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Formulation development of transdermal dosage forms: Quantitative structure-activity relationship model for predicting activities of terpenes that enhance drug penetration through human skin

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Abstract

Terpenes and terpenoids have been used as enhancers in transdermal formulations for facilitating penetration of drugs into human skin. Knowledge of the correlation between the human skin penetration effect (HSPE) and the physicochemical properties of these enhancers is important for facilitating the discovery and development of more enhancers. In this work, the HSPE of 49 terpenes and terpenoids were compared by the *in vitro* permeability coefficients of haloperidol (HP) through excised human skin. A first-order multiple linear regression (MLR) model was constructed to link the permeability coefficient of the drug to the lipophilicity, molecular weight, boiling point, the terpene type and the functional group of each enhancer. The Quantitative Structure-Activity Relationship (QSAR) model was derived from our data generated by using standardized experimental protocols, which include: HP in propylene glycol (PG) of 3 mg/ml as the donor solution containing 5% (w/v) of the respective terpene, the same composition and volume of receptor solution, similar human skin samples, in the same set of automated flow-through diffusion cells. The model provided a simple method to predict the enhancing effects of terpenes for drugs with physicochemical properties similar to HP. Our study suggested that an ideal terpene enhancer should possess at least one or combinations of the following properties: hydrophobic, in liquid form at room temperature, with an ester or aldehyde but not acid functional group, and is neither a triterpene nor tetraterpene. Possible mechanisms revealed by the QSAR model were discussed.

Keywords: Quantitative Structure-Activity Relationship (QSAR); Quantitative Structure-Property Relationship (QSPR); Terpenes; Penetration enhancement; *In vitro* permeation; Human skin

1. Introduction

Transdermal drug delivery systems offer many advantages over conventional dosage forms, which include controlled delivery, improved patient compliance and reduced side effects [1]. In many cases, chemicals that enhance skin permeability of these drugs have been included in drug formulations for enhancing the delivery of these drugs to reach the desired therapeutic levels [2,3]. Efforts have been directed at identifying safe and effective enhancers from both natural products and synthetic chemicals. In particular, terpenes from natural sources and laboratory-designed terpenoids have attracted great interest [4–6]. Terpenes are generally considered as less toxic and have less irritant effects compared to surfactants and other skin penetration enhancers, and some terpenes have been characterized as Generally Recognized As Safe (GRAS) by the US FDA [6,7]. The understanding of the physicochemical characteristics of terpenes that facilitate the enhancement of skin permeation of drugs can lead to identification or development of more safe and effective enhancers. Quantitative structure-activity relationship (QSAR) method [8] can be applied. One approach is to apply the quantitative correlation between the skin permeation enhancing effects and the physicochemical descriptors of terpenes. Apart from applications in such problems as drug design [9,10], ADMET (absorption, distribution, metabolism, elimination and

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toxicity) prediction [11,12], and environmental risk assessment [13], QSAR has been explored for relating skin permeation of compounds to their physicochemical properties [14]. In 1990, Flynn compiled 97 human skin permeability coefficients from 15 different literature sources and identified *LogP* as the most important factor for determining the permeability coefficients [15]. His study, and other subsequent QSAR studies raised interest in using QSAR for modeling skin permeation [14,16–26]. These QSAR models provided insights into the mechanism of skin penetration and guidance for permeation studies as well as for predicting permeability of new compounds.

While the relationship between the permeability of permeants and their physicochemical properties has been extensively investigated, some important issues remain to be resolved. For instance, the correlation between the enhancing effects of some monoterpenes and the permeation of 5-fluorouracil has been studied without establishing a QSAR model [27]. More recently, QSAR models have been constructed for a total of 34 terpenes, 16 pyrrolidinone derivatives and 7 N-acetylprolinate esters with respect to several drugs [28]. These QSAR models are based on data of different sources and skin types, including human, rats and hairless mouse skins, with each of them 3 types of enhancer exhibiting different penetration enhancing mechanisms. However, these data need more careful analysis when combined into a single dataset as it is difficult to explain the large variations, primarily due to inter-laboratory differences such as skin sample types and sources, solvent systems for the enhancers and experimental protocols.

The aim of this study is to investigate how the physicochemical properties of terpenes influence their enhancing effects on the permeation of a model drug through the human stratum corneum using 49 terpenes, the largest number of enhancers compared in a single study [28]. The permeability coefficients are determined with standardized methods using human skin samples from abdomen areas of 3 healthy female donors. A full spectrum of terpenes is selected to include monoterpene, sesquiterpene, diterpene, triterpene and tetraterpene with various functional groups ranging from hydrocarbons, alcohols, aldehydes, ester, ketones, to oxides, respectively (Fig. 1).

Multiple linear regression (MLR), which is one of the most common and simplest method for constructing QSAR models, was used in this study [29–31]. The advantage of MLR is that it is simple to use and the derived models are easy to interpret. A selected set of physicochemical properties of terpenes was used as the predictor variables and the permeability coefficients (K_p) of the model drug, haloperidol (HP), in solutions containing different terpenes, were chosen as the response variable. HP is a suitable candidate for transdermal delivery and there is a clinical need to develop such a dosage form [32,33]. By nature, it is a hydrophobic molecule with low molecular weight (Fig. 1). The only long-lasting formulation is its ester, the haloperidol decanoate, for intramuscular injection, which, however, has disadvantages such as injection pain, marked inter-individual variation and complex administration regime. It is important to develop an alternative for its maintenance therapy to prevent the relapse of psychosis. The solvent, propylene glycol (PG), is commonly used in skincare products.

