International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:9, No:7, 2015

Effect of Different Model Drugs on the Properties of Model Membranes from Fishes

M. Kumpugdee-Vollrath, T. G. D. Phu, M. Helmis

***Abstract*—**A suitable model membrane to study the pharmacological effect of pharmaceutical products is *human stratum corneum* because this layer of human skin is the outermost layer and it is an important barrier to be passed through. Other model membranes which were also used are for example skins from pig, mouse, reptile or fish. We are interested in fish skins in this project. The advantages of the fish skins are, that they can be obtained from the supermarket or fish shop. However, the fish skins should be freshly prepared and used directly without storage. In order to understand the effect of different model drugs e.g. lidocaine HCl, resveratrol, paracetamol, ibuprofen, acetyl salicylic acid on the properties of the model membrane from various types of fishes e.g. trout, salmon, cod, plaice permeation tests were performed and differential scanning calorimetry was applied.

***Keywords*—**Fish skin, model membrane, permeation, DSC, lidocaine HCl, resveratrol, paracetamol, ibuprofen, acetyl salicylic acid.

I. Introduction

SKINS from pig or mouse are much more difficult to obtain. Moreover, it is not necessary to wait for the shedding process of the snake. It was reported that some fish skins can be used as a model membrane instead of the human stratum corneum [1]-[5]. Therefore, this project focused on studying different kinds of fish skins which can be obtained in Germany e.g. trout, salmon, cod, plaice in order to use them as a model membrane for testing of pharmaceutical products. For this purpose, different model drugs i.e. lidocaine HCl, resveratrol, paracetamol, ibuprofen, acetyl salicylic acid were applied. Two techniques i.e. the permeation tests as well as the differential scanning calorimetry (DSC) were used to characterize the samples. The effects of the drugs will be shown in this article.

II. Materials and Methods

Different kinds of fishes were purchased from the local supermarket. The outer layer of fish skin was separated from its meat by a knife. Different drugs were purchased and used without treatment. The DSC technique was performed with DSC7, Perkin Elmer, USA. The samples were measured in a range from 30°C to 300°C with a heating rate of 10K/min. The permeation tests were performed with Franz diffusion cell as demonstrated in Fig. 1 (SES, Germany). The concentration of drug was measured by UV-VIS spectroscopy (Jasco V 630, Germany). The Flux "*J*" and the permeation coefficient "k_p" can be calculated by (1) and (2), respectively;

$$J = \frac{\Delta m}{\Delta t \cdot A} = \frac{D \cdot K \cdot C_D}{d} \quad (1)$$

$$k_p = \frac{D \cdot K}{d} = \frac{J}{C_D} \quad (2)$$

J = Flux, Δm = permeated drug in the time interval of Δt, D = diffusion coefficient, K = partition coefficient, C_D = concentration in the donor, A = diffusion area, d = membrane thickness, k_p = permeability coefficient.

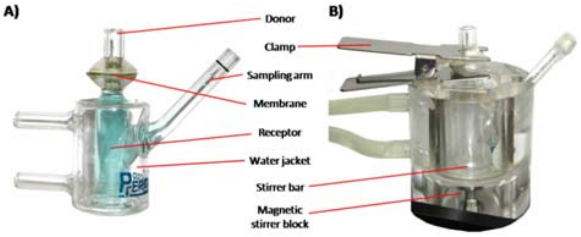

Fig. 1 Franz Diffusion Cell (A) cell outside thermostat and (B) cell with stirrer and jacket connected to the thermostat

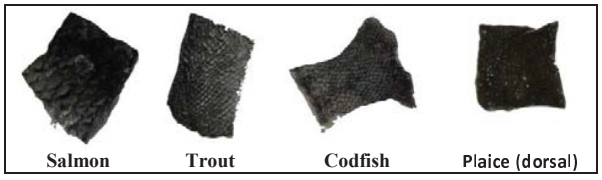

Fig. 2 Skins of salmon, trout, codfish and plaice

III. Results and Discussion

A. Calibration of Model Drugs

The fit of the calibration curve of lidocaine HCl with the equation "y = 1.5488x - 0.001" for lidocaine HCl soluble in mixture of de-ionized water and ethanol (1:1) showed a good correlation coefficient of $R^2 = 0.9999$. On the other hand, the equation for the drug resveratrol was " y = 0.1383x - 0.0008" with $R^2 = 0.9998$. The slope was used to determine the lidocaine concentration after permeation tests.

