dx.doi.org/10.14227/DT260419P18

Effect of Thyme Oil on the Transdermal Permeation of Pseudoephedrine HCl from Topical Gel

Rahman Gul[1,2*], Syed Umer Jan[2], Amanullah Khan[1], Nusrat Jahan[3], Rehana Rahman[4], Samiullah Sherani[1], and Nabeela Tariq[4]

[1]*Department of Health, Government of Balochistan, Pakistan.*
[2]*Faculty of Pharmacy and Health Sciences, University of Balochistan, Pakistan.*
[3]*Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan.*
[4]*Sardar Bahadur Khan Women's University, Quetta, Pakistan.*

e-mail: gul.dotani@yahoo.com

ABSTRACT

The aims of the current study were to prepare pseudoephedrine gel formulations for skin permeability and to assess the effect of thyme oil on the permeability of the formulations. Thyme oil was used in the gel formulations at a range of concentrations (0–3%) and its effects on pseudoephedrine permeation profiles in vitro were observed. Several physicochemical characteristics of the formulated gels were analyzed to assess their suitability for topical application. A standard pseudoephedrine calibration curve was prepared to analyze the quantity of drug released from the samples. In vitro drug permeability was studied using a Franz diffusion cell apparatus with a cellulose membrane and excised rabbit skin. Several kinetic models were used to assess the drug permeability behavior, and the Korsmeyer-Peppas kinetic model was found to correspond to the in vitro drug release profiles of the pseudoephedrine gels. The formulation was physiochemically stable, the drug was released, and thyme oil did enhance the permeation of the drug up to 69.87%.

KEYWORDS: Pseudoephedrine, thyme oil, topical gel, in vitro release kinetics, dissolution

INTRODUCTION

Topical drug delivery has been used since ancient times. Historically, thyme oil is used as both a topical and a traditional medicinal agent (*1*). Essential oils, such as thyme oil, are listed in different pharmacopoeia as phenolic ingredients (*2*). In addition, thyme is named in the American Botanical Council's Commission E list of official herbs. Therefore, aromatherapy is recognized, even approved, by the Commission E monographs (*3*).

The British Pharmacopoeia states that thyme oil is used in bronchitis, bronchitis catarrh, sore throat, and whooping cough, and that it is used in combination with other drugs recommended in the Monograph (*4, 5*). It is a fat-soluble and highly volatile substance, easily absorbed through the skin (*6*). Until the 19th century, it was considered that the skin is not permeable to drugs for absorption into the systemic circulation, but later in the 20th century, it was suggested that the outer layer of the skin acts as a permeable barrier (*7*). Transdermal drug delivery should result in low bioavailability because of the skin barrier (*8*), providing constant release of a drug and avoiding the first-pass effect. However, the bioavailability of drugs absorbed via this route is enough for pharmacological action (*9*). Many diseases can be treated topically, but administered drugs are often considered to have poor skin penetration. Overcoming these barriers using skin penetration enhancers is of interest in pharmaceutical research (*10*).

Pseudoephedrine HCl is an anti-asthmatic drug with a short half-life of 4-5 hours. Doses of 20 mg are therefore administered orally 3 to 4 times a day (*11*). However, frequent dosing may cause side effects, including tachycardia, hypotension, insomnia, and tremors (*12*). The objective of this study was to formulate a pseudoephedrine gel for transdermal administration and to assess the effect of thyme oil as an enhancer on the formulation.

MATERIALS AND METHODS

Pseudoephedrine HCl was purchased from Merck in Quetta, Pakistan, and triethanolamine, Carbopol 934P, and ethanol were from Martin Dow in Pakistan. Thyme oil was extracted from *Thymus serpyllum* L. (*5*).

*Corresponding author

Pseudoephedrine HCl Calibration Curve

To obtain the pseudoephedrine HCl standard curve, a stock solution was made by dissolving 10 mg pseudoephedrine HCl powder in 50 mL distilled water with agitation for several minutes and achieving a volume of 100 mL with phosphate buffer (pH 7.4). Dilutions of 0.025, 0.0125, 0.0062, 0.0031, and 0.0015 mg/mL were prepared from the stock solution, as shown in Table 1. The absorbance of all dilutions was measured at 242 nm using an ultraviolet (UV)-visible spectrophotometer (Shimadzu UV-1601, Japan). The linearity graph is shown in Figure 1.