2. Materials and methods

2.1. Materials

The following chemicals were purchased from Sigma-Aldrich Chemical Company (Steinheim, Germany): Haloperidol, droperidol, DL-lactic acid, anitibiotic antimycotic solution (100×), propylene glycol, terpinolene, α-phellandrene, ocimene, myrcene, (1R)-(−)-myrtenal, (S)-(−)-perillaldehyde, carvacrol, thymol, (R)-(−)-carvone, (1R)-(−)-myrtenol, (−)-α-thujone, (R)-(+)-pulegone, (+)-dihydrocarvone, (−)-carveol, citral, (−)-isopulegol, (+)-dihydrocarveol, (−)-dihydrocarveol, (S)-(−)-citronellal, geraniol, nerol, (±)-linalool, menthone, β-citronellol, L-(−)-menthol, cyclohexanemethanol, A-humulene, (−)-α-cedrene, (+)-β-cedrene, (+)-aromadendrene, (+)-longifolene, (−)-trans-caryophyllene, (−)-caryophyllene oxide, (−)-epiglobulol, (−)-guaiol, (+)-cedrol, (−)-isolongifolol, (−)-α-santonin, octisalate, (+)-cedryl acetate, retinol, phytol, squalene. The following terpenes were purchased from TCI chemical company (Kyoto, Japan): (±)-α-bisabolol, farnesol, (±)-nerolidol, eucarvone, retinoic acid, β-carotene. All other chemical reagents were of at least reagent grades and used as supplied without further purification.

2.2. Analytical method

Drug concentrations were determined by a reversed phase HPLC method (C_{18} column, Agilent, Germany) [32]. A photodiode array (PDA) detector was used to obtain the chromatographs corresponding to the wavelengths ranging from 170 to 800 nm. Mobile phase consisted of 0.05 M phosphate buffer (pH adjusted to 3) and acetonitrile at a ratio of 50:50. Droperidol was used as an internal standard. Flow rate was 1.3 ml/min and injection volume was 100 μl. Retention times of the internal standard and drug were approximately 4.9 and 6.7 min at 254 nm, respectively. Mean peak area ratios of the drug and internal standard in 0.03% (v/v) lactic acid were linearly related to the drug concentrations for the samples containing 20 to 1000 ng/ml ($r^2 = 0.9990$).

2.3. Solubility study of HP

30 mg of HP was added to 1 ml of 5% (w/v) terpene solution in PG in plastic cuvettes. The cuvettes were sonicated for 1 h in a water bath at 37 °C and kept at 37 °C for up to 72 h. The solution was then centrifuged at 16,000 rpm for 5 min and then 100 μl of the solution from PG phase was carefully withdrawn. The centrifugation time for β-carotene was 15 min to achieve better phase separation. The solution was diluted appropriately with the mobile phase solution before HPLC assay.

The results (Table 1) showed that out of 49 terpenes, only (R)-(−)-carvone (2.43 mg/ml) and terpinolene (2.30 mg/ml) made the solubility of HP slightly lower than 3 mg/ml. In order to use the same concentration gradient across the epidermis, i.e, 3 mg/ml, it is impossible to use the same thermodynamic activity, i.e., the saturated solution of HP, for all the terpenes. The choice for the permeation study is to make the

concentration gradient constant so that other unknown variables which also influence the permeation coefficient can be elucidated.

2.4. Preparation of human epidermis

Abdominal skin was obtained from 3 different Chinese female donors with informed consent after plastic surgery. Epidermal samples were prepared by immersing the whole skin in 60 °C water for 2 min, followed by careful removal of the epidermis from the connective tissues [34]. Samples were stored in plastic bags at −80 °C until use. Prior to permeation experiments, these epidermal samples with the stratum corneum sides up were equilibrated by allowing them to float over 0.9% (w/v) sodium chloride solution containing antibacterial anti-mycotic solution (1 in 100 dilution) at 22±1 °C for 2 h.

2.5. In vitro permeation study with human epidermis

Flow-through type diffusion cells were used for the permeation studies [35]. Human epidermis was mounted between donor and receptor compartments and excessive skin at the sides was trimmed off to minimize lateral diffusion. Stratum corneum faced towards the donor compartment and the circular skin area for

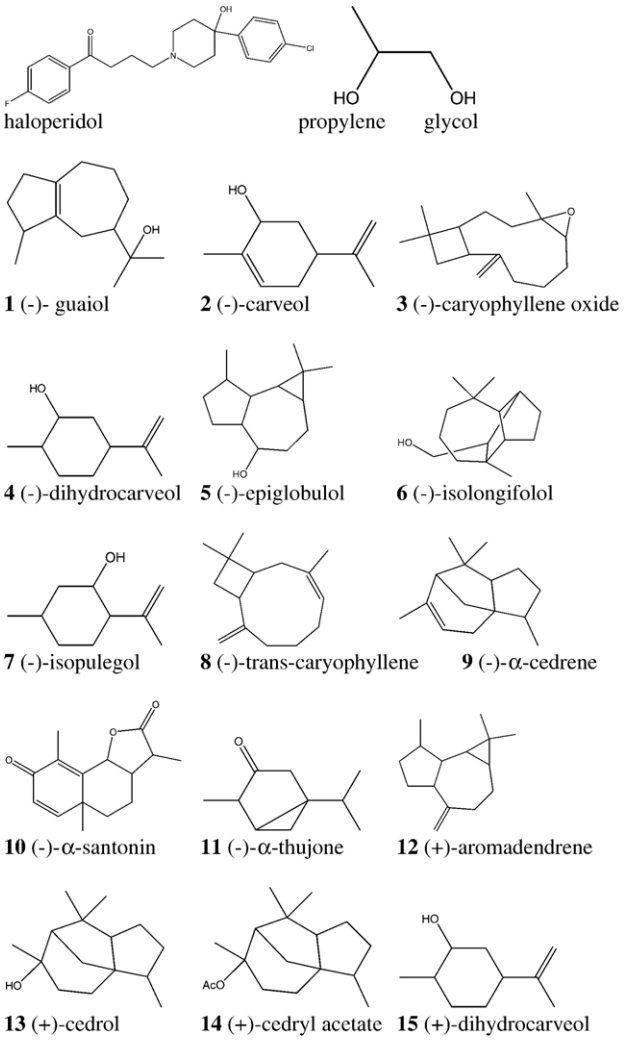

Fig. 1. The molecular structures of haloperidol, propylene glycol and 49 terpenes.