Mont Kumpugdee-Vollrath is professor at Beuth Hochschule für Technik Berlin - University of Applied Sciences, Faculty of Mathematics-Physics-Chemistry, Pharmaceutical and Chemical Engineering, Luxemburger Str. 10, D-13353 Berlin, Germany (phone: (+4930) 4504-2239; Fax: (+4930) 4504-2813; e-mail: vollrath@beuth-hochschule.de)

Tuyen Gila Duong Phu was a former Bachelor student and Mr. Mario Helmis is a scientific associate at Beuth Hochschule für Technik Berlin - University of Applied Sciences, Faculty of Mathematics-Physics-Chemistry, Pharmaceutical and Chemical Engineering, Luxemburger Str. 10, D-13353 Berlin, Germany.

B. Thickness of Different Fish Skins

The data of skin thickness measurements are shown in Tables I, II. The suitable fish skin for using as model membrane should have similar thickness as that of human stratum corneum.

TABLE I
THICKNESSES OF FISH SKINS USED FOR PERMEATION TESTS

No.	Thickness of skins with					
	Lidocain-HCl				Resveratrol	
	Salmon	Bio-Salmon	Trout	Codfish	Trout	Codfish
	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
1	0.634	0.678	0.442	0.607	0.321	0.478
2	0.697	0.604	0.364	0.495	0.298	0.487
3	0.633	0.701	0.309	0.598	0.282	0.452
4	0.569	0.708	0.375	0.563	0.319	0.535
5	0.543	0.704	0.362	0.490	0.420	0.477
6	0.590	0.706	0.353	0.760	0.332	0.481

TABLE II
AVERAGE THICKNESS OF ALL MEASURED FISH SKINS

Skins	Salmon	Bio-Salmon	Trout	Cod	Plaice (dorsal)	Plaice (ventral)	SC
	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]	[mm]
AT	0.580	0.684	0.342	0.478	0.431	0.573	0.619
SD	0.061	0.040	0.034	0.087	0.090	0.111	0.147
MI	0.016	0.017	0.007	0.018	0.032	0.039	0.085

SC = Stratum corneum, AT = average of thickness, SD = standard deviation, MI = measurement inaccuracy

C. Permeation Test Trough Different Skins

The permeation data were shown in Figs. 3-14. The cumulative of mass per area of each drug as well as lagtime was calculated.

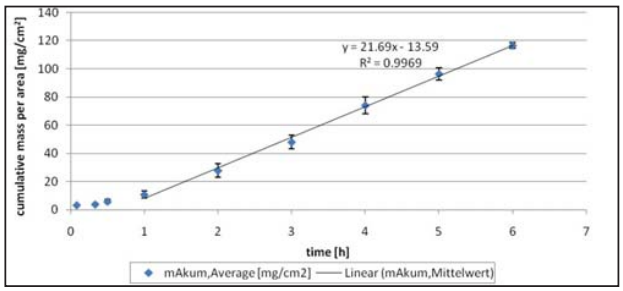

Fig. 3 Concentration of Lidocaine-HCl in acceptor chamber (n=3) tested on salmon

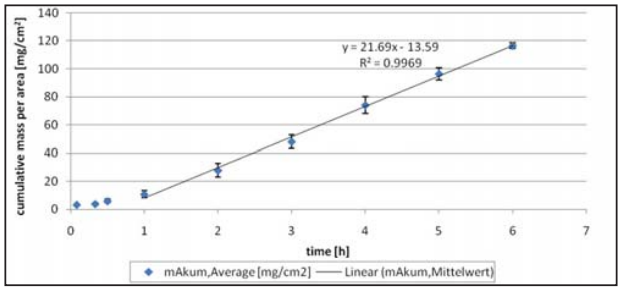

Fig. 4 Cumulative mass of Lidocaine-HCl per area of salmon skin against time (n=3)

