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In vitro release and permeation kinetics of *Melaleuca alternifolia* (tea tree) essential oil bioactive compounds from topical formulations

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KEYWORDS: tea tree oil, skin permeation, headspace solid phase microextraction

Abstract

Melaleuca alternifolia essential oil (tea tree oil, TTO) is well known for its antimicrobial, antifungal and anti-inflammatory properties, which it is currently used as an active ingredient in skin care products.

This study investigates and evaluates the permeation and release kinetics of TTO marker compounds from several semisolid formulations (creams, ointments, gels) containing TTO in different percentages (5 - 30% w/w).

In vitro permeation and release tests were run in Franz diffusion cells with synthetic and pig-skin membranes for different times (from 10 minutes to 50 hours). The recovered receiving phases were analyzed by Headspace-Solid Phase Micro-Extraction (HS-SPME) in combination with Gas Chromatography coupled with Mass Spectrometry (GC-MS). The method adopted is completely automatic and provides on-line monitoring of the release and permeation kinetics, while avoiding time-consuming solvent extraction. The study examined both the total amount of essential oil and some selected markers known to be responsible for TTO's biological activities, i.e. 1,8-cineole, 4-terpineol and α-terpineol.

The results of *in vitro* release and permeation tests demonstrated that all compounds show the same kinetics profiles, although amount released differs significantly. The markers were quantified by external calibration curves constructed through HS-SPME-GC-MS. Some preliminary experiments were also run to monitor the presence of the above markers within the skin.

Introduction

Tea tree essential oil (TTO) can be obtained by steam distillation of fresh leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden & Betche) Cheel, *Melaleuca linariifolia* Sm. or *Melaleuca dissitiflora* F.Muell., provided that the essential oil conforms to the specifications given in ISO 4730-2004 and the European Pharmacopoeia[1]. TTO composition may be affected by atmospheric oxygen or by exposure to light or relatively high temperatures.

TTO is recognized as a useful remedy for certain skin infections thanks to its several biological activities, which include antibacterial, antifungal, and anti-inflammatory properties [2]. Several formulations containing TTO are commercially available, including creams, ointments, and gels.

In 2008, the opinion on TTO of the European Scientific Committee on Consumer Products (SCCP) concluded that the lack of adequate dermal absorption studies makes the magnitude of systemic exposure to tea tree oil from cosmetic products uncertain [3]. To the best of the authors' knowledge, since 2008 few articles reporting TTO dermal permeation studies have been published[4-6], and none has made a comparison among different formulations or results on total TTO dermal absorption. Only Reichling *et al.* in 2006[2] studied different formulations, but considering only one of the TTO markers, i.e. 4-terpineol.

In response to the SCCP opinion, the aim of this study is an in-depth investigation of TTO *in vitro* release and permeation kinetics from different topical formulations, in order to evaluate their marketing potential. In particular, the following issues were investigated: 1) the influence of the formulations' chemical compositions and of the TTO marker partition coefficients on the release

and permeation profiles of those formulations, 2) the total amount of TTO released by the formulations and absorbed through the skin, 3) the presence of TTO marker compounds retained in the skin. Formulations, and receiving phases at different time-points, were sampled by Head Space Solid Phase Micro Extraction and analyzed by gas chromatography coupled with mass spectrometer (HS-SPME-GC-MS). Quantitation was by HS-SPME-GC-MS with in-matrix external standard calibration. The optimized method was validated in terms of precision, linearity, and LOD and LOQ values.

Experimental

Chemicals and samples

Tea tree essential oil was supplied by Witt (Poirino, Italy).

Studies on dermal absorption were carried out on seven formulations prepared by adding different percentages of TTO to a base cream (2.5, 5, and 10%), a base ointment (5, 15 and 30%), and a gel (5%) in Witt laboratories. Pure standard samples of 4-terpineol, α-terpineol, 1,8-cineole, α-pinene, β-pinene, α-terpinene, *p*-cimene, γ-terpinene were from Sigma Aldrich (Milan, Italy). Solvents (acetone, cyclohexane) were all HPLC-grade from Sigma Aldrich (Milan, Italy). Phosphate saline buffer and sodium dodecyl sulfate were also from Sigma Aldrich.