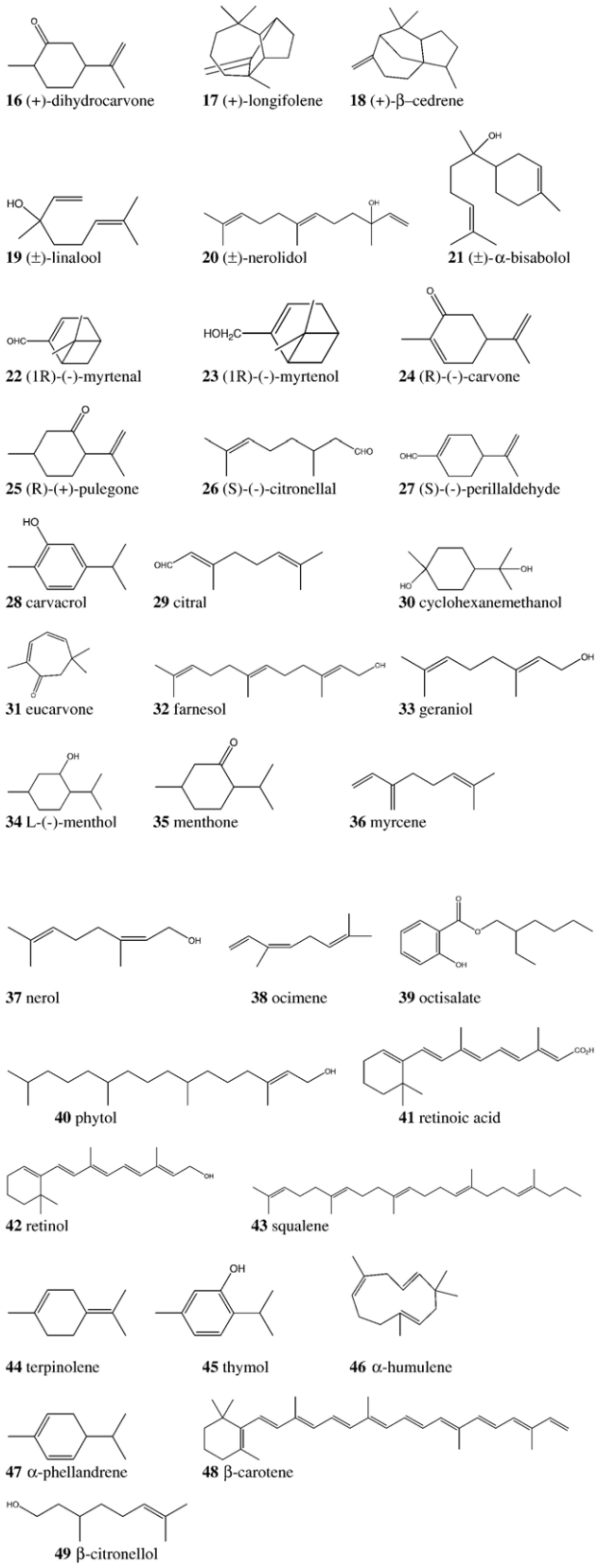

Fig. 1 *(continued)*.

Table 1
The second column is the name of each terpene, followed by its CAS entry and purity

