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RESEARCH ARTICLE

Diffusion properties of different compounds across various synthetic membranes using Franz-type diffusion cells

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Abstract Diffusion properties of typical functional cosmetic ingredients, niacinamide, ascorbic acid 2-glucoside, retinol and polyethoxylated retinamide, were evaluated across various synthetic membranes using Franz-type diffusion cells. Different kinds of artificial membranes available in the market were utilized for comparing how the functional ingredients diffuse through the membrane. Niacinamide and ascorbic acid 2-glucoside were resulted out similar diffusion pattern. On the other hands, retinol and polyethoxylated retinamide showed similar diffusion pattern. This might be due to their hydrophilic properties. Moreover, physicochemical properties of the membranes, pore size, and/or membrane thickness need to be considered as well. Solvents and composition of the donor and receptor compartment are the factors to investigate in detail to obtain systematic information regarding the diffusion properties. The results might help when to evaluate and analyze topical formulations in a faster and reproducible way.

Keywords Diffusion · Franz cell · Synthetic membrane · Niacinamide · Ascorbic acid 2-glucoside · Retinol · Polyethoxylated retinamide

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Introduction

When an active ingredient is applied topically as ointments or cosmetics, it must be released from the vehicle before it reaches the epidermal surface and be available for penetration into the stratum corneum and hence lower layers of the skin (Shah et al. 1999). In order to get information how a compound will interact with and permeate through the skin, the most relevant data needs to be obtained from in vivo in human (Feldmann and Maibach 1967). However, the procedures are expensive and time-consuming. Moreover, there is a wide biological variability and must meet with ethical approval (Hadgraft and Riduot 1987).

Alternatively, Franz-type diffusion cell with animal skin or a synthetic membrane can be utilized as it is not only a simple and relatively reproducible method but also cost/time effective (Guy and Hadgraft 1990). Even though this method has limited applicability for estimating the complex process of percutaneous absorption or skin permeation, it is useful as a screening tool for drug release (Smith and Haigh 1989; Clement et al. 2000). Moreover, there are significant advantages at using the synthetic membranes in Franz diffusion cell studies, simulation of the skin and quality control (Twist and Zats 1988; Twist and Zatz 1986; Corbo et al. 1993). The membranes which have hydrophobic property and possess rate-limiting property can simulate skin (Twist and Zatz 1986; Pellett et al. 1997). Synthetic membranes for quality control should have minimum diffusion resistance to compounds and only act as a support to separate the formulation from the receptor medium (Siewert et al. 2003; Shah et al. 1999; Ueda et al. 2009). However, it is still challenging to select which membrane is useful for a compound to replace animal test and also for the purpose of quality control since it depends

on a compound's physicochemical properties and the relationship between a membrane and a compound as well.

Two hydrophilic and two hydrophobic model compounds were selected for the diffusion study to evaluate the synthetic membranes with Franz cells: niacinamide and ascorbic acid 2-glucoside as hydrophilic ones and retinol and polyethoxylated retinamide as hydrophobic ones. They are functional compounds noticed by KFDA (Korea Food and Drug Administration). Niacinamide and ascorbic acid 2-glucoside have whitening effect and retinol is extensively used in functional cosmetics as its anti-wrinkle effect. Polyethoxylated retinamide, developed by LG Household and Healthcare Ltd., is also a functional compound possessing anti-wrinkle. This study evaluates the influence of different synthetic membranes on the release of the four active model compounds. The main goal is to compare the release pattern between different membranes with various properties and model compounds with different hydrophilicity to acquire information when to evaluate and develop topical and functional cosmetic formulations.

Materials and methods

Materials

The model active compounds, niacinamide, retinol, and ascorbic acid 2-glucoside were obtained from DSM NV (TE Heerlen, Netherlands), BASF (Ludwigshafen, Germany), and Hayashibara (Okayama, Japan), respectively. Polyethoxylated retinamide was kindly donated from LG Household and Heathcare Ltd. (Daejeon, Korea). Methanol and ethanol of HPLC grades were purchased from Honeywell Burdick & Jackson (NJ, USA). All other reagents were of analytical reagent grade and used as received.