In vitro release and permeation experiments

TTO release and permeation tests were carried out in static glass Franz diffusion cells mounted on a magnetic stirrer, using, respectively, synthetic cellulose membranes (Spectrapor, cut off 12000 Da) and ear pigskin slices isolated with a dermatome. The stirring rate was kept at 1000 rpm and the temperature at 25°C. Four receiving phases were prepared in a phosphate saline buffer 0.05M (pH 5.5) containing different solubilizing agents: sodium dodecyl sulphate 0.1%, TWEEN 80 1%, β-cyclodextrin 1% and N-methyl pyrrolidone 20%. The donor compartment, containing 1 gram of the above formulations ("infinite dose"), was sealed with Parafilm M® to prevent evaporation of the essential oil components. Eighteen different sampling times were investigated. At each times (0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 23, 24, 26, 28, 30, 32, 47, 48, 50 hours), the receptor phase (6 mL) was withdrawn and immediately replaced with an equal volume of fresh buffer. The stability of the formulations was verified by analyzing them before and after the *in-vitro* test.

Each experiment was repeated three times. Each pigskin specimen was from a different animal and was purchased from a local slaughterhouse.

SPME fibers

Polydimethylsiloxane (PDMS) and carboxen/divinylbenzene/PDMS (CAR/DVB/PDMS) SPME fibers were from Supelco Co. (Bellafonte, PA, USA). Before use, all fibers were conditioned as recommended by the manufacturer. Consistency of fiber performance was checked every 50 analyses through in-fiber external standardization with a standard mixture of hydrocarbons (C9-C25) in cyclohexane (1 µL of a 0.1 mg mL-1 solution) [7,8].

Sample preparation

Sampling conditions

For evaluation of release and permeation kinetics, at each sampling time, 1.8 mL of receiving phase were sampled in a 20 mL vial for 30 minutes at 35°C to mimic the skin temperature. For quantitation, for each sampling time, 10 µL of receiving phase were mixed in a 20 mL vial to evaluate the total amount of TTO released/permeated. Blank runs were carried out without detecting any carryover effects. After sampling, the fiber was automatically removed from the vapor phase, and inserted into the GC injection port, to desorb the sampled analytes thermally on-line into the GC column.

Fingerprints were normalized by the above in-fiber external standardization with a standard mixture of hydrocarbons (C9-C25) in cyclohexane (1 µL of a 0.1 mg mL-1 solution)[7,8].

Skin solvent extracts

Ear pigskin for the *in vitro* permeation tests was submitted to solvent extraction to evaluate the presence of TTO marker compounds. Extraction was carried out in an ultrasonic bath at 40°C for 15 minutes with 10 mL of acetone, repeating three times. The resulting extracts were dried and analyzed.

Analysis conditions

Analyses were carried out on an Agilent 6890 GC unit coupled to an Agilent 5973N MSD (Agilent, Little Falls, DE, USA) provided with a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed

GC conditions: injector temperature: 230°C, injection mode: split, ratio: 1/20; carrier gas: helium, flow rate: 1 mL min^{-1}; fiber desorption time and reconditioning: 5 min; column: Mega 5 column (95 % polydimethylsiloxane, 5 % phenyl) 25 m×0.25 mm dc ×0.25 µm df, from MEGA (Milan, Italy). Temperature program: from 50°C (1 min) to 105°C (0 min) at 3°C min^{-1}, then to 250°C (4 min) at 20°C min^{-1}.

MSD conditions: MS operated in EI mode (70 eV), scan range: 35 to 350 amu; target ions selected for quantitation are given in **Table 1**; dwell time 40 ms, ion source temperature: 230°C; quadrupole temperature: 150°C; transfer line temperature: 280°C. Marker compounds were identified by comparing their mass spectra and linear retention indices to those of authentic standards.

Quantitation

For essential oil quantitation and solvent extracts, a 5 mg mL^{-1} stock standard mixture of each pure standard of the selected markers in cyclohexane was prepared. For receiving phase quantitation, a stock standard mixture of the selected markers was prepared at 2 mg mL^{-1} of each pure standard in phosphate saline buffer 0.05M containing sodium dodecyl sulphate (0.1%). Suitable dilutions of each stock standard mixture were then prepared and renewed weekly. The resulting solutions (stock and diluted) were stored, respectively, at 0°C and 4°C. Two calibration curves were constructed by analyzing 5-7 sets of diluted mixtures at different concentrations (concentration ranges: 0.01-5 mg mL^{-1} for the essential oil, and 0.005-2 mg mL^{-1} for the receiving phases).

Calibration curves were constructed by direct injection, for essential oil and solvent extract quantitation, and by headspace sampling for the receptor phase.