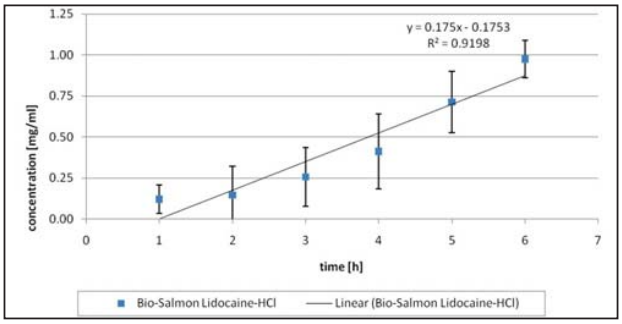

Fig. 5 Concentration of Lidocaine-HCl in acceptor chamber (n=3) tested on bio-salmon

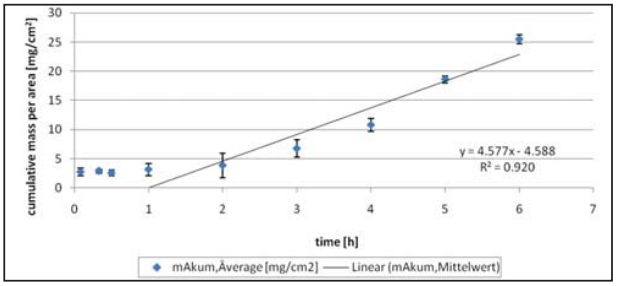

Fig. 6 Cumulative mass of Lidocaine-HCl per area of bio-salmon skin against time (n=3)

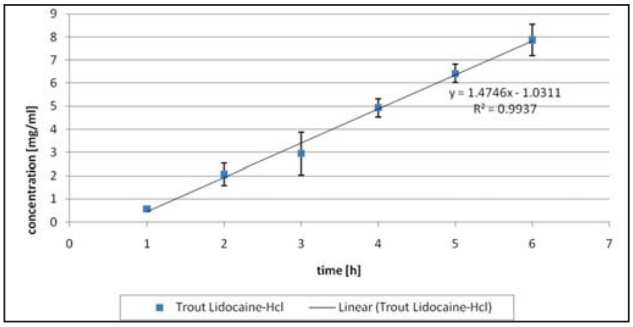

Fig. 7 Concentration of Lidocaine-HCl in acceptor chamber (n=5) tested on trout

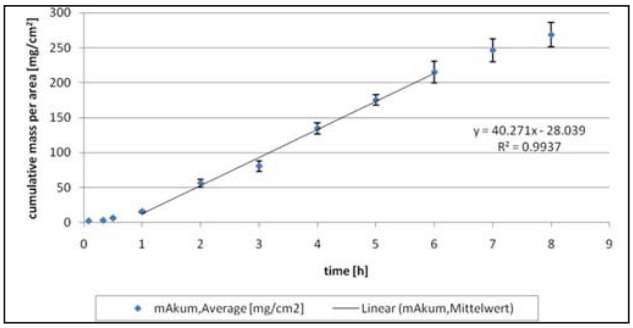

Fig. 8 Cumulative mass of Lidocaine-HCl per area of trout skin against time (n=5)

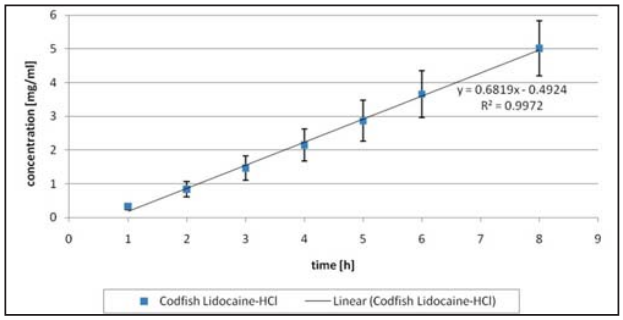

Fig. 9 Concentration of Lidocaine-HCl in acceptor chamber (n=3) tested on codfish