Synthetic membranes

The synthetic membranes which are commonly used for filtration and also commercially available were selected for the study. Their basic properties and product information were given in Table 1. Even though there are a lot of membranes in the market, membranes which are easy to obtain and relatively cheaper ones were purchased and they were divided into two groups either cellulosic or polymeric (Ng et al. 2010). Cellulose-based membranes may include cellulose acetate, cellulose ester, and/or cellulose nitrate. Polymer-based membranes are polycarbonate, polytetrafluoroethylene, polytetrafluoroethylene, polyethersulfone, and/or polysulfone.

Diffusion of active compounds is certainly dependent on the membrane components and the pore size of the membrane as well. In order to compare the effects of pore size on diffusion, different sizes of membranes were also included as shown in Table 1 (Advantec 0.2/0.45 and NL16/NL17). The membrane should have such properties as inert holding surface for the test solution, but not a barrier. Moreover, there is no physicochemical interaction between the membrane and the test solution (Thakker and Chern 2003). The synthetic membranes that contain pores act as 'continuous' medium of the receptor medium (Ng et al. 2010).

In vitro diffusion with membranes

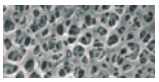
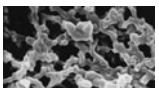
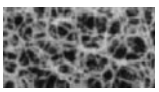
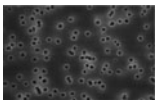
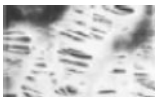
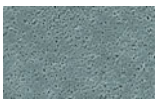
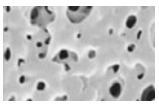
In vitro diffusion studies were carried out using Franz-type diffusion cells with a receptor compartment volume of 12.6 mL and an effective diffusion area of 1.82 cm^2. Since some membranes had different size to others, they were trimmed into circular discs (diameter 25 mm) and all the membranes were soaked in receptor medium for 30 min before each experiment. For donor compartment, ascorbic acid 2-glucoside and niacinamide were dissolved in water. On the other hand, retinol and polyethoxylated retinamide were dissolved in ethanol with the concentration of 0.2 %. PBS (phosphate buffered saline, pH 7.4) was used as the receptor medium in case of ascorbic acid 2-glucoside and niacinamide. On the other hand, 60 % ethanol was used as the receptor medium for retinol and polyethoxylated retinamide to maintain skin condition in the receptor compartment. The receptor was continuously stirred with a magnetic bar at 480 rpm and thermostated at 32 ± 0.5 °C with circulating jacket. At predetermined time intervals (0.5, 1, 2, 4, and 6 h), samples were withdrawn from the receptor compartment and replaced with an equal volume (0.5 mL) of fresh buffer. The content of the model active compounds in each sample was analyzed by HPLC. The cumulative amounts released of the compound are plotted against time.

Chromatographic conditions and method validation

Analytical experiments were carried out using a HPLC system (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA, USA). A C18 reverse phase column was used and maintained at approximately 30 °C. Each analytical sample was collected in Eppendorf tubes, centrifuged at 12,000 rpm for 1.5 min, and then 10 μL (30 μL in case of polyethoxylated retinamide) of each sample's supernatant was injected into the HPLC. The chromatographic condition of each compound was provided in Table 2.