Method validation

The method was validated on a three-week protocol, over three-months, and the following parameters were characterized: precision, linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ). Repeatability was evaluated by replicating HS-SPME sampling on a formulation every day for one week. Intermediate precision (inter-week precision) was measured by replicating HS-SPME sampling on a standard mixture of hydrocarbons (C9-C25) in cyclohexane (1 µL of a 0.1 mg mL^{-1} solution) every week over a period of six months. Linearity was assessed through linear regression analyses within the working range, over at least six different concentrations.

The Limit of Quantification (LOQ) was determined experimentally by analyzing decreasing concentrations of TTO in the receiving phase, sampled by HS-SPME; each sample was analyzed in triplicate, and the LOQ was the lowest concentration for which the instrumental response reported an RSD% of below 30% across replicate analyses. The LOD of each analyte was calculated from the average "peak to peak" noise value sampled in the analyte's region of elution in the chromatogram, with a coverage factor of 3.

Results and discussion

Essential oil quantitation

The tea tree essential oil used to prepare the formulations examined in this study was analyzed by GC-MS, and its markers were quantified by the external standard calibration approach. **Table 1** lists the tabulated and experimental retention indices of all TTO markers identified, the diagnostic ions used for their quantitation, partition coefficient (Log P) values, relative % abundances (area %), and true concentrations expressed as mg/g. The relative % abundances confirmed that the composition of the TTO complies with the ISO norm and that of the European Pharmacopoeia.

Sampling optimization

HS-SPME sampling and GC analysis conditions were optimized to obtain a fully automatic procedure for on-line monitoring the *in vitro* release and permeation kinetics. Two fiber coatings were tested to obtain a representative profile of the volatile fraction emitted by the receiving phase containing the released and permeated essential oil, in particular, polydimethylsiloxane 100 μm (PDMS), adopted by Gabbanini *et al.*[9], and carboxen/divinylbenzene/ polydimethylsiloxane (CAR/DVB/PDMS). **Figure 1** reports the HS-SPME-GC/MS profiles obtained by sampling the selected receiving phase containing TTO, with PDMS and CAR/DVB/PDMS respectively, under the same conditions. The results show that the PDMS fiber clearly discriminated the recovery of the TTO oxygenated monoterpenes (i.e. 4-terpineol and α-terpineol), while the CAR/DVB/PDMS fiber provided a reliable profile, qualitatively "superimposable" upon that of the essential oil (although, of course, they are not directly comparable). Moreover, the CAR/DVB/PDMS coating was also chosen for this study because it was more effective/sensitive than PDMS for the recovery of markers at trace levels.

All sampling parameters (vial volume, sample volume, sampling time) were carefully optimized to determine the best conditions giving a representative and reproducible HS-SPME-GC/MS profile.

Receiving phase selection

Four 0.05M phosphate saline buffer based receiving phases (pH 5.5) were prepared by adding appropriate amounts of different solubilizing agents so as to improve solubility of the TTO marker compounds, i.e. 0.1% of sodium dodecyl sulphate, 1% of TWEEN 80, 1% of β-cyclodextrin, and 20% of N-methyl pyrrolidone. These four receiving phases were tested to find the best compromise for the dual purpose of solubilizing the released and permeated EO components, and recovering them by headspace sampling. The best compromise was obtained with sodium dodecyl sulphate at 0.1%, since i) TWEEN 80 and β-cyclodextrin kept EO components in solution, making their headspace sampling impossible, and ii) N-methyl pyrrolidone was present in massive amounts in the headspace, although it provided a more favorable headspace/solution partition coefficient of the EO components (data not shown).

***In vitro* TTO release from topical formulations**

In vitro TTO release was evaluated by comparing the kinetics of different topical formulations, in order to find that giving maximum release at the minimum TTO concentration. The formulations were analyzed before and after the *in vitro* release test, to verify their stability; no modifications were observed during the tests.

In vitro TTO kinetic release from the above seven selected formulations was measured by monitoring the total TTO released into the receiving phase through a synthetic membrane at different times. TTO was expressed as the sum of the normalized peak areas of each marker divided by the membrane surface area (i.e. norm peak area/cm^2). All markers identified in the TTO profile were released into the receptor phase, although to different extents, the oxygenated monoterpenes being released in the largest amounts (i.e. 1,8-cineole, 4-terpineol, and α-terpineol) and the hydrocarbons only at trace levels (see **Figure 2)**.