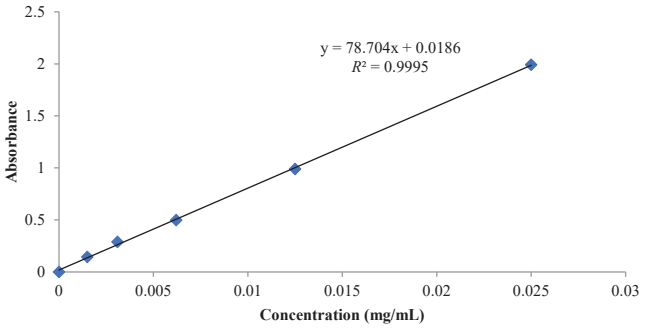

Figure 1. Calibration curve of pseudoephedrine.

Pseudoephedrine Gel Development

Pseudoephedrine gel, in the presence and absence of thyme oil, was formulated in the laboratory to assess the permeation of pseudoephedrine from the gel and to examine the effect of thyme oil on pseudoephedrine permeation across both cellulose membrane and rabbit skin. The pseudoephedrine gel was developed by dissolving 1 g carbopol 934P polymer in 50 mL distilled water while stirring until a consistent dispersion was prepared. A continuously stirred, homogenous solution of 1 g pseudoephedrine in 10 mL ethanol was added drop wise to the carbopol solution, stirring constantly. The enhancer, thyme oil, was slowly added to the gel formulation at different concentrations to prepare seven different formulations: a blank (with no enhancer); and six formulations containing 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% thyme oil (Table 2). Exactly 1 mL of triethanolamine was added to the 3% solution. The final volume of each (100 mL) was achieved by adding a sufficient quantity of distilled water and again stirred until a homogenous transparent gel was obtained.

Table 1. Formulation of 2.0% (w/v) Pseudoephedrine Gel

Sample No.	Pseudoephedrine HCl (gm)	Carbopol (gm)	Ethanol (mL)	TEA (mL)	Thymol Oil (mL)	Distilled water (mL)
1	2.0	1.0	10.0	1.0	Blank	100
2	2.0	1.0	10.0	1.0	1.0	100
3	2.0	1.0	10.0	1.0	2.0	100
4	2.0	1.0	10.0	1.0	3.0	100
5	2.0	1.0	10.0	1.0	4.0	100
6	2.0	1.0	10.0	1.0	5.0	100
7	2.0	1.0	10.0	1.0	6.0	100

HCl, Hydrochloric acid; w/v, weight/volume; TEA, triethanolamine

Table 2. Physical Parameters Values for Pseudoephedrine Gel Formulations

Pseudoephedrine Concentration (%)	pH	Spreadability (g/cm/s)	Homogeneity	Skin irritation	Drug content (%)
0.5	5.5	4.6	Good	NO	99.10
1.0	5.9	4.8	Good	NO	98.09
1.5	6.0	4.9	Good	NO	99.31
2.0	5.8	5.1	Good	NO	99.80
2.5	6.4	5.2	Good	NO	99.78
3.0	6.5	5.7	Good	NO	98.21

Physicochemical Determination of Pseudoephedrine Gel

To assess the suitability of the pseudoephedrine gel for transdermal use, its physicochemical characteristics were determined as described below.

pH: The pH values of the pseudoephedrine gels were assessed using a calibrated pH meter (*13*).

Consistency: The pseudoephedrine gel consistency was assessed by the dropping cone technique. In this technique, a cone attached to a holding rod was dropped from a 10-cm space in the middle of a gel-filled cup. The distance covered within the gel cup was observed after 50 seconds to determine the consistency (*13*).

Spreadability: The spreadability of all formulations was assessed by determining the diameter of a 0.5-g formulation after its compression between two 10-g glass slides (*14*).

Homogeneity: The homogeneity of the pseudoephedrine gel samples was assessed by visual observation. Transparent narrow glass tubes were filled with gel and observed under light to check for any lumps or particulates (*13*).

Drug Content: To measure the pseudoephedrine quantity in the prepared gels, a 10-mg sample of each was dissolved, with stirring, in 100-mL HCl solvent, filtered through a 0.2-µm membrane filter, and assessed using a calibrated UV-visible spectrophotometer. The pseudoephedrine percentage was calculated (*13*).

