Journal of Pharmacy and Pharmacology 11(1): 001-008, March 2024
DOI: 10.15413/ajpp.2024.0114
ISSN 2315-7828

Academia Publishing

Research Paper

An evaluation of collagen peptide for transdermal delivery using Strat-M® membrane and excised mouse skin

Zheng Yan[1], Peng Dong[1,2*], Bin Jiang[1], Xiao-Qing Zhang[2],Li-Ge Liu[2], and Hai-Yan Wang[2]

[1]College of Bioengineering, Beijing Polytechnic, Beijing 100176, China.
[2]Beijing Semnl Biotechnology Co., Ltd., Beijing 100062, No. 9, Street 1, Liangshuihe, Development Area, Beijing 100176, China.

Corresponding author. E-mail: dongpeng87@163.com. Tel: +86-10-87226057. Fax: +86-10-87226057

Accepted 16th February, 2024

ABSTRACT

In the field of medicine and cosmetics, transdermal experiments are commonly used to evaluate the transdermal absorption of topical formulations. In this study, we applied Strat-M® membrane to investigate the *in vitro* transdermal properties of collagen peptide with different average molecular weight, and compared them with the transdermal absorption through excised mouse skin *in vitro*. The results showed that the transdermal rate of collagen peptide through Strat-M® and excised mouse skin increased linearly with time, and the different concentrations of collagen peptide solutions affected the final cumulative transmission. The cumulative transmission per unit area of collagen peptides through Strat-M® had a good correlation with that through excised mouse skin, $R^2 > 0.98$. Strat-M® can replace the excised mouse skin in vitro to carry out the transdermal experiment of collagen peptide.

Key words: Collagen peptide, Strat-M®, *in vitro*, transdermal absorption, molecular weight distribution.

INTRODUCTION

Collagen peptide (CP) is the hydrolysis product of collagen, one of the main structural protein of the different connective tissues, such as skin, bone, cartilage and tendons in mammals (Khatri et al., 2021). CPs play a critical role in the fields of food, medicine and cosmetics due to its natural, safe, and easy compatibility characteristics (Song and Li, 2018). Recent years, more and more studies have shown that CPs has a positive impact on skin health and appearance, as well as promote wound healing and hair growth (Hwang et al., 2018; Zhao et al., 2022). Besides oral, topically applied to skin is another route of administration. But unlike oral, in order to exert efficacy, CPs must get through the skin, the natural protective barrier of human body. The penetration of CPs is influenced by factors such as the physical properties, including its lipophilicity, solubility and molar mass (Klebeko et al., 2021). Up to now, there are few reports on the skin permeability and mechanism of action of topical CPs of different molecular weight.

In the field of medicine and cosmetics, the parallel artificial membrane permeability assay (PAMPA) is often used for screening of active components and studies on penetration in topical preparations (Sinkó et al., 2021). The Skin-PAMPA can be a good alternative to skin permeability study, and the selection of membrane is an imperative aspect affecting the overall permeation parameters. *In vitro* permeation experiments using excised human and animal skins are very useful for understanding the skin permeation profiles and skin concentrations of topically applied chemicals, but ethical and economic reasons pose a major problem to the availability and use of human skin. *In vitro* methods without the use of animal tissues have gained increasing attention for evaluating the safety and efficacy of cosmetic ingredient (Kaur et al., 2018). So replacements for skin membranes involving the use of artificial membranes (e.g., Strat-M®, silicone membrane) designed to mimic human and animal skin offer a competent alternative to estimate permeation of drugs through skin (Todo, 2017).

In this study, our aims were to investigate the permeability of different molecular weight CPs. So CPs with different average molecular weight were selected as experimental subjects. Strat-M® and mouse abdominal skin

were applied to study the CPs release and permeation using the skin-PAMPA method. The usefulness and membrane-permeation characteristics of Strat-M® were investigated by comparing these parameters with mouse abdominal skin.