Several mathematical models were tested to evaluate the kinetics of diffusion: i) cumulative normalized peak area per unit surface area (norm peak area/cm^2) plotted against time (hours) for

zero-order kinetics; ii) cumulative normalized peak area per unit surface area (norm peak area/cm^2) plotted against the square root of time, in line with Higuchi's model (pseudo-first-order model); iii) log of the cumulative normalized area per unit surface area (log norm peak area/cm^2) plotted against time (hours) for first-order kinetics[10].

The calculated coefficients of determination (R^2) were considered to be linear if their value was above 0.97. **Table 2** reports the in-vitro TTO release regression equations and R^2 values obtained applying the above three mathematical models. The R^2 values clearly indicate that almost all formulations follow zero-order kinetics, and thus the release profiles may be considered linear, with the exception of the 2.5% cream, the 5% ointment, and the 5% gel, which fit Higuchi's model more closely.

Figure 3 reports the TTO *in vitro* kinetic release profiles obtained for all the formulations under investigation, applying zero-order kinetics. As expected, for creams and ointments, the release rate increases with the increase of TTO concentration, showing the highest release with the 30% ointment. For the different formulations with the same TTO concentration (i.e. 5%), the fastest release is achieved using the gel formulation, while the slowest is given by the ointment. These results can be explained by the Log P values reported in **Table 1**. Almost all TTO markers are characterized by very high Log P values (above 4), indicating that they are released from a hydrophilic formulation, such as a gel, faster than they are from lipophilic ointments. These considerations highlight the fact that release is closely influenced by the physico-chemical properties of both formulations and bioactive markers, in particular by Log P.

Moreover, because of the low affinity of TTO compounds for gel, the release clearly shows that gel provides faster release than either ointment or cream, although the latter are formulated with higher TTO concentrations. This means that gels make it possible to use less concentrated formulations while keeping the same local/topical efficacy.

In vitro TTO percutaneous permeation

In vitro TTO kinetic permeation was evaluated by monitoring the total TTO permeated through the ear pigskin in the receiving phase (expressed as the sum of the normalized peak areas of each marker compound divided by the membrane surface area, i.e. norm peak area/cm^2) at different times. The formulations were also analyzed before and after the *in vitro* permeation tests, and no modifications were observed.

All TTO markers considered permeated the skin, although Cross et al.[4] reported that only? 4-terpineol and α-terpineol penetrated the epidermal membrane. As previously observed for release profiles, the oxygenated monoterpenes (i.e. 1,8-cineole, 4-terpineol, and α-terpineol) preferentially diffused through the skin, while hydrocarbons were only present at trace levels.

Figure 4 shows the TTO *in vitro* kinetic permeation profiles obtained for all formulations investigated, applying zero-order kinetics, while **Table 2** reports *in vitro* TTO permeation regression equations and R^2 values calculated with the above three mathematical models. In this case, too, the R^2 values clearly indicate that the permeation profiles of all formulations are linear, following zero-order kinetics. The fastest permeation rate was with the 5% gel, followed by the 30% ointment.

Figure 5 shows the correlation between release and permeation kinetics of the seven formulations investigated. Almost all of them present linear relationships, indicating that release and permeation have the same trend (i.e. permeation increases linearly as the release rate increases). The only exception is the gels, for which a potential regression fit the data more closely, and a high permeation rate was only achieved with a relatively high release.

Quantitation of released and permeated TTO components

The total amount of each marker released and permeated during 50 hours was determined quantitatively, with the external standard calibration approach combined with HS-SPME sampling. Standard solutions at different concentrations were prepared directly in a blank (TTO free)

receiving phase, to bypass the strong matrix effect due to the presence of the surfactant. **Table 3** reports the total absolute amount initially present in the formulation, and the percentage of that total released and permeated, for 1,8-cineole, 4-terpineol, and α-terpineol. The results show that the amount released ranged, for the different markers, a) from 5% (5% ointment) to 32% (30% ointment) for 1,8-cineole, accounting for 88 and 3642 μg/cm^2 respectively, b) from 2% (5% ointment) to 44% (5% gel) for 4-terpineol, corresponding to 277 and 5437 μg/cm^2, and c) from 1% (5% ointment) to 52% (5% gel), for α-terpineol, that is 20 and 941 μg/cm^2. The amount permeated ranged a) from 2% (almost all creams and ointments) to 14% (5% gel) for 1,8-cineole, corresponding to 29 and 235 μg/cm^2 respectively, b) from 0.4% (5% ointment) to 15% (5% gel) for 4-terpineol, that is 71 and 2103 μg/cm^2, and c) from 0.2% (5% ointment) to 15% (5% gel) for α-terpineol, that is 5 and 312 μg/cm^2.