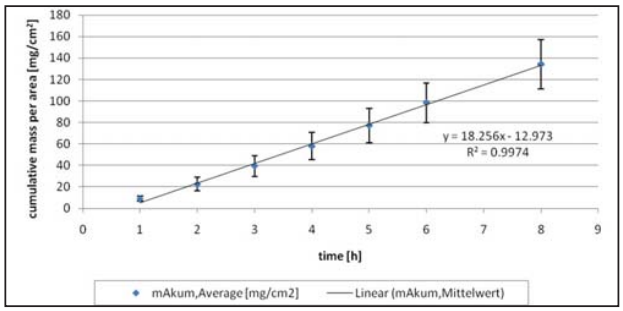

Fig. 10 Cumulative mass of Lidocaine-HCl per area of codfish skin against time (n=3)

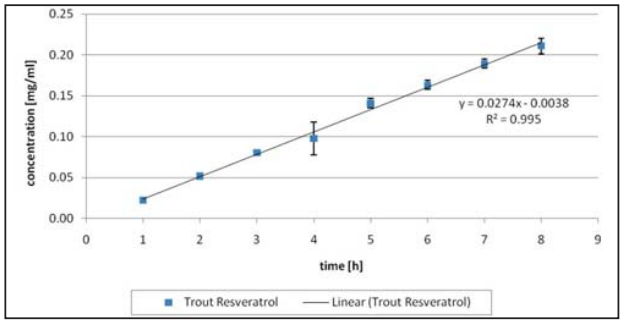

Fig. 11 Concentration of resveratrol in acceptor chamber (n=4) tested on trout

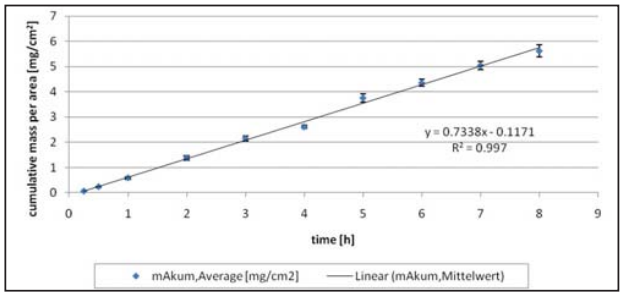

Fig. 12 Cumulative mass of resveratrol per area of trout skin against time (n=4)

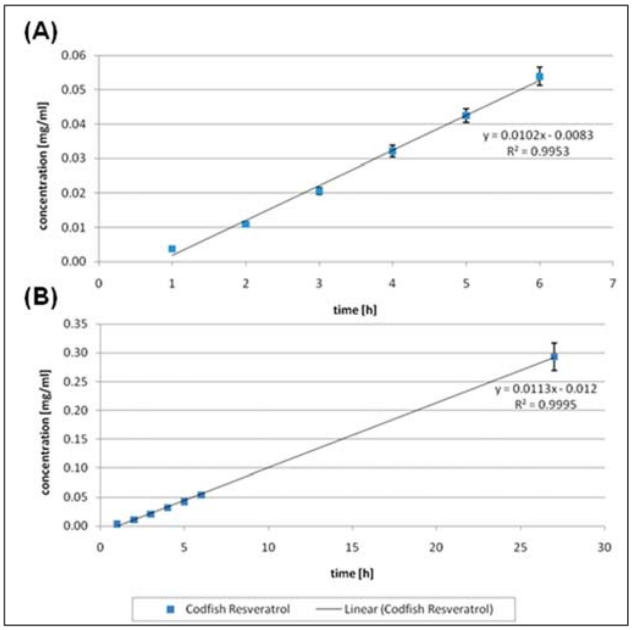

Fig. 13 Concentration of resveratrol in acceptor chamber (n=3) tested on codfish until (A) 6h and (B) 27h