MATERIALS AND METHODS

Four commercial CPs (m.w.500, m.w.1000, m.w.2000, m.w.3000) were supplied by Beijing SEMNL Biotechnology Co., Ltd (Beijing, China).Hydroxyproline (Hyp) and 2,4-dinitrophenylhydrazine were purchased from Sigma-Aldrich (St.Louis, MO, USA). Acetonitrile (HPLC grade) was purchased from J.T. Baker (Deventer, Netherlands). All other chemicals and reagents used were of analytical grade.

Mice skin preparation

Mice weighting 20-25 g, were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). After removing the abdominal villi, the mice were fed overnight to promote the absorption and metabolism of the depilatory creams. Before the experiment, the mice were killed by removing cervical vertebrae. The abdominal skin of 2.5cm × 2.5cm was removed, and the subcutaneous adipose tissue and connective tissue were scraped off. The upper skin was washed with normal saline and soaked for 30 min.

Determination of molecular weight distribution

Molecular weight distribution of CPs were determined according to the method described by Yang et al. (2011) using an Agilent 1260 high performance liquid chromatography (HPLC) system (Agilent Technologies). The CPs were loaded onto TSK gel G2000 SWXL column (300 × 7.8 mm, Tosoh, Tokyo, Japan) and eluted with 45% (v/v) acetonitrile containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.5 mL/min. The elution was monitored by recording the absorbance at 220 nm. A molecular weight calibration curve was prepared from the average elution volume of the following standards: Cytochrome C (12384 Da), aprotinin (6512 Da), zinc bacitracin (1423 Da), Gly-Gly-Tyr-Arg (451 Da) and Gly-Gly-Gly (189 Da) (Sigma Co., USA).

Determination of hydroxyproline (Hyp) content

According to the method described by Wang et al. (2022), CPs (10 - 20 mg) was mixed with 10 mL HCl (6 mol/L) and hydrolyzed at 110°C for 22 h. Then the samples were cooled, dried and redissolved with deionized water. The Hyp content of each sample was analyzed by an Amino Acid Analyzer (S-433D, Sykam, Germany).

Transdermal test

The artificial membrane Strat-M® membrane (Merck Millipore, Tullagreen, Carrigtwohill, Ireland) was used directly, and the soaked mouse skin was wrapped in filter paper. The *in vitro* permeation studies were carried out in a Franz Cell system (Jingtuoyq, Tianjin, China) with a diffusion area of 1.54 cm^2 and capacity of 17 mL for the receptor medium, physical saline solution 0.9% (w/v). The Franz Cell system was maintained at a constant temperature of 37±0.5°C, while the receptor medium was stirred constantly at 300 rpm during the experiment.

For each CP permeating through Strat-M® or the mouse skin, an assay was performed with six diffusion cells. Each membrane was carefully fixed between the sample cell and the receiving cell, with 1 mL of the normal saline solution with 10% (w/v) CPs placed in the sample pool, and saline solution 0.9% (w/v) was used as blank control. Aliquots of 1 mL were taken from the receptor pool at 2, 4, 6, 8 and 24 h, respectively, and the pool was refilled with 1 mL fresh saline. The content of Hyp in each aliquot was determined as follows.

The cumulative amount of the CPs permeated through the membrane (Q_n) and the transdermal rate constant per unit area (J) were calculated according to the following formulas:

$$Q_n = \frac{C_n \times 10 \times V + \sum_{i=1}^{n-1} C_i \times 10 \times V_0}{A} \quad (1)$$

Where, C_n is the Hyp concentration measured at the n-th sampling point, in μg/mL; C_i is the Hyp concentration measured at the i-th sampling point, in μg/mL; 10 is the conversion coefficient between Hyp content and CPs content; V is the capacity of the receiving cell, which is 17 mL; V_0 is the aliquot volume at each sampling point, which is 1 mL; A is the permeation area, which is 1.54 cm^2

$$J = \frac{dQ}{dt} \quad (2)$$

The slope of the equation is the constant of transdermal rate per unit area (J, μg/cm^2/h).

RESULTS

Molecular weight distribution of CPs

The molecular weight distribution of the four CPs was

Table 1: Molecular weight distribution of CPs.