The linearity of the calibration curve was obtained by plotting the nominal concentration of the active compound (y) versus the peak area (x) in the tested concentration range. The intra-day accuracy and precision of the method were determined by analyzing three samples six times on

Table 1 Basic properties of the synthetic membranes with their product information

Membrane	Material	Pore size (μm)	Thickness (μm)	Source	Batch no.	Image
Cellulose-based						
Advantec	Cellulose acetate	0.2	125	Advantec	81216306	
Advantec	Cellulose acetate	0.45	125	Advantec	90401306	
Metricel	Mixed cellulose esters	0.45	152	Pall	T10896	–
MF	Cellulose acetate/nitrate mixtures	0.45	150	Millipore	R0PA12528	
MRC RC	Regenerated cellulose	0.45	160	Chm	0800243	
S-Pak	Mixed cellulose esters	0.45	–	Millipore	F0KA39876	–
Polymeric-based						
Isopore	Isopore track-etched polycarbonate	0.4	7–22	Millipore	R1DA59364	
MTF PTEF	Polytetrafluoroethylene	0.45	65–100	Chm	0904460	
NL16	Polyamide	0.2	110	Whatman	9156262	
NL17	Polyamide	0.45	110	Whatman	9063230	
Supor	Polyethersulfone	0.2	145	Pall	T03752	–
Tuffryn	Polysulfone	0.45	145	Pall	T91318	

the same day. The analytical assay was also performed repeatedly on three consecutive days to validate the inter-day accuracy and precision. The accuracy is expressed as a percentage by comparing the mean measured active compound concentration to the nominal concentration in the sample. The precision was calculated using the relative standard deviation (RSD) of the measured each compound concentrations in each sample.

Table 2 Chromatographic conditions to analyze the model ingredients in this study

Model ingredients	Solvent	Mobile phase	Detector (UV) (nm)	Flow rate (mL/min)	Column
Ascorbic acid 2-glucoside	H_2O	0.2 % TFA	245	0.8	Phenomenex C18, 5 μm, 4.6*150 mm
Niacinamide	MeOH	100 % MeOH	263	0.8	
Retinol	EtOH	MeOH:H_2O (90:10)	325	1.0	
Polyethoxylated retinamide	MeOH	H_2O:acetonitrile (25:75)	350	2.0	XDB-C18, 3.5 μm, 4.6*50 mm

TFA trifluoroacetic acid

Calculation of the cumulative amount released

The cumulative amount (Q) of the active compound released per surface area of membrane was obtained using the following equation (Thakker and Chern 2003):

$$Q = \left\{ C_n V + \sum_{i=1}^{n-1} C_i S \right\} / A$$

where Q is the cumulative amount of the compound released per surface area of the membrane (μg/cm^2), C_n is the concentration of the compound (μg/mL) determined at nth sampling interval. V is the volume of individual Franz diffusion cell and $\sum_{i=1}^{n-1} C_i$ is the sum of concentrations of the compound (μg/mL) determined at sampling intervals 1 through n − 1. S is the volume of sampling aliquot (0.5 mL) and A is the surface area of sample well. In this study, the surface area was 1.82 cm^2.

Results and discussion

Validation of the analytical methods

The samples showed decent symmetrical peaks. The calibration curves of each model compound showed good linearity ($R^2 \geq 0.999$) in the tested ranges (Table 3). The limit of detection (LOD) and limit of quantification (LOQ) of the compounds are following: ascorbic acid 2-glucoside (1.757 and 5.325 μg/mL), niacinamide (0.880 and 2.667 μg/mL), retinol (0.026 and 0.122 μg/mL), and polyethoxylated retinamide (0.453 and 1.371 μg/mL).

Table 3 summarizes the intra- and inter-day precision/accuracy at low, medium, and high active compounds' concentrations. The intra- and inter-day precisions of the ingredients were more than 98 %, and the intra-and inter-day accuracies ranged less than 6 %.