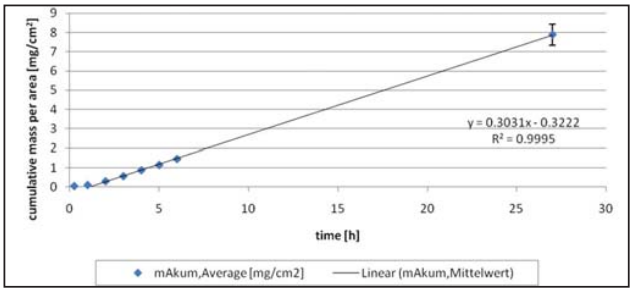

Fig. 14 Cumulative mass of resveratrol per area of codfish skin against time (n=3)

The results of permeation studies are summarized in Table III.

D.DSC

The DSC data were shown in Figs. 15-20. The calculated values were shown in Table IV.

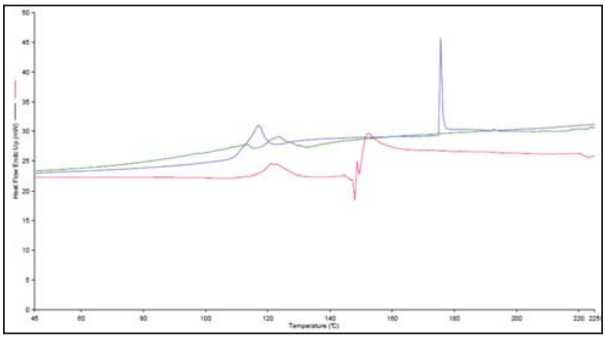

Fig. 15 DSC Thermogram of plaice dorsal side without drug

TABLE III
RESULTS OF PERMEATION STUDIES

Drug / Fish	Lidocain-HCl				Resveratrol		
Value	Salmon	Bio-Salmon	Trout	Codfish	Trout	Codfish (6 h)	Codfish (27 h)
$J\left[\frac{mg}{cm^2 \cdot h}\right]$	21.69	4.58	40.48	18.26	0.74	0.3	0.3
$u\left[\frac{mg}{cm^2 \cdot h}\right]$	0.44	0.25	1.40	17.55	0.02	0.01	0.01
$k_P\left[\frac{cm}{h}\right]$	2.73E-02	5.76E-03	5.09E-02	2.30E-02	3.72E-02	1.51E-02	1.51E-02
$u_{kp}\left[\frac{cm}{h}\right]$	6.11E-04	3.17E-04	1.83E-03	2.21E-02	8.49E-04	5.27E-04	5.27E-04
t_{lag} [min]	38	60	42	43	5	31	53
$c_{p,6h}$ [%]	0.4	0.1	1.1	0.6	0.7	0.3	0.3

With Flux J, inaccuracy u, which is standard deviation divided with the square of number of testings u ($u = \left[\frac{s}{\sqrt{n}}\right]$), permeability coefficient k_p, lag time t_{lag}, which describes the drug release after a certain time and $c_{p,6h}$ the amount of drug diffused after 6 h, assuming donor concentration c_D as 100%.

TABLE IV
SUMMARY OF DSC-RESULTS

Drug	Temp [°C]	Plaice	Salmon	Trout	Codfish	SC
no	T o	115.4+2	125.4+1	145.8+1	160.9+7	130.6+12
	T p	120.4+2	130.4+0	149.7+1	162.9+6	146.2+7
ASS	T o	147.6+13	185.7			
	T p	154.3+7	189.5			
Ibu	T o	131.4+6	130.7+7			
	T p	135.5+6	134.0+8			
Para	T o	122.5+2	135.3+2	169.5+1	169.3+0	167.7+1
	T p	125.6+1	139.1+1	171.1+1	170.7+0	169.0+1
Lido	T o			77.1		
	T p			80.5		

ASS = acetyl salicylic acid, Ibu = ibuprofen, Para = paracetamol, Lido = lidocaine HCl, SC = stratum corneum, To = T_{onset}, Tp = T_{peak}