No.	Terpene Name, [CAS] and purity / %	T	MW	mp/°C	bp/°C	*LogP*	Sol/mg.ml^{-1}	*Log*K_p /cm h^{-1}
0	Haloperidol	–	–	–	–	3.36	3.08±0.28	−9.04±0.06
1	(−)-guaiol [489-86-1] 99	2	222.37	90	288	4.75	4.73±0.31	−8.88±0.61
2	(−)-carveol [99-48-9] 97	1	152.23	Liquid	232	2.68	6.32±0.59	−6.45±0.15
3	(−)-caryophyllene oxide [1139-30-6] 99	2	220.35	63	280	4.57	4.49±0.38	−6.78±0.68
4	(−)-dihydrocarveol [20549-47-7] 97	1	154.25	Liquid	220	2.92	5.89±0.45	−8.87±0.10
5	(−)-epiglobulol [88728-58-9] 95	2	222.37	Liquid	294	4.81	4.95±0.38	−4.41±0.01
6	(−)-isolongifolol [1139-17-9] 99	2	222.37	112	–	4.05	4.63±0.19	−8.55±0.06
7	(−)-isopulegol [89-79-2] 99	1	154.25	Liquid	197	2.93	6.60±0.49	−8.35±0.21
8	(−)-trans-caryophyllene [87-44-5] 99	2	204.35	Liquid	268	6.78	5.09±0.02	−7.28±0.02
9	(−)-α-cedrene [469-61-4] 99	2	204.35	Liquid	263	6.38	4.62±0.10	−6.89±0.03
10	(−)-α-santonin [481-06-1] 98	2	246.30	171	423	1.80	5.71±0.38	−7.58±0.30
11	(−)-α-thujone [76231-76-0] 96	1	152.23	Liquid	206	1.90	6.83±0.08	−8.52±0.14
12	(+)-aromadendrene [489-39-4] 97	2	204.35	Liquid	258	6.41	4.77±0.15	−7.40±0.08
13	(+)-cedrol [77-53-2] 99	2	222.37	84	277	4.77	4.35±0.12	−7.96±0.00
14	(+)-cedryl acetate [77-54-3] 95	2	264.40	45	292	5.67	5.76±0.35	−5.52±0.33
15	(+)-dihydrocarveol [22567-21-1] 97	1	154.25	Liquid	220	2.92	6.28±0.53	−8.71±0.08
16	(+)-dihydrocarvone [7764-50-3] 98	1	152.23	Liquid	222	2.47	6.92±0.18	−7.17±0.05
17	(+)-longifolene [475-20-7] 99	2	204.35	Liquid	252	6.39	4.55±0.18	−7.42±0.01
18	(+)-β-cedrene [546-28-1] 97	2	204.35	Liquid	263	6.37	4.72±0.13	−7.01±0.06
19	(±)-linalool [78-70-6] 96	1	154.25	Liquid	199	3.28	5.05±0.13	−8.97±0.29
20	(±)-nerolidol [7212-44-4] 97	2	222.37	Liquid	276	5.31	5.10±0.20	−4.59±0.05
21	(±)-α-bisabolol [515-69-5] 99	2	222.37	Liquid	315	5.01	6.26±0.48	−5.25±0.30
22	(1R)-(−)-myrtenal [564-94-3] 98	1	150.22	Liquid	216	2.52	7.27±0.16	−5.29±0.06
23	(1R)-(−)-myrtenol [515-00-4] 95	1	152.23	Liquid	225	2.64	5.51±0.05	−828±0.04
24	(R)-(−)-carvone [6485-40-1] 98	1	150.22	Liquid	231	2.27	2.43±0.19	−7.56±0.11
25	(R)-(+)-pulegone [89-82-7] 98	1	152.23	Liquid	229	2.56	3.53±0.07	−6.63±0.25
26	(S)-(−)-citronellal [5949-05-3] 96	1	154.25	Liquid	208	3.48	9.43±0.61	−4.83±0.03
27	(S)-(−)-perillaldehyde [18031-40-8]	1	150.22	Liquid	238	2.81	6.34±0.06	−6.59±0.09
28	Carvacrol [499-75-2] 98	1	150.22	3.5	237	3.28	5.84±0.26	−8.44±032
29	Citral [5392-40-5] 96	1	152.23	Liquid	229	3.17	6.33±0.62	−5.08±0.03
30	Cyclohexanemethanol [565-50-4] 99	1	172.76	117	265	1.07	5.16±0.14	−8.08±0.55
31	Eucarvone [503-93-5]	1	150.22	Liquid	227	2.21	5.47±0.02	−7.60±0.04
32	Farnesol [4602-84-0] 97	2	222.37	Liquid	283	5.31	5.65±0.26	−6.72±0.36
33	Geraniol [106-24-1] 98	1	154.25	Liquid	230	3.28	6.11±0.69	−7.43±024
34	L-(−)-menthol [2216-51-5] 98	1	156.27	43	215	3.20	5.11±0.51	−7.34±0.05
35	Menthone [14073-97-3] 90	1	154.25	Liquid	209	2.63	7.53±0.08	−8.72±0.05
36	Myrcene [123-35-3] 95	1	136.23	Liquid	167	4.58	6.03±0.66	−5.43±020
37	Nerol [106-25-2] 97	1	154.25	Liquid	230	3.28	5.54±0.20	−7.80±0.01
38	Ocimene [3338-55-4] 70	1	136.23	Liquid	175	4.70	7.74±0.70	−5.41±0.01
39	Octisalate [118-60-5] 99	2	250.33	Liquid	332	5.77	3.14±0.34	−5.19±014
40	Phytol [7541-49-3] 97	3	296.53	Liquid	336	8.66	4.77±0.31	−5.13±0.02
41	Retinoic acid [302-79-4] 98	3	300.44	146	463	6.83	8.79±1.46	−12.13±0.90
42	Retinol [68-26-8] 97	3	286.45	63	421	6.84	7.11±0.23	−6.71±0.06
43	Squalene [111-02-4] 97	4	410.72	Liquid	429	13.09	3.91±0.16	−8.56±0.07
44	Terpinolene [586-62-9] 97	1	136.23	Liquid	182	4.67	2.30±0.10	−7.01±0.48
45	Thymol [89-83-8] 98	1	150.22	51	233	3.28	6.69±0.55	−8.29±0.10
46	α-humulene [6753-98-6] 99	2	204.35	Liquid	276	7.03	5.28±0.43	−6.23±0.03
47	α-phellandrene [99-83-2] 92	1	136.23	Liquid	171	4.43	4.83±0.21	−4.96±0.00
48	β-carotene [7235-40-7] 102.8	5	536.87	181	655	15.51	18.6±1.60	−11.15±0.19
49	β-citronellol [106-22-9] 95	1	156.27	Liquid	225	3.38	5.29±0.20	−7.66±0.26

The third column T indicates the terpene category. Key: 1 monoterpene, 2 sesquiterpene, 3 diterpene, 4 triterpene, 5 tetraterpene. The fourth to seventh columns are molecular weight, melting point, boiling point and *LogP* of each terpene, respectively. The boiling point of (−)-isolongifolol is not available and is estimated at 300 °C to be similar to the boiling points of other sesquiterpenes. The eighth column, Sol, is the solubility of HP in PG at 37 °C with or without 5% (w/v) enhancer. The last column K_p is the *in vitro* permeability coefficient of HP though human stratum corneum. Data are given as Mean±SD. For column 8 and 9, the data were determined experimentally in the lab. The other data were obtained from SciFinder Scholar® and original product information.

