate (C_d) and the

concentration of extracellular fluid is referred to a relative recovery (RR), which is an important parameter to describe the efficacy of microdialysis, and has been used for calibration *in vitro* and *in vivo* studies (Fig. 1).

$$RR = \frac{C_d - C_p}{C_m - C_p} \quad (1)$$

When the concentration of drug in the medium surrounding the probe is greater than the concentration in the perfusate, drug molecules will travel along the concentration gradient and perfuse into the probe; conversely, drug molecules will access to the medium around the probe, for which the relative recovery is often called delivery. It can be defined by the following equation:

$$\text{Delivery} = \frac{C_d - C_p}{C_p} \quad (2)$$

Drug solutions with series of concentrations, which were higher or lower than the extracellular drug concentration were prepared and perfused into the probe. Set the concentration of drug in perfusate as abscissa, the variation of drug concentration before and after perfusion as the vertical axis for linear regression, and the intersection of the horizontal axis is considered to be the medium concentrations. Therefore, the slope of the line above horizontal axis is considered as the relative recovery; and the slope of the line below horizontal axis is the delivery. According to the assumption that diffusions across the probe membrane are quantitatively equal in both directions, retrodialysis has been widely used in *in vivo* microdialysis calibration.

4.3.1. In vitro solubility determination

The equilibrium solubility of MF was evaluated in Ringer's solution, 40%PEG400 (mixture of PEG400 and normal saline in a ratio of 2:3), 5% fat emulsion (mixture of 20% fat emulsion and 20% fat emulsion and normal saline in a ratio of 1:3) and 5% fat emultion. Mometasone furoate was suspended in 30 mL of different medium, and then stirred at 200 rpm constantly untill a steady-state concentration was attained. The supernatant was filtered with a nylon membrane filter (0.45 μm) and analyzed using the HPLC method described before.

4.3.2. Effect of different perfusates on relative recovery

Microdialysis probes were perfused with 40%PEG400, 5% fat emultion and 20% fat emulsion containing various concentrations of mometasone furoate (0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 $\mu g \cdot mL^{-1}$) at a flow rate of 1.0 $\mu L \cdot min^{-1}$, meanwhile the probe was immersed in a 0.4 $\mu g \cdot mL^{-1}$ mometasone furoate solution. All *in vitro* studies were conducted at $37 \pm 1\,^{\circ}C$, using a heating magnetic stirrer (85-2A, Jiangsu Jintan Instrument Company). The systems was stirred at 300 rpm, in order to reduce solution boundary layer effects and convection (Le et al. 1995), and was allowed to equilibrate for 40 min until mometasone furoate could be detected. Samples were collected once every 30 min from the dialysis system. The collected emulsion dialysates were treated with acetonitrile at a ratio of 1:1 to break down the emulsion, then centrifuged at 15000 rpm for 20 min (LeiBoEr, Beijing) (Tre et al. 2012). The supernatant (20 μL) was analyzed with the HPLC method as described before. The collected samples from 40% PEG400 were analyzed directly without treatment.

4.3.3. Influence of perfusion flow rate on in vitro recovery rate

With 5% fat emulsion as the perfusate, the recoveries of mometasone furoate were evaluated at different flow rates (0.5, 1.0, 2.0, and 4.0 $\mu L \cdot mL^{-1}$). A 0.4 $\mu g \cdot mL^{-1}$ mometasone furoate was used in the microdialysis experiment to determine the recovery rate.

4.4. In vivo ***microdialysis studies***

4.4.1. Relative recovery of momentasone furoate in different tissues

Based on the result from the *in vitro* studies, 5% fat emulsion was selected as the perfusate to evaluate *in vivo* mometasone furoate recovery by a retrodialysis method, Mometasone furoate concentration was used to calculate relative recovery, since the delivery could be represented by recovery in *in vivo* studies (Ding et al. 2000).

$$Cm = Cd/RR \quad (3)$$

The rats were anesthetized with 20% urethane solution (i.p., 6.5 mL/kg) and shaved the abdominal hair by Oster electric clipper (Oster Model 5-01). The rats were fixed on the rat bed.

Under visual inspection, each rat was implanted with three microdialysis probes in its dermis, subcutaneous and muscle tissue respectively. Immediately after probe implantation, perfusion (1 $\mu L \cdot min^{-1}$) was initiated with normal saline for 1.5 h to relieve skin irritation. Then the probes were perfused with 5% fat emulsion containing different concentrations of mometasone furoate (0.05, 0.1, 0.2, 0.4, 0.8, and 1.0 $\mu g \cdot mL^{-1}$). The systems were allowed to equilibrate for 40 min until mometasone furoate was detected. The samples were collected at 30 min intervals for 10 h and submitted to HPLC as described before.