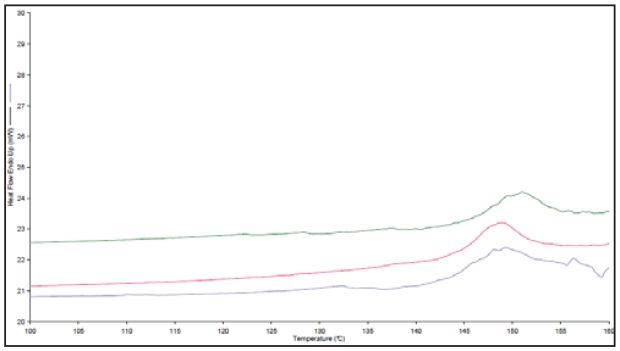

Fig. 16 DSC Thermogram of trout without drug

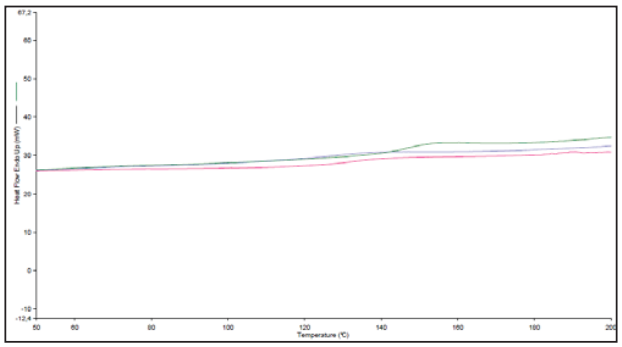

Fig. 17 DSC Thermogram of human stratum corneum without drug

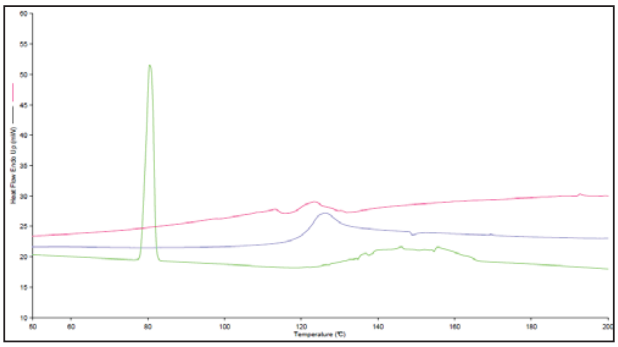

Fig. 18 DSC Thermogram of (green) ibuprofen (red) plaice dorsal (blue) plaice + ibuprofen

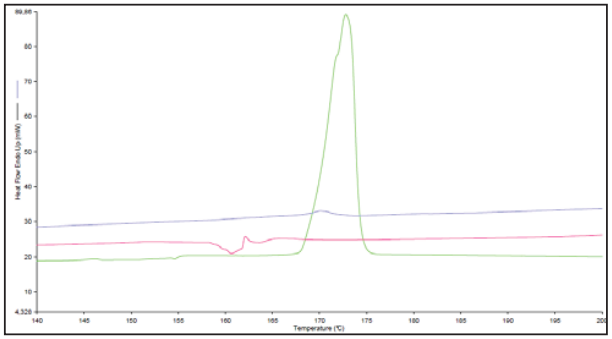

Fig. 19 DSC Thermogram of (green) paracetamol (red) codfish (blue) codfish + paracetamol

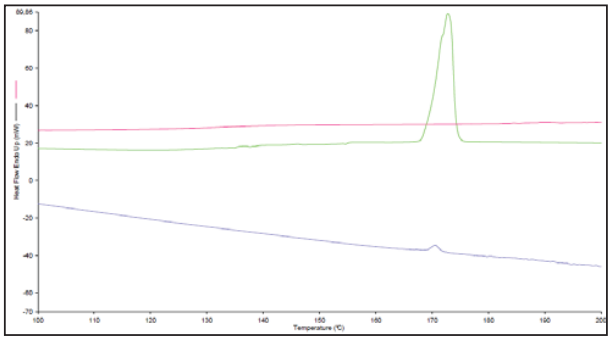

Fig. 20 DSC Thermogram of (green) paracetamol (red) human stratum corneum (blue) human stratum corneum + paracetamol

The DSC results show that the DSC measurement with plaice (Fig. 15) did not give in reproducible data. However, the measurement with trout or human stratum corneum results in better data. Some drugs cannot penetrate into the skins e.g. ibuprofen; some drugs, however, can well penetrate e.g. paracetamol.