Skin Irritation Study

The animal study was approved by the ethics committee in the faculty of pharmacy and alternative medicine at The Islamia University of Bahawalpur, Pakistan. The formulated pseudoephedrine gels were applied to the shaved rabbit skin over an area of almost 6 cm^2 and covered with a semi-occlusive gauze patch for 1 hour. After this period, the residual gel was removed with no damage to the integrity of the skin. Observations are listed as in Table 2. The application was repeated once a day for 7 days, and any response or sensitivity was observed and recorded (*15*).

Excised Rabbit Skin Preparation

Ex vivo permeation studies of the pseudoephedrine gels were performed on prepared rabbit skin. The animals were anesthetized with chloroform, the dorsal region was shaved, and the skin was washed using surgical gauze and cotton swabs. After a 24-hour recovery period, the rabbit was sacrificed and the skin was excised. The epidermis was removed by soaking the skin in water at 60 °C and teasing and separating the dermis from the two layers. The epidermal layer was wrapped in aluminum foil and frozen at –50 °C until further utilization (*16–18*).

In Vitro Diffusion Study Protocol

Franz diffusion cell equipment (Perm Gear, USA) was used for the pseudoephedrine gel cellulose membrane and rabbit skin in vitro diffusion studies. The membranes were set in the receptor and donor holders of the Franz diffusion cell equipment. The receptor chamber was filled with 5 mL phosphate buffer (pH 7.4), and the sample chambers were filled with the prepared pseudoephedrine gels: a blank (with no enhancer) and the gels containing 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% thyme oil. The solvent temperature was a constant 37 °C throughout the study. At 0.5, 1, 1.5, 2, 3, 4, 8, 12, 16, 20, and 24 hours, samples of 2.0 mL were taken from the receptor cells, and the cells were immediately refilled with fresh buffer at 37 °C (*16*). The samples were filtered via Millipore filters (Whatman, Germany; 150 mm), and the pseudoephedrine concentration was assessed using the UV-visible spectrophotometer at 242 nm.

Kinetic Analysis of Pseudoephedrine Release In Vitro

The quantity of pseudoephedrine released in the in vitro diffusion studies was calculated, and the drug release parameters and linear regression analyses for all formulations were assessed. The correlation coefficient (R^2) was calculated for all formulations, to determine which of the given kinetic models corresponded to the release of the drug via the cellulose membrane and the rabbit skin. The kinetics equations given below were used for the calculations (*19*).

Zero-order model: $A = K_0 t + A_0$ Eq. (1)

Where A is the amount of drug released at time t and K_0 is the zero-order release rate constant.

First-order model: $\text{Log } A = Kt/2.3 + \text{Log } A_0$ Eq. (2)

Where A_0 is the amount of drug release at time t and K is the first-order rate constant.

Higuchi-diffusion model: $Q = K_H t^{1/2}$ Eq. (3)

Where Q is the amount of drug released to the membrane (in mg) at time t in minutes, and K_H is the Higuchi square root of time release constant.

Korsmeyer-Peppas model: $C_t/C_\infty = Kt^n$ Eq. (4)

Where C_t/C_∞ is a fraction of drug released at time t, K is the release constant, and n is the release exponent.

Hixson-Crowell diffusion release model:
$Q_t/Q = Kt^n$ Eq. (5)

Where Q_t/Q is the amount of released drug at time t, K is the constant comprising the structural and geometric characteristics of the formulations, and n is the release of exponent.

Stability Studies

The stability of the pseudoephedrine gel formulations under different storage conditions was studied. Samples were analyzed after 3 months of storage at 0 ± 1 °C (freezer), 8 ± 0.1 °C (refrigerator), 25 ± 0.1 °C (incubator), and 40 ± 0.1 °C (incubator). All formulations were tested for changes in appearance, pH, consistency, and homogeneity (*13, 18*).

Statistical Analysis

Statistical analyses of the kinetic results were performed on DD Solver (Microsoft Excel 2007) (*19, 20*). SPSS (version 18.0, IBM, USA) was used for ANOVA calculations (*21*). All formulation data were applied to the kinetics model, and flux (*J*) was calculated as µg/h/cm^2 (*22*).