In vitro diffusion through synthetic membranes

The cumulative amount (Q) of the each compound released per unit surface area of each membrane was shown in Fig. 1. In case of relatively hydrophilic compounds such as niacinamide and ascorbic acid 2-glucoside, they showed similar release patterns with a shape of increasing concave-down. Hydrophobic MTF PTFE (polytetrafluoroethylene) membrane did not allow the diffusion of the hydrophilic model compounds as no detection was observed in the receptor compartment after 6 h of the test (Table 4). The

Table 3 Validation results of the model ingredients using the chromatographic conditions

Compound	Range (μg/mL)	Linearity	Accuracy		Precision		LOD (μg/mL)	LOQ (μg/mL)
			Intra-day (n = 6)	Inter-day (n = 3)	Intra-day (n = 6)	Inter-day (n = 3)		
Ascorbic acid 2-glucoside	50–240	$R^2 \geq 0.999$	150 μg/mL:0.09 %	150 μg/mL:4.39 %	150 μg/mL:99.2 %	150 μg/mL:98.4 %	1.757	5.325
			200 μg/mL:0.04 %	200 μg/mL:4.63 %	200 μg/mL:99.2 %	200 μg/mL:97.8 %		
			240 μg/mL:0.14 %	240 μg/mL:5.29 %	240 μg/mL:99.3 %	240 μg/mL:97.2 %		
Niacinamide	50–240	$R^2 \geq 0.999$	150 μg/mL:0.08 %	150 μg/mL:5.78 %	150 μg/mL:99.8 %	150 μg/mL:99.7 %	0.880	2.667
			200 μg/mL:0.06 %	200 μg/mL:5.77 %	200 μg/mL:99.5 %	200 μg/mL:99.5 %		
			240 μg/mL:0.12 %	240 μg/mL:5.61 %	240 μg/mL:99.4 %	240 μg/mL:99.5 %		
Retinol	3.75–18	$R^2 \geq 0.999$	12 μg/mL:0.20 %	12 μg/mL:1.67 %	12 μg/mL:100.9 %	12 μg/mL:101.6 %	0.026	0.122
			15 μg/mL:0.16 %	15 μg/mL:1.14 %	15 μg/mL:100.8 %	15 μg/mL:100.6 %		
			18 μg/mL:0.23 %	18 μg/mL:1.86 %	18 μg/mL:100.9 %	18 μg/mL:102.0 %		
Polyethoxylated retinamide	6.25–30	$R^2 \geq 0.999$	18.75 μg/mL:0.32 %	18.75 μg/mL:1.42 %	18.75 μg/mL:100.2 %	18.75 μg/mL:100.9 %	0.453	1.371
			25 μg/mL:0.21 %	25 μg/mL:1.20 %	25 μg/mL:99.4 %	25 μg/mL:100.4 %		
			30 μg/mL:0.17 %	30 μg/mL:1.34 %	30 μg/mL:99.0 %	30 μg/mL:99.2 %		