The quantitative data confirmed that the 5% gel is very effective, in general giving the highest release and permeation percentages.

Skin retention of TTO marker compounds

The results showed that only 4-terpineol and α-terpineol are retained in the skin. The highest retention was observed with the 30% ointment that, respectively, gave 0.52 μg/cm^2 for 4-terpineol and 0.41 for μg/cm^2 α-terpineol. On the contrary, the 5% gel showed the lowest retention, giving, respectively, 0.09 μg/cm^2 for 4-terpineol and 0.15 for μg/cm^2 for α-terpineol. This is in agreement with permeation kinetics. In this case, retention was positively affected by the greater affinity of ointments for the skin compared to gels. However, retention uptake was in all cases negligible, being below 0.1%. Further studies are under way to optimize the extraction procedure.

Method validation

The results show very high precision, the average RSD% for each TTO marker being in all cases below 10%. The intermediate precision was also satisfactory, since RSD% on the standard C9-C25 mixture never exceeded 20%, ranging from 8.5% for C9 to 18.8% for C25.

The LOD values were 25 ppb for 4-terpineol, 40 ppb for α-terpineol, and 15 ppb for 1,8-cineole, while their LOQ values were 125 ppb, 200 ppb and 75 ppb, respectively.

Linearity within the working calibration range was satisfactory, R^2 values always being above 0.99.

Conclusions

The HS-SPME/GC-MS method adopted here is a useful approach for on-line monitoring *in-vitro* release and permeation kinetics, thanks to careful optimization that sought to reduce both sampling and GC analysis times. The method can be further speeded up to make it applicable to on-line monitoring in the short run, by adopting automatic high speed sampling and fast GC.

The results of this study demonstrate that both matrix composition and Log P of the TTO markers markedly affect markers' release and permeation rates. The gel formulations showed the highest percentages of marker release and permeation compared to creams and ointments, including at lower TTO concentrations. This suggests that gels make it possible to use less concentrated formulations while keeping local/topical activity constant. The most released and permeated compounds are 4-terpineol, α-terpineol and 1,8-cineole; the other TTO markers are released and permeated at trace levels.

Skin retention may be considered negligible, being in all cases below 0.1% of the total amount present in the formulation.

The above results demonstrate the key role played by the composition of topical formulations in TTO release and permeation kinetics profiles. As a general consideration, in selecting a suitable vehicle as a function of local activity, creams should chiefly be proposed for cosmetic applications, and gel for dermatological uses.

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Caption to Figures

Figure 1. HS-SPME-GC/MS profiles obtained by sampling the selected receiving phase containing TTO, respectively, with PDMS and CAR/DVB/PDMS. Legend: 1) α-pinene, 2) α-terpinene, 3) *p*-cymene, 4) 1,8-cineole, 5) γ-terpinene, 6) α-terpinolene, 7) 4-terpineol, 8) α-terpineol.

Figure 2. HS-SPME-GC/MS profiles obtained by sampling the selected receiving phase, collected from *in vitro* release (synthetic membrane) and permeation (ear pigskin) test, respectively.

Figure 3. TTO *in vitro* kinetic release profiles obtained for all formulations under investigation, applying zero-order kinetics.

Figure 4. TTO *in vitro* kinetic permeation profiles obtained for all formulations under investigation, applying zero-order kinetics.

Figure 5. Correlation between release and permeation kinetics for the seven formulations considered.

Table 1. List of TTO marker compounds identified, together with their tabulated and experimental retention indices, marker diagnostic ions used for quantitation, Log P values, relative % abundances (area %) and true concentrations expressed as mg/g

α-pinene	939	938	93	4.37	6.0	71
α-terpinene	1018	1018	93	5.52	7.5	62
p-cymene	1026	1028	119	4.02	2.4	22
1,8-cineole	1033	1034	43	2.82	5.0	33
γ-terpinene	1062	1062	93	4.36	19.8	239
α-terpinolene	1088	1089	93	4.67	1.8	22
4-terpineol	1177	1178	71	2.99	43.7	450
α-terpineol	1189	1191	59	2.79	6.4	65

Table 2. Regression equations and R^2 values obtained applying three different mathematical models (zero-order kinetics, Higuchi model, and first-order kinetics) to *in vitro* TTO release and permeation tests data.