RESULTS AND DISCUSSION

The pH, consistency, spreadability, homogeneity, skin irritation, and drug content of the pseudoephedrine gel formulations were characterized (see Table 2). There was no difference between the gels in terms of their liquefaction, color, and phase separation, and in additional characteristics, such as the pH. The gels all had a pH between 5.5 and 6.5, consistent with the pH seen in a prior study of topical dosage formulations of flurbiprofen gel and with normal human skin pH (range, 4.5–6.5) (*23, 24*). Consistency and spreadability were observed over a period of 90 days. The spreadability varied from 4.6 to 5.7 g/cm/s, indicating that the gels were spreadable with slight shearing pressure. Good homogeneity was observed in all gel formulations, with no lumps or visual particles. Application of the gels to rabbit skin for 7 days resulted in no redness, lesions, or itching, indicating that they were not irritating to the skin. The drug content of pseudoephedrine gel was in the range of 98.09–99.80%, and the gels demonstrated good uniformity. All physical parameters examined indicated that the pseudoephedrine gels are suitable for transdermal application.

In Vitro Drug Diffusion Study

All formulation data were applied to the Korsmeyer-Peppas permeation model. The release of pseudoephedrine from the transdermal gel, and its permeability via the cellulose membrane, are shown in Figure 2. The results indicated that the amount of drug released from the gel with no thyme oil was lowest, with only 23.61% recovered in the receptor solvent in the cellulose membrane study after 24 hours. After the addition of 0.5% thyme oil, the amount of recovered drug released from the pseudoephedrine gel and permeating the membrane reached 50.66%. Drug release and permeation increased with increasing concentration of the enhancer, thyme oil, and in the pseudoephedrine gel containing 3% thyme oil, the recovered amount of drug in the receptor solvent was 69.85%. Other studies have indicated that the presence of an essential oil can cause an increase in drug diffusion (*25*). The release of pseudoephedrine from the gel and its permeability via excised rabbit skin in the presence of different concentrations of thyme oil over 24 hours is shown in Figure 3. Permeation across rabbit skin was also lowest in the absence of thyme oil. In the gel formulations containing thyme oil, the maximum amount of drug recovered was 66%, higher than that without thyme oil, and there was a trend towards increasing permeability with increasing amount of thyme oil. The comparative effect of thyme oil as enhancer on in vitro drug permeation of pseudoephedrine across cellulose membrane and rabbit skin is shown in Figure 4.

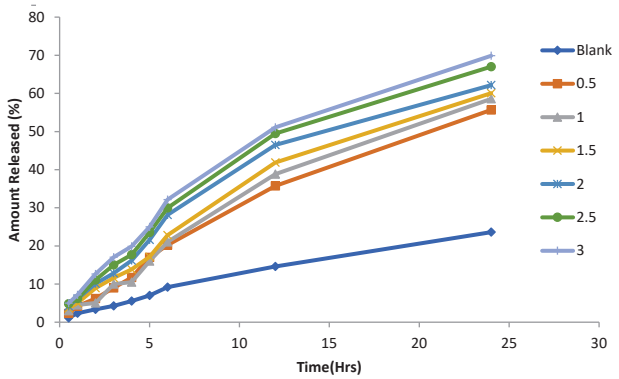

Figure 2. Release of pseudoephedrine gel 2% (w/v) via cellulose membrane in phosphate buffer pH 7.4. w/v, weight/volume.

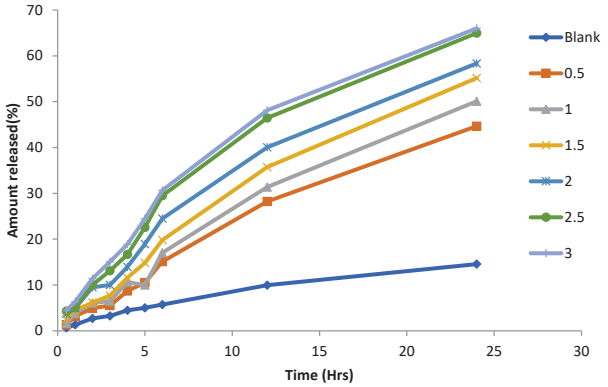

Figure 3. Release of pseudoephedrine gel 2% (w/v) via rabbit skin in phosphate buffer pH 7.4. w/v, weight/volume.