permeation was 0.785 cm^2. Since the solubility of HP in 0.03% (v/v) lactic acid solution is approximately 1 mg/ml, the receptor solution of 500 ml of 0.03% (v/v) lactic acid solution containing 1% (v/v) antibacterial antimycotic solution was placed in the reservoir bottle and allowed to flow through the receptor compartment at 0.75 ml/h. The pH of the receptor solution was approximately 3.3 but that did not affect the integrity of the epidermis [32]. Receptor solution was thoroughly degassed to prevent the formation of bubbles beneath the epidermis. An antibacterial and antimycotic solution was added to the receptor solutions to minimize the microbial contamination in samples during analysis. Solutions of HP (3 mg/ml) in PG with 5% (w/v)

enhancer or without enhancer (control) were prepared. When the solubility of HP fell below 3 mg/ml, the solution used was at the actual concentration, for example, the concentration of HP in PG with 5% (w/v) (R)-(−)-carvone is 2.43 mg/ml (Table 1). A 1-ml solution was added to the donor compartment and covered with Parafilm® to minimize the contamination of the solution. Ambient temperature of the cells was controlled at 37 °C by a heater/circulator (Haake, Germany). The receptor solutions were pumped by a 16-channel peristaltic cassette pump (Ismatec, Switzerland) continuously through the receptor compartment and drained into test-tube located in the fraction collector (ISCO Retriever IV, US). Cumulated receptor liquid samples were taken at 6-h intervals for HPLC assay. The permeation study was conducted continuously for 48 h.

Table 2
The second column is the lag time of the permeation and the third column is the correlation coefficient of the linear portion of the permeation profiles

No.	Terpene name, [CAS] and purity/%	L_t/h	r^2
0	Haloperidol	17.72±1.72	0.999±0.000
1	(−)-guaiol [489-86-1] 99	18.54±5.90	0.999±0.000
2	(−)-carveol [99-48-9] 97	21.83±6.65	1.000±0.000
3	(−)-caryophyllene oxide [1139-30-6] 99	24.94±18.4	0.989±0.012
4	(−)-dihydrocarveol [20549-47-7] 97	18.17±9.44	0.997±0.000
5	(−)-epiglobulol [88728-58-9] 95	54.13±39.6	0.993±0.002
6	(−)-isolongifolol [1139-17-9] 99	14.09±0.76	0.994±0.000
7	(−)-isopulegol [89-79-2] 99	26.98±7.62	0.997±0.000
8	(−)-trans-caryophyllene [87-44-5] 99	18.13±1.03	0.998±0.000
9	(−)-α-cedrene [469-61-4] 99	16.59±2.41	0.999±0.000
10	(−)-α-santonin [481-06-1] 98	19.82±4.22	0.999±0.000
11	(−)-α-thujone [76231-76-0] 96	23.18±7.03	1.000±0.000
12	(+)-aromadendrene [489-39-4] 97	18.10±0.56	0.997±0.001
13	(+)-cedrol [77-53-2] 99	19.98±1.32	0.991±0.006
14	(+)-cedryl acetate [77-54-3] 95	20.74±4.93	0.996±0.005
15	(+)-dihydrocarveol [22567-21-1] 97	23.73±1.83	0.998±0.000
16	(+)-dihydrocarvone [7764-50-3] 98	29.53±2.21	0.998±0.001
17	(+)-longifolene [475-20-7] 99	24.10±0.07	0.997±0.000
18	(+)-β-cedrene [546-28-1] 97	15.84±2.25	0.999±0.001
19	(±)-linalool [78-70-6] 96	21.29±5.45	0.989±0.007
20	(±)-nerolidol [7212-44-4] 97	45.91±4.80	0.999±0.000
21	(±)-α-bisabolol [515-69-5] 99	42.87±9.73	0.992±0.004
22	(1R)-(−)-myrtenal [564-94-3] 98	39.27±0.46	1.000±0.000
23	(1R)-(−)-myrtenol [515-00-4] 95	34.37±0.70	0.999±0.000
24	(R)-(−)-carvone [6485-40-1] 98	13.37±1.58	0.997±0.004
25	(R)-(+)-pulegone [89-82-7] 98	49.48±10.7	0.985±0.001
26	(S)-(−)-citronellal [5949-05-3] 96	21.33±1.60	0.971±0.011
27	(S)-(−)-perillaldehyde [18031-40-8]	24.46±2.30	0.999±0.000
28	Carvacrol [499-75-2] 98	13.37±1.58	0.997±0.004
29	Citral [5392-40-5] 96	16.90±2.08	0.991±0.011
30	Cyclohexanemethanol [565-50-4] 99	33.41±7.44	0.999±0.000
31	Eucarvone [503-93-5]	17.43±1.08	0.999±0.000
32	Farnesol [4602-84-0] 97	29.25±6.09	0.989±0.000
33	Geraniol [106-24-1] 98	29.02±2.85	0.996±0.001
34	L-(−)-menthol [2216-51-5] 98	28.49±7.82	0.983±0.007
35	Menthone [14073-97-3] 90	13.84±0.57	1.000±0.000
36	Myrcene [123-35-3] 95	3.645±0.21	0.988±0.005
37	Nerol [106-25-2] 97	34.82±2.56	0.997±0.004
38	Ocimene [3338-55-4] 70	2.271±0.05	0.962±0.037
39	Octisalate [118-60-5] 99	39.20±4.85	0.996±0.004
40	Phytol [7541-49-3] 97	4.246±0.02	0.998±0.002
41	Retinoic acid [302-79-4] 98	24.04±6.85	0.979±0.008
42	Retinol [68-26-8] 97	20.81±0.79	0.998±0.003
43	Squalene [111-02-4] 97	37.32±15.9	0.997±0.002
44	Terpinolene [586-62-9] 97	5.109±4.55	0.998±0.004
45	Thymol [89-83-8] 98	51.34±6.76	0.995±0.002
46	α-humulene [6753-98-6] 99	26.57±3.71	0.997±0.001
47	α-phellandrene [99-83-2] 92	3.642±1.84	0.984±0.004
48	β-carotene [7235-40-7] 102.8	19.41±3.05	0.975±0.005
49	β-citronellol [106-22-9] 95	16.82±5.93	0.998±0.002