Samples	<500 Da	500-1000Da	1000-2000 Da	2000-3000 Da	3000-5000 Da	>5000 Da	Average
CP500	54.71±0.21	30.47±0.63	11.50±0.37	2.26±0.12	0.87±0.09	0.00	610.12±4.88
CP1000	28.67±0.04	34.01±0.72	23.83±0.32	7.56±0.10	4.84±0.15	0.00	1078.31±3.73
CP2000	17.17±1.00	22.39±0.79	24.33±0.64	13.56±0.36	13.57±0.55	1.33±0.16	2105.58±80.21
CP3000	6.79±0.08	13.52±0.18	20.32±0.45	14.70±0.17	18.76±0.14	4.60±0.04	3553.76±26.73

Table 2: The cumulative transmission per unit area of the CPs of different concentrations.

Samples	C(%)	Q_n(μg/cm²)		
		4 h	8 h	24h
CP500	1	1688	2889	4353
	5	2896	4782	6240
	10	3469	5575	7504
CP1000	1	829	1601	2468
	5	1308	2406	3966
	10	1523	2659	4454
CP2000	1	468	839	1758
	5	758	1259	2572
	10	921	1497	3079
CP3000	1	208	766	1205
	5	267	783	1302
	10	292	863	1518

measured, and the results are shown in Table 1. The average molecular weight of CP500, CP1000, CP2000 and CP3000 were 610.12±4.88 Da, 1078.31±3.73 Da, 2105.58±80.21 Da and 3553.76±26.73 Da, respectively. CP500 was observed to have the highest percentage (54.71%) for < 500 Da fraction. CP1000 was observed to have the highest percentage (34.01%) for 500-1000 Da fraction. CP2000 was observed to have the highest percentage (24.33%) for1000-2000 Da fraction. CP3000 had the highest percentage of >5000 Da, 3000-5000and 2000-3000 fractions than CP2000, CP1000 and CP500. However, the percentages of its < 500 Da and 500-1000 fractions were lower.

In vitro permeation of the CPs with different mass concentrations using Strat-M®

To study the effects of different concentrations on the penetration rates of the CPs through Strat-M®, the CPs of different concentrations (1%, 5% and 10%) were prepared for transdermal experiment. The results are shown in Table 2. As shown in the table, the cumulative transmissions per unit area of the four CPs increased with the increase in concentrations. Moreover, the cumulative transmissions per unit area of each concentration of the four CPs increased over time. The CP500 had the highest cumulative transmission per unit area among the four CPs, while the CP3000 had the lowest.

In vitro transdermal rate of the CPs using Strat-M®

To compare the transdermal rates of the four CPs, 10% normal saline solutions of the CPs penetrated through Strat-M®, and the cumulative transmissions per unit area are shown in Figure 1. As shown in the figure, the cumulative transmissions per unit area of the four CPs increased over time, and the transdermal rates of the CPs varied greatly with different average molecular weights. The transdermal rate of CP500 was the highest among the four CPs, and the cumulative transmission per unit area reached (7504 ± 1584) μg/cm² at 24 h.However, the transdermal rate of CP3000 was the lowest.

Ex vivo transdermal rate of the CPs using excised mouse skin

To compare the transdermal rates of the four CPs, 10% normal saline solutions of the CPs that penetrated through mouse abdominal skin were measured, and the cumulative transmissions per unit area are shown in Figure 2. When using the excised mouse skin, the cumulative transmissions