4.4.2. Distribution of mometasone furoate in different tissues

After the microdialysis probe was implanted and washed out, normal saline was used to replace 5% fat emulsion as the perfusate, and equilibrated for 1 h. Then the rats received mometasone furoate creams (mometasone furoate dose 0.2 mg/cm^2) on the skin area (5 × 1.5 cm) just above the dialysis membrane. Occlusion covering with dressing tapes was maintained for 10 h. Samples were collected at 30 min intervals within 10 h, and then analyzed by HPLC.

4.5. Data analysis

Data were expressed by mean ± standard deviation (SD). The data of *in vitro* solubility, *in vitro* relative recovery, *in vitro* delivery, *in vivo* relative recovery and mometasone furoate *in vivo* concentrations were compared by analysis of variance. Differences were considered statistically significant at p-values less than 0.05 on a two-tail test.

References

Araujo BV, Silva CF, Haas SE, Dalla Costa T (2008) Microdialysis as a tool to determine free kidney levels of voriconazole in rodents: A model to study the technique feasibility for a moderately lipophilic drug. J Pharm Biomed 47: 876–881.

Bungay PM, Morrison PF, Dedrick RL (1990) Stead -statet heory for qua ntitativemicrodialysis of solutes and water *in vivo* and *in vitro*. Life Sci 46: 105–119.

Chen T, Hui M (2004) Research development of fat emulsion preparation. Digest of the World Latest Medical Information 3: 1295–1298.

Ding P, Xu H, Zheng J (2000) Microdialysis sampling coupled to HPLC for transdermal delivery study of ondansetron hydrochloride in rats. Biomed Chromatogr 14: 141–143.

Groth L, Jùrgensen A (1997) *In vitro* microdialysis of hydrophilic and lipophilic compounds. ANAL Chim Acta 355: 75–83.

Holmgaard R, Benfeldt E, Nielsen JB, Gatschelhofer C, Sorensen JA, Hoefferer C, Bodenlenz M, Pieber T R, Sinner F (2012) Comparison of open-flow microperfusion and microdialysis methodologies when sampling topically applied fentanyl and benzoic acid in human dermis ex *vivo*. Pharm Res 29: 1808–1820.

Holmgaard R, Nielsen JB, Benfeldt E (2010) Microdialysis sampling for investigations of bioavailability and bioequivalence of topically administered drugs: Current state and future perspectives. Skin Pharmacol Phys 23: 225–243.

Khramov AN, Stenken JA (1999) Enhanced microdialysis extraction efficiency of ibuprofen *in vitro* by facilitated transport with β-cyclodextrin. Anal Chem 71: 1257–1264.

Kurosaki Y, Nakamura S, Shiojiri Y, Kawasaki, H (1998) Lipomicrodialysis: A new microdialysis method for strdying the pharmacokinetics of lipophilic substances. Biol Pharm Bull 21: 194–196.

Le QA, Dupin S, Genissel P, Saivin S, Marchand B, Houin, G (1995) Microdialysis probes calibration: Gradient and tissue dependent changes in no net flux and reverse dialysis methods. J Pharmacol Toxicol 33: 11–16.

Morgan CJ, Renwick AG, Friedmann PS (2003) The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. Brit J Dermatol 148: 434–443.

Nelson T, Thomas L, Lo G, Gyorgy MV (1998) Recent trends in the application of microdialysis in bioprocesses. Anal Chim Acta 374: 111–135.

Schnetz E, Fartasch M (2001) Microdialysis for the evaluation of penetration through the human skin barrier - a promising tool for future research. Eur J Pharm Sci 12: 165–174.

Surber C, Wilhelm KP, Bermann D, Maibach, HI (1993) *In vivo* skin penetration of acitretin in volunteers using three sampling techniques. Pharm Res 10: 1291–1294.

Tre ES, Patel C, Aghara S, Yadav C, Stagni, G (2012) Optimization of perfusate ph to improve microdialysis recovery of lipophilic compounds. J Pharmacol Toxicol 66: 276–280.

Wang D, Shi L, Hu J (2008) Impact factors on microdialysis probe recovery: research development. International J Pharm Res 4: 261–266.

Wang H, Aslanian R, Madison VS (2008) Induced-fit docking of mometasone furoate and further evidence for glucocorticoid receptor 17α pocket flexibility. J Mol Graph Model 27: 512–521.

Ward KW, Medina SJ, Portelli ST, Doan KM, Spengler MD, Ben MM, Lundberg David, Levy MA, Chen EP (2003) Enhancement of *in vitro* and *in vivo* microdialysis recovery of sb-265123 using intralipid and encapsin as perfusates. Biopharm Drug Dispos 24: 17–25.

William J. T, Donald W. M (2003) Use of osmotic agents in microdialysis studies to improve the recovery of macromolecules. J Pharm Sci-US 92: 1419–1427.