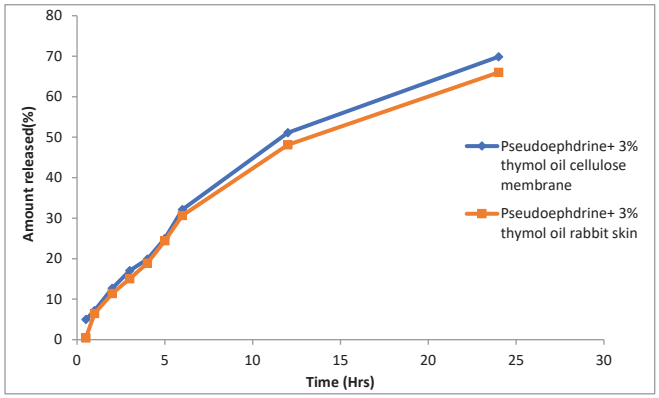

Figure 4. Comparative release of pseudoephedrine gel 2% (w/v) via cellulose membrane and rabbit skin in phosphate buffer pH 7.4. w/v, weight/volume.

Stability Studiess

The stability of the pseudoephedrine gels, stored at temperatures of 0 , 8, 25, and 40 °C for a period of up to 3 months, was studied. No previous data is available on the stability of pseudoephedrine when prepared in a topical gel formulation. In topical dosage formulations, stability is the main quality concern (*23*). Our formulations were stored under conditions which limited potential oxidation. All the formulations were found stable.

CONCLUSIONS

Pseudoephedrine gel was successfully formulated with a high in vitro release rate. Thyme oil effectively enhanced the penetration of pseudoephedrine into the skin and was most efficacious as an enhancer of drug permeation via the transdermal route at higher concentrations. Further studies are necessary to evaluate the efficacy of the pseudoephedrine gel in vivo.

ACKNOWLEDGEMENTS

We thank the members of the Faculty of Pharmacy and Health Sciences at the University of Balochistan and Health Department Government of Balochistan for study leave and their timely support to complete this research.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

REFERENCES

1. Jane, B. Aromatherapy. What Is It? In *HerbalGram, The Journal of the American Botanical Council*, Austin, TX: American Botanical Council, 2003, 57; p. 50.
2. Hatcher, R.; Eggleston, C. Useful Drugs. A List of Drugs Selected to Supply the Demand for a Less Extensive Materia Medica with a Brief Discussion of Their Actions, Uses and Dosage. *The American Medical Association Chicago*, **1935**, *189* (5). DOI 10.1097/00000441-193505000-00043.
3. The Commission E Monographs: Thyme. American Botanical Council website. (Last updated December 2, 1992). http://cms.herbalgram.org/commissione/Monographs/Monograph0357.html (accessed September 25, 2019).
4. *The British Pharmacopoeia*. Commission Secretariat of the Medicines and Healthcare Products Regulatory Agency. The Stationary Office, London, 2015.
5. Gul, R.; Jan, U. S.; Ahmad, M.; Akhtar, M.; Faridullah. Use of traditional herbal medicines for the treatment of asthma in Balochistan. *Curr. Res. J. Biol. Sci.*, **2017**, *9*, 16–22. DOI: 10.19026/crjbs.9.5138.
6. Pisseri, F.; Bertoli, A.; Pistelli, L. Essential oils in medicine: principles of therapy, *Parassitologia*. **2008**, *50*, 89–91.
7. Morrow, D. I. J.; McCarron, P. A.; Woolfson, A. D.; Donnelly, R. F. Innovative strategies for enhancing topical and transdermal drug delivery. *Open Drug Deliv. J.* **2007**, *1*, 36–59. DOI: 10.2174/1874126600701010036.
8. Francesco, C.; Paola, M.; Antonella, C.; Leila, T.; Stefania. P.; Luisa, M. Polymethacrylates as crystallization inhibitors in monolayer transdermal patches containing ibuprofen. *Eur. J. Pharm. Biopharm*. **2005**, *60*, 61–66. DOI: 10.1016/j.ejpb.2005.02.001.
9. Syed, N. H. S.; Mahboob, R.; Muhammad, F. A. Study of percutaneous absorption of diclofenac diethylamine in the presence of cetrimide through hairless rabbit skin. *J. Res. Science* **2006**, *17*, 45–51.
10. Sinha, V. R.; Kaur, M. P. Permeation enhancers for transdermal drug delivery. *Drug Dev. Ind. Pharm*. **2000**, *26*, 1131–1140. DOI: 10.1081/DDC-100100984.
11. Satoskar, R. S.; Bhandarkar S. D. *Pharmacology and Pharmacotherapeutics*, 7th ed.; Popular Prakashan: Bombay, 1980; pp. 204–206.
12. *Martindale: The extra pharmacopoeia*, 28th ed.; Reynolds, J. E.