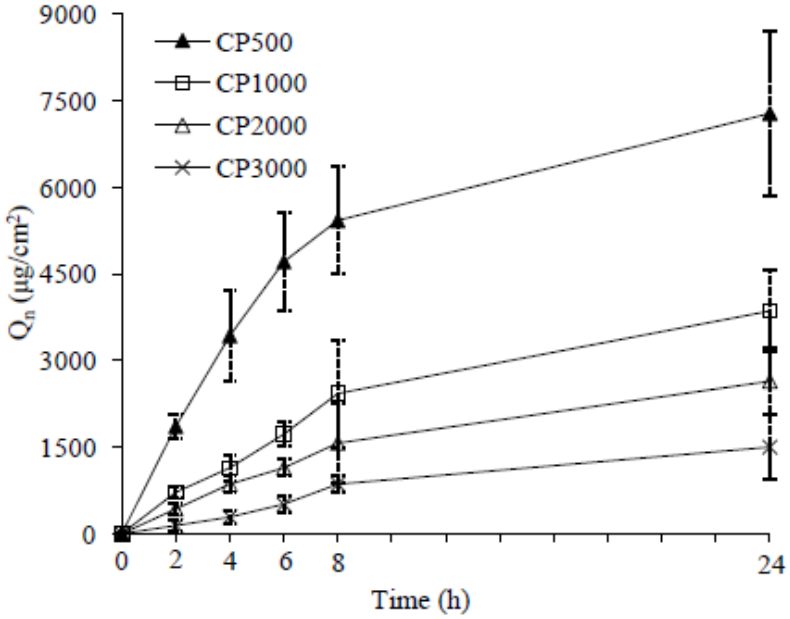

Figure 1: The cumulative transmissions of the CPs through Strat-M®.

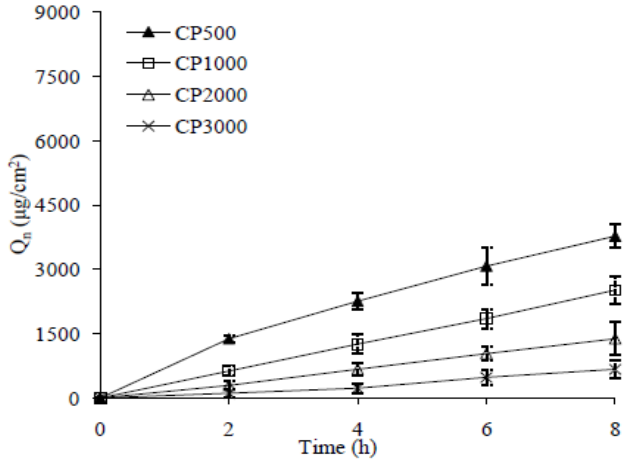

Figure 2: The cumulative transmission of the CPs through excised mice skin.

per unit area of the four CPs increased over time. Among them, the rate of CP500 was the highest, the cumulative penetration per unit area (Q_n) was (3773 ± 275) μg/cm² at 8 h. Whereas, CP3000 was the minimum, and Q_n was (693 ± 132) μg/cm² at 8 h. The average molecular weight of the CPs had the same trend of influence using Strat-M®membrane and excised mouse abdominal skin.

Comparison of transdermal properties of the CPs uing Strat-M® and excised mouse skin

The kinetic parameters of the CPs that penetrated through Strat-M® and excised mouse skin are shown in Tables 3 and 4. By comparing Tables 3 and 4, the kinetic parameters of the four CPs through Strat-M® and excised mouse skin increased linearly with transdermal time, and followed the zero-order kinetics equation. The transdermal absorption coefficient (J), or the transdermal rate constant per unit area across both membranes is ordered in the same way: J(CP500) > J(CP1000) > J(CP2000) > J(CP3000).

The results of comparison of the cumulative penrgouetration amount per unit area of the CPs through Strat-M® and excised mouse skin are shown in Figure 3. All of the correlation coefficients (r^2) were greater than 0.98, indicating that the two have a high correlation with the

Table 3: The kinetic parameters of the CPs penetrating through Strat-M®.

Samples	Q-t (Strat-M®)	r^2 (M)	J (Strat-M®) /(μg/cm²/h)
CP500	Q = 706.0 t + 333.6	0.9689	706.0
CP1000	Q = 308.0 t – 18.0	0.9940	308.0
CP2000	Q = 204.2 t + 2.4	0.9984	204.2
CP3000	Q = 104.1 t – 55.2	0.9353	104.1

Table 4: The kinetic parameters of the CPs penetrating through excised mouse skin.