2.6. QSAR model construction

The permeability coefficient K_p was experimentally determined and calculated by means of a nonlinear regression model. Details of the calculation have been described in our earlier publication [36]. In short, the cumulative amount of permeated drug, Q, is expressed as a function of time t, i.e., $Q=f(t)$, which is used to calculate the permeability coefficient K_p and the lag-time L_t by the relation of $K_p=K'D'$ and $L_t=1/(6D')$ where K' and D' are parameters obtained by nonlinear regression analysis. The calculated results of K_p and L_t are shown Tables 1 and 2, respectively. The average of L_t was about 24 h, higher than that of HP (about 17 h), suggesting that terpenes may interact with SC so that a longer time was required to achieve steady state. For the individual permeation curve, the correlation coefficient of the linear portion of the curve, r^2, is also given in Table 2, which showed that steady states were achieved.

The results showed that for most enhancers, less than 1 mg of HP penetrated after 48 h. So the remaining amount of HP is about 2 mg. Considering the fact that significant amount of PG also penetrated through the skin, the assumption of constant donor concentration of HP was valid.

The solubility of HP in PG was also determined experimentally. Other descriptors of terpenes including the molecular weight, melting point, boiling point and *LogP* were collected from SciFinder Scholar® (American Chemical Society, USA) and original product information literature. These data are tabulated in Table 1. Then, the QSAR model was fitted using Minitab 14® (State College, USA). The multiple linear regression equation was determined by a stepwise selection procedure.

3. Results and discussion

3.1. Regression model and predictor selection

The best regression equation based on stepwise selection is:

$$\begin{aligned}\text{Log } K_p = {} & -9.13 + 0.344\,\text{Log}P + 0.616\text{Liquid} - 4.84\,\text{Tri} \\ & -7.37\text{Tetra} + 2.03\text{Aldehyde} + 1.49\text{Ester} - 5.36\text{Acid} \\ n = 98, r^2 = 0.621, q^2 = 0.553, \text{SD} = 1.036, F = 21.03 \quad & (1)\end{aligned}$$

Abbreviations were listed as follows: K_p, permeability coefficient; *LogP*, logarithm of the octanol–water partition coefficient; n, number of observations; r^2, coefficient of determination; q^2, cross-validated correlation coefficient; SD, standard deviation; F, Fisher's statistic; MW, molecular weight; HBonds, hydrogen bonds; Liquid, Tri, Tetra are indicator variables, standing for liquid terpene, triterpene and tetraterpene, respectively; Aldehyde, Ester and Acid are also indicator variables, standing for terpenes with aldehyde, ester or acid functional groups, respectively.

Earlier studies have found that MW and HBonds are important factors affecting the permeability coefficients of permeants through the skin [37,38]. However, addition of these two descriptors to Eq. (1) did not improve the current regression model.

$$\begin{aligned}\mathrm{Log}\,K_p = {} & -9.70 + 0.280\ \mathrm{Log}\,P + 0.774\mathrm{Liquid} - 5.15\ \mathrm{Tri} \\ & -7.91\mathrm{Tetra} + 2.04\mathrm{Aldehyde} + 1.34\mathrm{Ester} - 5.53\ \mathrm{Acid} \\ & +0.00392\mathrm{MW} \\ \mathrm{n} = 98, \mathrm{r}^2 = 0.624, \mathrm{q}^2 = 0.544, \mathrm{SD} = 1.037, \mathrm{F} = 18.45 \end{aligned} \quad (2)$$

$$\begin{aligned}\mathrm{Log}\,K_p = {} & -9.27 + 0.356\ \mathrm{Log}\,P + 0.652\mathrm{Liquid} - 4.89\ \mathrm{Tri} \\ & -7.40\mathrm{Tetra} + 2.05\mathrm{Aldehyde} + 1.41\mathrm{Ester} - 5.44\ \mathrm{Acid} \\ & +0.047\mathrm{HBonds} \\ \mathrm{n} = 98, \mathrm{r}^2 = 0.621, \mathrm{q}^2 = 0.543, & \\ \mathrm{SD} = 1.041, \mathrm{F} = 18.24 & \end{aligned} \quad (3)$$

$$\begin{aligned}\mathrm{Log}\,K_p = {} & -9.69 + 0.252\ \mathrm{Log}\,P + 0.775\mathrm{Liquid} - 5.17\ \mathrm{Tri} \\ & -8.01\mathrm{Tetra} + 2.02\mathrm{Aldehyde} + 1.39\mathrm{Ester} - 5.49\ \mathrm{Acid} \\ & +0.00490\mathrm{MW} - 0.049\mathrm{HBonds} \\ \mathrm{n} = 98, \mathrm{r}^2 = 0.624, & \\ \mathrm{q}^2 = 0.539, \mathrm{SD} = 1.042, \mathrm{F} = 16.24 & \end{aligned} \quad (4)$$

A possible explanation is that the current descriptor subset in Eq. (1) encodes some of the information provided by the two descriptors, for example, triterpenes and tetraterpenes are generally larger in size than the other types of terpenes, therefore, MW may be at least partially redundant because the indicator variables for these two types of terpenes already encode some information about their respective sizes. Moreover, MW and *LogP* exerted some degree of collinear correlation at least for the enhancers considered here, and it is impossible to differentiate the effect of *LogP* from that of MW. As for HBonds, it was found that liquid terpenes generally have poorer ability to form hydrogen bonds than solid terpenes. The average numbers of hydrogen bonds that can be formed by liquid and solid terpenes are 1.1 and 2.0, respectively ($p<0.05$). As such, the indicator variable for liquid terpenes may encode the effects of hydrogen bonds, rendering the descriptor HBonds unnecessary.