F.; Prasad, A. B.; Eds. The Pharmaceutical Press: London, 1982; p. 11. DOI: 10.1002/jps.2600730653.

13. Shivhare, U. D.; Jain, K. B.; Mathur, V. B.; Bhusari, K. P.; Roy, A. A. Formulation development and evaluation of diclofenac sodium gel using water-soluble polyacrylamide polymer. Dig. *J. Nanomater. Biostruct*. **2009**, *4*, 285–290.
14. Desai, K. G. H. Enhanced skin permeation of rofecoxib using topical micro emulsion gel. *Drug Dev. Res*. **2004**, *63*, 33–40. DOI: 10.1002/ddr.10386.
15. Sidramappa, B.; Shirsand, G. R. K.; Ganesh, G. K.; Sharanabasappa, S. B.; Padivala V. S. Formulation and evaluation of clotrimazole niosomal gel for topical application. *RGUHS J. Pharm. Sci*. **2015**, *5*, 32–38. DOI: 10.5530/rjps.2015.1.4.
16. Gul, R.; Jan, U. S.; Ahmad, M.; Akhtar, M.; Faridullah, S. Formulation, characterization, in vitro and ex vivo release of Ephedra extract from topical preparations using dialysis membrane and rabbit skin. *Dissolution Technol*. **2017**, *24*, 24–30. DOI: 10.14227/DT240417P24.
17. Ozgüney, I. S.; Karasulu, H. Y.; Kantarci, G.; Sözer, S.; Güneri, T.; Ertan, G. Transdermal delivery of diclofenac sodium through rat skin from various formulations. *AAPS PharmSciTech*. **2006**, *7*, E39–E45. DOI: 10.1208/pt070488.
18. Shah, V. P.; Williams, R. L. Importance of in vitro drug release. In *Topical Drug Bioavailability, Bioequivalence, and Penetration*; Springer: New York, NY, 2014; pp. 61–67. DOI: 10.1007/978-1-4939- 1289-6_4.
19. Gul, R.; Jan, U. S.; Ahmad, M.; Mukhtiar, M. Formulation of Hedera helix L. in topical dosage forms: in vitro and ex vivo evaluation. *Dissolution Technol*. **2018**, *25*, 24-30. DOI: 10.14227/DT250218P40.
20. Zhang, Y.; Huo, M.; Zhou, J. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. *AAPS J*. **2010**, *12*, 263–271. DOI: 10.1208/s12248-010-9185-1.
21. Yuksel, N.; Kanik, A. E.; Baykara, T. Comparison of in vitro dissolution profile by ANOVA-based, model-dependent and -independent methods. *Int. J. Pharm*. **2000**, *209*, 57–67. DOI: 10.1016/S0378-5173(00)00554-8.
22. Kreilgaard, M.; Pedersen, E. J.; Jaroszewski, J. W. NMR characterisation and transdermal drug delivery potential of microemulsion system. *J. Control. Release* **2000**, *69*, 421–433. DOI: 10.1016/S0168-3659(00)00325-4.
23. Abid, H.; Gul, M. K.; Syed, U. J.; Shefaat, U. S.; Kifayatullah, S.; Muhammad A.; Nouman, R.; Asif, N.; Abdul, W. Effect of olive oil on transdermal penetration of flurbiprofen from topical gel as enhancer. *Pak. J. Phar. Sci*. **2012**, *25*, 365–369.
24. Schmid-Wendtner, M. H.; Korting, H. C. The pH of the skin surface and its impact on the barrier function. *Skin Pharmacol. Physiol*. **2006**, *19*, 296–302. DOI: 10.1159/000094670.
25. Naseem, A. C.; Areeg, A. A. S.; Kanchan, K.; Krishna, P.; Ziyaur, R. Improvement in bioavailability of transdermally applied flurbiprofen using Tulsi (Ocimum sanctum) and turpentine oil. *Colloids Surf. B: Biointerfaces* **2008**, *65*, 300–307. DOI: 10.1016/j.colsurfb.2008.05.001.