Samples	Q-t (mouse)	r^2 (mouse)	J (mouse) / (μg/cm²/h)
CP500	Q = 461.9 t + 244.6	0.9760	461.9
CP1000	Q = 252.4 t – 13.2	0.9997	252.4
CP2000	Q= 173.1 t - 20.8	0.9986	173.1
CP3000	Q= 87.65 t - 50.8	0.9534	87.65

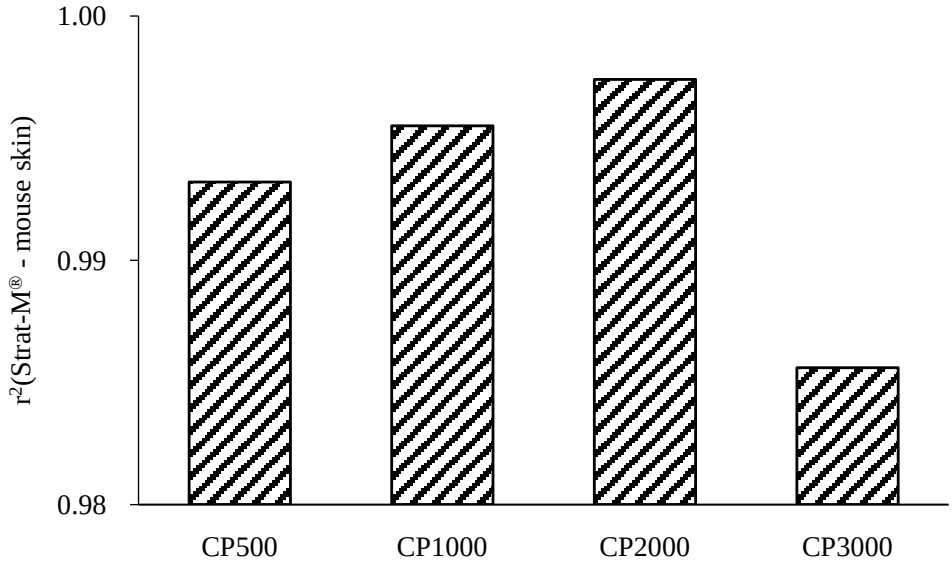

Figure 3: Correlation between penetration of CPs through Strat-M® and excised mouse skin.

percutaneous permeability of the collagen peptides. Strat-M® can be used as a substitute of excised mouse skin in transdermal experiment.

DISCUSSION

In vitro permeation studies are meant to evaluate the transdermal diffusion of compounds across the skin layers. The Strat-M®, specifically designed for skin penetration studies, consists of two layers of polyethersulfone in the outer layer, and a more diffusive polyolefin layer in the bottom layer (Arce et al., 2020). In addition, it contains simulated skin lipids found in the human stratum corneum (Mijaljica et al., 2024; Uchida et al., 2015). The Strat-M® can be applied as an inexpensive, stable and simple membrane in diffusion studies of active ingredients, excipients, and finished products for both topical and transdermal formulations. Advantages include that it is easy to use, requires no pretreatment, shows lot-to-lot consistency, and does not require any special storage (Simon et al., 2016). In this experiment, the cumulative transmission per unit area of the CPs with average molecular weight of 500, 1000, 2000 and 3000 Da increased over time and followed the zero-order kinetics equation. The CPs of different average molecular weights penetrated through Strat-M® at different rates. These results are in line with those who used excised mouse skin *in vitro*. This study shows how Strat-M® makes it easy to optimize the receiving medium, compatibility materials and dosage form for external collagen peptide preparation.

The permeability of CPs plays a very important role in

their efficacy and activity when applied topically to the skin. Therefore, the selection of an appropriate molecular weight may be the key to increasing penetration and achieving a fast therapeutic effect in the tissues under the skin. Due to the barrier effect of skin stratum corneum, substances with smaller molecular weight were easier to penetrate through stratum corneum, while those with higher molecular weight were more difficult to be absorbed (Chai et al., 2010; Ossowicz-Rupniewska et al., 2021; Prausnitz and Linger, 2008). Our study data showed that the cumulative transmission per unit area decreased as the molecular weight increased. Strat-M® can differentiate the transdermal rates of CPs with different molecular weights, which may be related to its porous and multi-layer structure with different diffusivities (Neupane et al., 2020). There was a linear relationship between the cumulative penetration amount of CPs and transdermal time, which was significantly different from that reported by Liu (2014), who concluded that the transdermal rate of CPs within 2 h was much greater than that after 2 h. This could be because the CPs used in the literature had a narrow molecular weight distribution range, whereas the CPs used in this experiment had a wide molecular weight distribution range. This could be interpreted as the weighted average rate of the CPs in different parts used in the literature, and CPs with different rates of molecular weight ranges led to different penetration speeds. Chai et al. (2010) also reported that the transdermal rate of polypeptides with different molecular weight after fractionation by intercepting 5, 3, 1 and 0.5 kDa membrane was significantly higher than that of the mixed tilapia skin collagen peptide before grading.