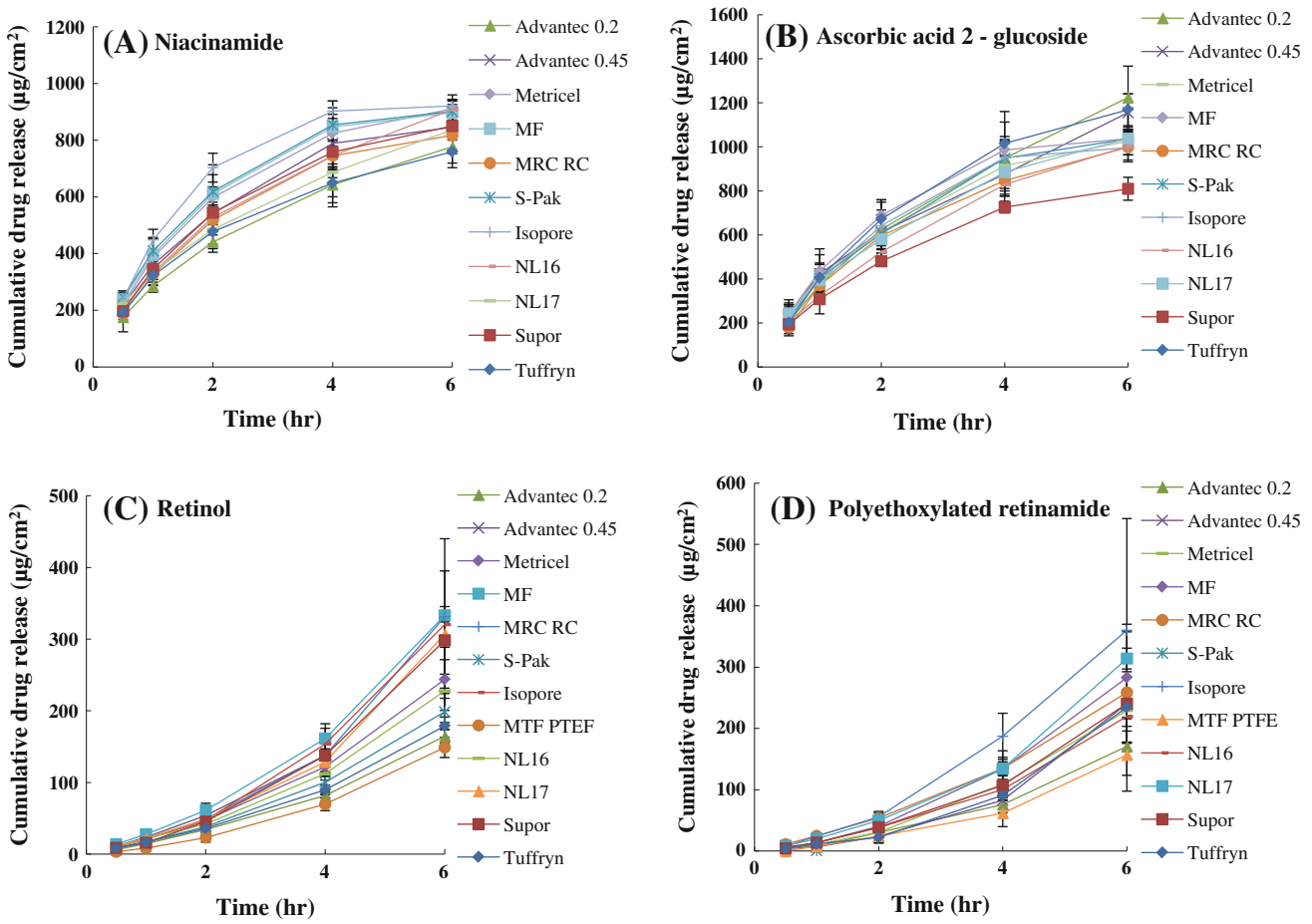


Fig. 1 Diffusion of model compounds across different kinds of synthetic membranes **a** niacinamide, **b** ascorbic acid 2-glucoside, **c** retinol, and **d** polyethoxylated retinamide

overall diffusion pattern of the other synthetic membranes was pretty much similar.

On the other hand, diffusion pattern of the hydrophobic compounds such as retinol and polyethoxylated retinamide showed a shape of increasing concave-up. The compounds were also detected in the receptor compartment as the MTF PTFE membrane was inserted between the diffusion cells. Even though there was difference in the lag time, the overall diffusion pattern was similar (Fig. 1). Based on the observation, drug diffusion through synthetic membranes might be strongly dependent on the physicochemical properties of the compound and the membranes as well.

Apparently, it is not easy to present the differences among the membranes in this study. The effects of membranes on the drug diffusion are difficult to predict and development and evaluation of formulations is on a 'case-by-case' basis with limited systematic consideration. Differences in the release with each membrane might be interpreted in terms of affinity of the compound for the membrane (Clement et al. 2000). Niacinamide and ascorbic acid 2-glucoside are hydrophilic and hence better affinity is expected in hydrophilic membranes compared to hydrophobic ones. On the other hand, hydrophobic compounds such as retinol and polyethoxylatedretinamide might have better affinity in hydrophobic membrane as shown in the results of Fig. 1.

No flux was calculated on the synthetic membranes since the drug release patterns were not linear. The releases from different synthetic membranes are similar each other in case of the same compound. Both niacinamide and ascorbic acid 2-glucoside were released after being solubilized in water and their release pattern became slower over time approaching a plateau. On the other hand, the release pattern of retinol and polyethoxylated retinamide solubilized in ethanol showed different pattern becoming faster over time.