Eq. (1) provides a simple way to predict the enhancing ability of terpene enhancers for drugs with physicochemical properties similar to HP. All the descriptors of Eq. (1) are readily available and the predictions are within the reasonable error range (Fig. 2). Compared with another reported model [28], which requires complicated descriptors such as electrotopological index and the lowest atomic charge in the molecule, Eq. (1) requires only the *LogP* value as the input variable, allowing for easier prediction of the penetration enhancing effects of other terpenes.

3.2. Coefficient of variation (cv) and coefficient of determination (r^2)

Among all the descriptive statistics, the coefficient of variation (cv) is defined as the ratio of standard deviation (SD) to the mean [39]. In this study, the cv of $LogK_p$ is 22.49%,

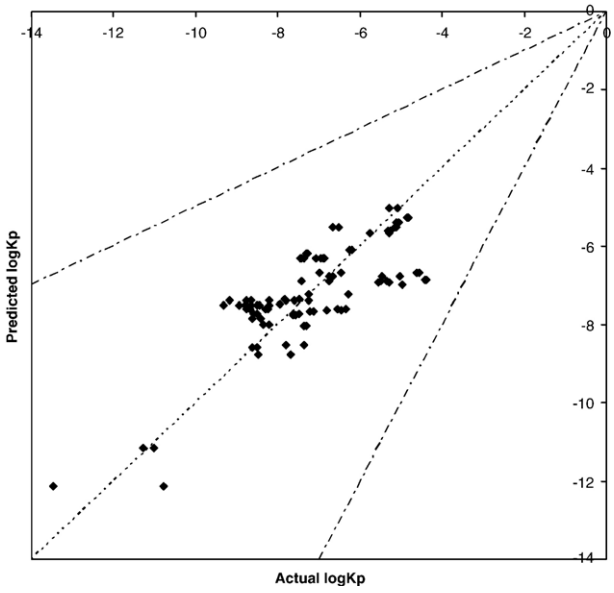

Fig. 2. Plot of predicted $LogK_p$ vs observed $LogK_p$. The dotted line represents line of unity. The area between the two dotted–dash lines represents an area within two-fold error respectively.

if $LogK_p$ was considered to be a random variable, influenced by the terpene enhancers and other factors. On the other hand, in linear regression analysis, the coefficient of determination (r^2) is defined as the ratio of regression sum of squares (SSR) to total sum of squares (SSTO), where r is known as correlation coefficient. The only link connecting cv and r^2 is SD, i.e., $\mathrm{SD} = \sqrt{\mathrm{SSTO}/(n-1)}$, where n is the number of data points. Although both cv and r^2 indicate variations, they are completely different statistics and by no means additive as argued in several publications [20,37,40]. The former is the measure of total variation of a certain data set, useful for comparing variation in data sets with markedly different means, or data expressed in different units of measurement, while the latter is the proportionate reduction of total variation associated with the use of predictor variables in linear regression analysis. For the current model, the r^2 is 0.621, which indicates that 62.1% of the total variation was reduced by introducing the 7 predictor variables in Eq. (1). Therefore, it is inappropriate to explain that the remaining 37.9% was caused by the 22.49% dataset variability. This can be exemplified by a simple linear regression where all observations fall on the fitted regression line. As such, the r^2 is unity and there is no remaining variations left to be accounted by the cv of the data set.

While the r^2 cannot be compared with cv, it is interesting to compare r^2 from the regression model with that from the one-way ANOVA model. For the current data set, the r^2 from the one-way ANOVA model is 97.63%, if the data were grouped by individual terpenes. It indicates that out of an SSTO of 254.42, 97.63% resulted from terpenes and the variation within group accounts for less than 3%. This infers that the terpene is the single important factor affecting $LogK_p$. The regression model shown in Eq. (1) could explain only 62% with the 7 factors, and although it led a smaller r^2 than ANOVA models, it yielded valuable information.

3.3. Predicted $LogK_p$ vs observed $LogK_p$ values

A plot of the predicted $LogK_p$ values from Eq. (1) against the observed values, as given in Fig. 2, shows that the predicted $LogK_p$ values for all the terpenes are less than two-fold from the observed $LogK_p$ values. This suggests that the model does not have a tendency to overpredict or underpredict the $LogK_p$ values and thus is useful for the prediction of $LogK_p$ values of terpenes. The predictive correlation coefficient, i.e., q^2, was obtained by cross-validation using a leave-one-out deletion pattern. For the current model shown in Eq. (1), q^2 is larger than 0.5, which also suggests it has good predictive ability.

3.4. The mechanistic implications of the model

For all the seven predictors from Eq. (1), *LogP*, Liquid, Aldehyde and Ester have positive coefficients while Tri, Tetra and Acid have negative coefficients. These suggest that, first, liquid terpenes tend to produce better enhancing effects than solid terpenes; second, triterpenes and tetraterpenes generally have poorer penetration enhancer effects than other terpenes; and third, terpenes with aldehyde and ester functional groups tend to increase $LogK_p$ while those with acid functional groups tend to decrease $LogK_p$.

Among all the predictors, *LogP* is the most interesting one, which consistently appears in many QSAR models including skin permeation models [14,37]. As a ratio of two concentrations at equilibrium, *LogP* represents collective effects of all intermolecular forces between a solute and the two types of media. From Eq. (1), it can be seen that terpenes with larger *LogP* values are more effective enhancers than those with smaller *LogP*. This may be explained by the fact that it is easier for the lipophilic terpenes to be mixed with stratum corneum intercellular lipids so as to extract lipids or induce lipid phase transition, the net effect offering a more permeable SC. Interestingly, it is also true that compounds with large *LogP* permeate faster than those with small *LogP*.