These results suggest that skin permeation by CPs maybe predicted according to their permeability coefficients through Strat-M®. Our results can be used to guide formulators in selecting vehicles in early development in the pharmaceutical, personal care and cosmetic industries. The *in vitro* permeation studies give significant insight into the behaviour of formulations *in vivo*.

CONCLUSION

In this study, we applied Strat-M® membrane to investigate the *in vitro* transdermal properties of collagen peptide with different average molecular weight, and compared them with the transdermal absorption through excised mouse skin *in vitro*. The results showed that the transdermal rate of collagen peptide through Strat-M® and excised mouse skin increased linearly with time, and the different concentrations of collagen peptide solutions affected the final cumulative transmission. The cumulative transmission per unit area of collagen peptides through Strat-M® had a good correlation with that through excised mouse skin, $R^2 > 0.98$. Strat-M® can replace the excised mouse skin *in vitro* to carry out the transdermal experiment of collagen peptide.

ACKNOWLEDGEMENT

This project was supported by the Beijing Polytechnic (2022X001-KXD, 2023X024-KXZ), the Research on National Reference Material and Product Development of Natural Products (SG030801).

REFERENCES

Arce FJ, Asano N, See GL, Itakura S, Todo H, Sugibayashi K (2020). Usefulness of artificial membrane, Strat-M®, in the assessment of drug permeation from complex vehicles in finite dose conditions. Pharmaceutics. 12(2):173. doi: 10.3390/pharmaceutics12020173

Arce FJ, Asano N, See GL, Itakura S, Todo H, Sugibayashi K (2020). Usefulness of artificial membrane, Strat-M®, in the assessment of drug permeation from complex vehicles in finite dose conditions. Pharmaceutics. 12(2):173. doi: 10.3390/pharmaceutics12020173.

Chai HJ, Li JH, Huang HN, Li TL, Chan YL, Shiau CY, Wu CJ (2010). Effects of sizes and conformations of fish-scale collagen peptides on facial skin qualities and transdermal penetration efficiency. J. Biomed. Biotechnol. 2010: 757301. doi: 10.1155/2010/757301.

Edgar S, Hopley B, Genovese L, Sibilla S, Laight D, Shute J (2018). Effects of collagen-derived bioactive peptides and natural antioxidant compounds on proliferation and matrix protein synthesis by cultured normal human dermal fibroblasts. Sci. Rep. 8: 10474. doi: 10.1038/s41598-018-28492-w

Hwang SB, Park HJ, Lee BH (2022). Hair-growth-promoting effects of the fish collagen peptide in human dermal papilla Cells and C57BL/6 mice modulating Wnt/β-Catenin and BMP Signaling Pathways. Int. J. Mol. Sci. 23(19):11904. doi: 10.3390/ijms231911904.

Kaur L, Singh K, Paul S, Singh S, Singh S, Jain SK (2018). A mechanistic study to determine the structural similarities between artificial membrane Strat-M™ and biological membranes and its application to carry out skin permeation study of amphotericin B nanoformulations. AAPS Pharm. Sci. Tech. 19: 1606-1624. doi: 10.1208/s12249-018-0959-6.

Khatri M, Naughton RJ, Clifford T, Harper LD, Corr L (2021). The effects of collagen peptide supplementation on body composition, collagen synthesis, and recovery from joint injury and exercise: a systematic review. Amino Acids. 53(10): 1493-1506. doi: 10.1007/s00726-021-03072-x.