IV. Summary

The results show that the skin of trout has the highest permeability. This skin shows a permeability coefficient (k_P) of ($5.09 \times 10^{-2} \pm 1.83 \times 10^{-3}$) cm/h for lidocaine HCl and ($3.72 \times 10^{-2} \pm 8.49 \times 10^{-4}$) cm/h for resveratrol. The permeability coefficient for trout is in both cases twice as large as that for codfish. Furthermore, the skin of bio-salmon has the smallest permeability. Compared to the salmon from the conventional breeding the permeability is smaller by the factor 5. In general, it can be summarized that the thicker the skin, the lower the permeability. In addition, skins from the lateral organ usually have the lowest permeability than other part of the fish. The DSC measurements of the untreated fish skins show no reproducible results. Among different drugs used in this experiment i.e. acetyl salicylic acid, ibuprofen, lidocaine HCl and paracetamol, paracetamol is the one with the most reproducible results. Paracetamol can diffuse better into the cells of the stratum corneum and of the skins from fishes than the other drugs. The DSC-studies show a characteristic peak (phase transition) for paracetamol in the skins of trout, cod and human stratum corneum. This means that if the effect of drug paracetamol was studied, the use of skins from trout or cod instead of human stratum corneum will be suitable.

Acknowledgment

The authors would like to thank Prof. Dr. H. Hungerbühler and Mr. E. Leo for DSC-experiments; Ms. L. Mehr for the introduction of permeation experiments. Thanks are also given to the Zentrales Innovationsprogramm Mittelstand (ZIM) for the financial support. Thanks are also given to Ms. Z. Kazemi for experimental help.

References

[1] M. Masson, S.D. Sigfusson, T. Loftsson "Fish Skin as a Model Membrane to Study Transmembrane Drug Delivery with Cyclodextrins", *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, vol. 44, issue 1-4, pp 177-182, 2002.

[2] F. Konradsdottir, T. Loftsson, S. D. Sigfusson "Fish skin as a model membrane: structure and characteristics", *Journal of Pharmacy and Pharmacology*, vol. 61, issue 1, pp 121-124, 2009.

[3] M. Kumpugdee-Vollrath "Determination of secondary nanostructure of model membrane from local fishes for application in pharmaceutical field", *Acta Physica Polonica A*, accepted for 2015.

[4] M. Kumpugdee-Vollrath, T. Subongkot, T. Ngawhirunpat "Model Membrane from Shed Snake Skins", *World Academy of Science, Engineering and Technology*, vol. 82, pp 1140-1147, 2013.

[5] N. Wonglertnirant, T. Ngawhirunpat, M. Kumpugdee-Vollrath "Evaluation of skin enhancing mechanism of surfactants on the biomembrane from shed snake skin", *Biological & Pharmaceutical Bulletin*, vol. 35, issue 4, pp 1-9, 2012.

Prof. Dr. Mont Kumpugdee-Vollrath was born in Bangkok/Thailand. She was graduated Bachelor of Science in Pharmacy in 1993, Faculty of Pharmacy, Mahidol University, Thailand, Master of Sciences in Pharmacy in 1996 from Mahidol University in collaboration with the Institute of Pharmacy, Christian-Albrechts-University of Kiel, Germany, and the Doctoral Degree in Natural Sciences in 2002 from Institute of Pharmacy, Faculty of Chemistry, University of Hamburg, Germany. 2002-2005 Scientist at GKSS Research Center, Germany. 2004-2005 Part-time lecturer at the Technical University Carolo-Wilhelmina of Braunschweig, Germany. Since 2005 Professor of Pharmaceutical Technology at Beuth Hochschule für Technik Berlin - University of Applied Sciences, Berlin, Germany. Since 2007 Head of Laboratory Chemical and Pharmaceutical Technology. She is reviewer for many journals and funding programs.