Apart from *LogP*, all the other predictors are qualitative variables. For Tri, Tetra and Acid, there is only one compound related to each of them, which are squalene, β-carotene and retinoic acid, respectively (Fig. 1). Although squalene is in liquid form at room temperature with a large *LogP*, its enhancing effect is poor, which made the coefficient of the descriptor of Tri negative. β-carotene and retinoic acid are both solids and retarded the permeation of the drug and have negative coefficients. It is found that liquid terpenes were more effective than solid terpenes, which may be explained by the difference in the numbers of HBonds that liquid and solid terpenes can form with the intercellular lipids of the SC. On average, liquid terpenes form fewer HBonds than solid terpenes. Therefore, it is easier for these liquid terpenes to pass through the lipid passages within the stratum corneum, where two of three major lipid components, i.e., ceramides and cholesterol, have the capacities to form HBonds [41,42]. One mechanism of facilitated penetration is the formation of micelles or other complexes by the enhancers with the permeants. These reversible complexes, which may decompose after passing though the stratum corneum, can permeate through SC lipid passages at a faster rate than the permeants alone, for the same reason that terpene enhancers can pass through the skin more rapidly than HP [43].

It is also noted that aldehyde and ester functional groups were found in terpenes, which are better enhancers. The boiling points of aldehydes are lower than those of corresponding alcohols due to the absence of HBonds. Their water solubility values are higher because of low molecular weights and large dipole interactions compared to the other groups. For esters, their boiling points are comparable to those of aldehydes but lower than those of acids and alcohols of comparable size due to the lack of HBonds between ester molecules. These relatively weak intermolecular forces may allow terpenes with aldehyde or ester functional groups to act more effectively as penetration enhancers than those with other functional groups.

3.5. Other deselected predictors

Although the solubilizing ability of terpenes for HP has been considered to be a very important factor for influencing skin permeation [41], the results from this study showed otherwise. It should be noted that the drug concentration was fixed at similar drug concentration gradients in order to compare the enhancing ability of terpenes. In general, greater solubilization is advantageous in transdermal drug delivery since a higher drug concentration creates a higher concentration gradient across skin, driving more drug through the skin. Typically, terpenes increase the solubility of HP to more than 3 mg/ml (Table 1). In this permeation study, HP at 3 mg/ml was the concentration in the donor cells so that the concentration gradients are similar across the SC. However, when formulating the dosage form, saturated solutions of the drug may be used to achieve better permeation.

Boiling point, a characteristic index of intermolecular interaction, was also deselected in this study. A possible explanation may be that it describes the properties at high temperature range while the current study is conducted at room temperature. Other predictors such as the types of terpene or functional groups were deselected because they did not show enough statistical significance.

3.6. Statistical estimation and theoretical relationship

By definition, $K_p = KD/l$. Taking logarithmic conversion on both sides of the equation, $LogK_p$ is a sum of $LogK$ and $\mathrm{Log}(D/l)$. The best regression equation included a most significant term of *LogP* (partition coefficient between octanol and water for terpenes) among all, which could be considered to be closely resemble to the *LogK* term (partition coefficient between vehicle and skin for HP). It indicates that the model constructed for QSAR is predictable by the theoretical relationship of $LogK_p = LogK + \mathrm{Log}(D/l)$. As a result, QSAR could be more accurately evaluated based on the quantitative influence of terpenes on these two terms, $LogK$ and $\mathrm{Log}(D/l)$. Although both P and K describe the partition between hydrophilic phase and hydrophobic phase but for terpenes and HP, respectively, the presence of terpenes in the hydrophilic phase (as vehicle) should

lead to a deviation of p value from K value with the extent dependent on the solubilizing effect of terpenes on HP in the vehicle. In light of this finding, the solubility (Sol) was forcibly added to the other predictor variables shown in Eq. (1) for another regression analysis. The result showed that the value of r^2 increased only by 0.1%. However, the positive coefficient of Sol suggested that higher solubility of HP could lead to an increased permeation. The reason may be because terpenes formed complexes with HP, which, in turn, increased *LogK*.

The second term Log(D/l) in the relationship of $LogK_p$= Log+Log(D/l) is considered to influence the diffusion pathway of terpenes, including diffusion resistance (D) and length of path (l). For a hydrophobic compound like HP, the former can be related to the easiness of diffusion across the lipid region and the latter is to describe the modification of diffusion path involving lipid region. Therefore, the perturbation of terpenes on the structure domain of lipid region should give different extent of the influence on this term, in turn affecting the resulting K_p value. As expected, these structural descriptors, including Triter, Tetrater, Ester, Aldehyde, and Acid, could have some influence on the Log (D/l) term resulting in their inclusion in the best regression model. However, instead of using qualitative descriptor, a quantitative term that can be related to the perturbation on the lipid region of skin, such as the phase transition temperature of lipid structure that modified by the presence of terpenes could be considered for future study.

4. Conclusion

Our study suggests that terpenes which possess one or combinations of the specific properties related to the level of hydrophobicity, phase (liquid state), appearance of specific functional groups (ester or aldehyde but not acid), and chemical types (not a triterpene or tetraterpene) may be better enhancers for drug permeation through skin. This knowledge is useful for the design of new terpene enhancers and for preliminary screening of terpenes as penetration enhancers. The established quantitative structure-activity relationship models allow the prediction of HSPE of other terpenes and terpenoid compounds for drugs with physicochemical properties similar to HP without the need to conduct *in vitro* experiments with scarce human skin samples. Moreover, in terms of methodology for QSAR study, it is inappropriate to partition the coefficient of the MRL model using the coefficient of variation of the data set.

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