The cumulative amount of the each compound released per unit surface area through membranes with different pore sizes is shown in Fig. 2. As already introduced in Table 1, 0.2 and 0.45 mean the pore size of the membranes 0.2 and 0.45 μm, respectively. NL 16 and 17 have the pore size of 0.2 and 0.45 μm, respectively. In general, smaller

Table 4 Total amount diffused after 6 h from different synthetic membranes

Membrane	Total diffusion after 6 h (μg/cm^2)			
	Niacinamide	Retinol	Ascorbic acid 2-glucoside	Polyethoxylated retinamide
Advantec 0.2 μm	776.73 ± 73.71	164.39 ± 9.37	1223.59 ± 143.06	170.59 ± 6.78
Advantec 0.45 μm	845.67 ± 80.53	293.13 ± 8.49	1155.66 ± 85.41	240.50 ± 117.00
Metricel	911.05 ± 29.63	243.73 ± 52.33	1026.67 ± 63.16	229.41 ± 25.95
MF	897.55 ± 40.11	333.23 ± 61.88	1037.11 ± 36.82	282.88 ± 87.08
MRC RC	817.26 ± 64.81	331.24 ± 7.29	998.00 ± 33.37	258.50 ± 25.15
S-Pak	901.61 ± 58.48	198.65 ± 18.79	1036.10 ± 42.60	172.58 ± 28.51
Isopore	920.69 ± 23.32	320.32 ± 119.79	996.86 ± 64.78	360.03 ± 182.14
MTF PTEF	na	148.87 ± 14.07	na	156.81 ± 59.02
NL16	907.78 ± 36.26	227.82 ± 3.81	1003.18 ± 62.90	219.45 ± 43.65
NL17	836.73 ± 71.54	306.94 ± 18.22	1037.78 ± 41.20	313.98 ± 16.96
Supor	850.61 ± 15.67	298.28 ± 47.17	809.57 ± 51.93	240.53 ± 22.50
Tuffryn	759.79 ± 40.58	178.85 ± 4.75	1168.53 ± 71.92	234.30 ± 58.15