Klebeko J, Ossowicz-Rupniewska P, Nowak A, Janus E, Duchnik W, Adamiak-Giera U, Kucharski Ł, Prowans P, Petriczko J, Czapla N, Bargiel P, Markowska M, Klimowicz A (2021). Permeability of ibuprofen in the form of free acid and salts of L-valine alkyl esters from a hydrogel formulation through Strat-M™ membrane and human skin. Materials (Basel). 14(21): 6678. doi: 10.3390/ma14216678.

Liu CY, Sui HS, Che S, Yu JW, Wang CW, Zou PF (2017). Investigation of transdermal absorption performance of collagen peptides from jellyfish, bovine bone and cod skin. China Surfact. Det. & Cosmetics, 47(03): 164-167+177. doi:10.13218/j.cnki.csdc.2017.03.009.

Mijaljica D, Townley JP, Spada F, Harrison IP (2024). The heterogeneity and complexity of skin surface lipids in human skin health and disease. Prog. Lipid Res. 93:101264. doi: 10.1016/j.plipres.2023.101264.

Neupane R, Boddu Sai HS, Renukuntla J, Jayachandra Babu R, Tiwari Amit K (2020). Alternatives to biological skin in permeation studies: current trends and possibilities. Pharmaceutics. 12(152): 12020152. doi: 10.3390/pharmaceutics12020152.

Ossowicz-Rupniewska P, Nowak A, Klebeko J, Janus E, Duchnik W, Adamiak-Giera U, Kucharski Ł, Prowans P, Petriczko J, Czapla N, Bargiel P, Markowska M, Klimowicz A (2021). Assessment of the effect of structural modification of ibuprofen on the penetration of ibuprofen from Pentravan® (Semisolid) formulation using human skin and a transdermal diffusion test model. Materials (Basel). 14(22): 6808. doi: 10.3390/ma14226808.

Prausnitz MR, Langer R (2008). Transdermal drug delivery. Nat. Biotechnol. 26(11): 1261-1268. doi: 10.1038/nbt.1504.

Simon A, Amaro MI, Healy AM, Cabral LM, Sousa VP (2016). Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin within vivo-in vitro correlation. Int. J. Pharm. 512(1): 234-241. doi: 10.1016/j.ijpharm.2016.08.052.

Sinkó B, Bárdos V, Vesztergombi D, Kádár S, Malcsiner P, Moustie A, Jouy C, Takács-Novák K, Grégoire S (2021). Use of an in vitro skin parallel artificial membrane assay (Skin-PAMPA) as a screening tool to compare transdermal permeability of model compound 4-phenylethyl-resorcinol dissolved in different solvents. Pharmaceutics. 13(11): 1758. doi: 10.3390/pharmaceutics13111758.

Song HD, Li B (2018). Beneficial effects of collagen hydrolysate: areview on recent developments. Biomed. J. Sci. Tech. Res.1(2): 458-461. doi: 10.26717/BJSTR.2017.01.000217

Todo H (2017). Transdermal permeation of drugs in various animal species. Pharmaceutics. 9(3): 33. doi: 10.3390/pharmaceutics9030033.

Uchida T, Kadhum WR, Kanai S, Todo H, Oshizaka T, Sugibayashi K (2015). Prediction of skin permeation by chemical compounds using the artificial membrane, Strat-M®. Eur. J. Pharm. Sci. 67: 113-118. doi: 10.1016/j.ejps.2014.11.002.

Wang JP, Liu JM, John A, Jiang YM, Zhu H, Yang B and Wen LR (2022). Structure identification of walnut peptides and evaluation of cellular antioxidant activity. Food Chem. 388(18): 132943.doi: 10.1016/j.foodchem.2022.132943.

Yang B, Yang HS, Li J, Li ZX, Jiang YM (2011). Amino acid composition, molecular weight distribution and antioxidant activity of protein hydrolysates of soy sauce lees. Food Chem. 124(2): 551-555. doi:10.1016/j.foodchem.2010.06.069.

Zhao XC, Zhang XJ, Liu DY (2021). Collagen peptides and the related synthetic peptides: A review on improving skin health. J. Funct. Foods. 86: 104680. doi:10.1016/J.JFF.2021.104680.