na not available

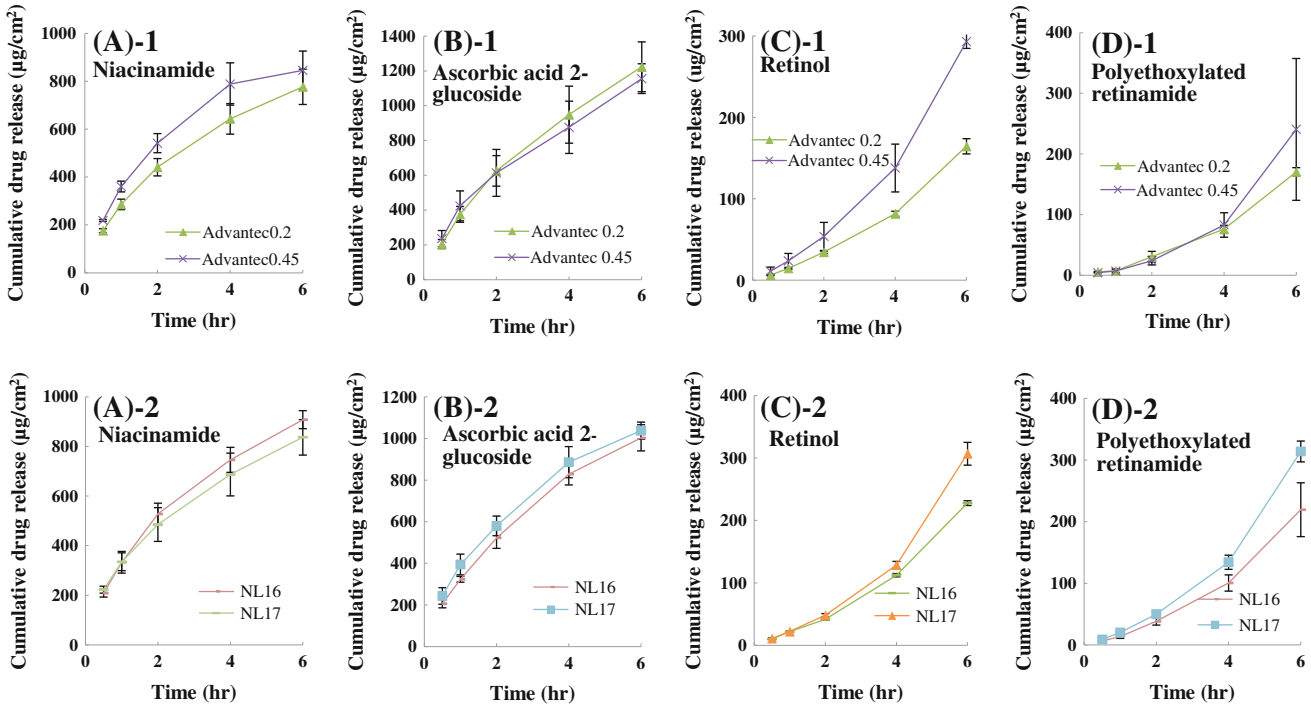


Fig. 2 Diffusion of model compounds across synthetic membranes with different pore size **a** niacinamide, **b** ascorbic acid 2-glucoside, **c** retinol, and **d** polyethoxylated retinamide

pore size may hinder the diffusion of compounds through the membrane since the smaller size can increase the resistance against diffusion as provided in the Fig. 2. Compared to the hydrophilic compounds (niacinamide and ascorbic acid 2-glucosie), hydrophobic ones (retinol and polyethoxylated retinamide) showed wider difference between the two sizes as diffusion time went by. Since the aqueous ethanol was used for the solvent of the hydrophobic compounds, diffusion through the membranes might be accelerated with the help of the solvent. Given the steeper slope over time on the Fig. 2, it seems that the aqueous ethanol acts as an accelerant for the release. Therefore, if a dissolving solvent diffuses well, the diffusion amount through a membrane would increase more.

According to the Table 4, retinol had the total diffusion amount in the order of MF, MRC RC, and Isopore

membrane after 6 h of experiment. Advantec 0.2, MTE PTEF, and Tuffryn membranes were following with relatively low diffusion properties. In case of polyethoxylated retinamide, MTE PTEF membrane had the smallest. Ascorbic acid 2-glucoside has the largest diffusion amount in Advantec membrane while Supor membrane has the smallest. Again, ascorbic acid 2-glucoside and niacinamide could not diffuse through the MTF PTFE at all and hence it may not be an appropriate membrane to evaluate skin permeation.

Isopore membrane showed superior diffusion properties in all the four compounds. This might be due to the much thinner membrane, which means that the traveling distance of a compound from donor to receptor is shorter. Given the thickness in Table 1, it can be expected according to the Fick's diffusion law that diffusion is inversely proportional to membrane thickness.

Based on the results, a lot of factors need to be considered together including physicochemical properties of the membranes, pore size, and/or membrane thickness to evaluate diffusion or topical permeation through synthetic membranes. The well-investigated membranes might be utilized alternatively to replace animal tests. Moreover, solvents and composition of the donor and receptor compartment need to be evaluated in detail to obtain systematic information regarding the diffusion properties and hence optimum formulations can be evaluated by selecting appropriate synthetic membranes through the screening strategy.

Conclusions

This study evaluates the influence of different synthetic membranes on the release of the four model active compounds. By comparing the release pattern between different membranes with various properties and model compounds with different hydrophilicity, various data could be obtained and be utilized to perform diffusion test for quality control and to replace animal test. However, scientists need to pay attention when to select which membrane is useful as it depends on a compound's physicochemical properties and also the relationship between a membrane and a compound.

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