Formulation	Mathematical model	Equation	R^2
In vitro release kinetics			
2.5% cream	zero-order kinetics	y = 779560x + 2E+06	0.9807
	Higuchi model	y = 6E+06x - 4E+06	**0.9832**
	first-order kinetics	y = 0.0291x + 6.4142	0.7397
5% cream	zero-order kinetics	y = 2E+06x + 5E+06	**0.9848**
	Higuchi model	y = 1E+07x - 9E+06	0.9710
	first-order kinetics	y = 0.026x + 6.8883	0.7841
10% cream	zero-order kinetics	y = 3E+06x + 7E+06	**0.9752**
	Higuchi model	y = 2E+07x - 2E+07	0.9364
	first-order kinetics	y = 0.0265x + 7.0797	0.7782
5% ointment	zero-order kinetics	y = 859811x + 3E+06	0.9352
	Higuchi model	y = 7E+06x - 5E+06	**0.9730**
	first-order kinetics	y = 0.03x + 6.4354	0.7278
15% ointment	zero-order kinetics	y = 4E+06x + 5E+06	**0.9885**
	Higuchi model	y = 3E+07x - 3E+07	0.9555
	first-order kinetics	y = 0.0312x + 7.0188	0.7755
30% ointment	zero-order kinetics	y = 8E+06x + 2E+07	**0.9813**
	Higuchi model	y = 6E+07x - 5E+07	0.9584
	first-order kinetics	y = 0.0281x + 7.4551	0.7629
5% gel	zero-order kinetics	y = 4E+06x + 3E+07	0.9533
	Higuchi model	y = 3E+07x - 5E+06	**0.9838**
	first-order kinetics	y = 0.0196x + 7.4896	0.7464
In vitro permeation kinetics			
2.5% cream	zero-order kinetics	y = 342153x - 181162	**0.9731**
	Higuchi model	y = 3E+06x - 3E+06	0.9034
	first-order kinetics	y = 0.0222x + 6.7312	0.5370
5% cream	zero-order kinetics	y = 505615x + 356925	**0.9871**
	Higuchi model	y = 4E+06x - 5E+06	0.9731
	first-order kinetics	y = 0.0408x + 5.587	0.6731
10% cream	zero-order kinetics	y = 929375x - 1E+06	**0.9776**
	Higuchi model	y = 7E+06x - 1E+07	0.9314
	first-order kinetics	y = 0.0582x + 5.3173	0.6345
5% ointment	zero-order kinetics	y = 306873x - 428493	**0.9920**
	Higuchi model	y = 2E+06x - 3E+06	0.9403
	first-order kinetics	y = 0.0491x + 5,0931	0.7086
15% ointment	zero-order kinetics	y = 971481x - 564075	**0.9932**
	Higuchi models	y = 7E+06x - 1E+07	0.9503
	first-order kinetics	y = 0.0403x + 5.9957	0.7597
30% ointment	zero-order kinetics	y = 2E+06x - 2E+06	**0.9855**
	Higuchi model	y = 1E+07x - 2E+07	0.9485
	first-order kinetics	y = 0.0539x + 5.7358	0.6545
5% gel	zero-order kinetics	y = 3E+06x - 4E+06	**0.9884**
	Higuchi model	y = 2E+07x - 3E+07	0.9407
	first-order kinetics	y = 0.043x + 6.1425	0.5976

Table 3. Total absolute amount and percentage (of the total amount initially present in the formulations) released and permeated for 1,8-cineole, 4-terpineol and α-terpineol.

Formulation	Compound	released amount		permeated amount	
		μg/cm^2	%	μg/cm^2	%
5% gel	1,8-cineole	236	17	235	14
	4-terpineol	5437	44	2103	15
	α-terpineol	941	52	312	15
2.5% cream	1,8-cineole	72	9	74	9
	4-terpineol	354	5	182	3
	α-terpineol	38	4	14	1
5% cream	1,8-cineole	137	8	31	2
	4-terpineol	874	6	84	1
	α-terpineol	102	5	6	0.3
10% cream	1,8-cineole	318	7	93	2
	4-terpineol	1648	4	248	1
	α-terpineol	190	3	21	0.4
5% ointment	1,8-cineole	88	5	29	2
	4-terpineol	277	2	71	0.4
	α-terpineol	20	1	5	0.2
15% ointment	1,8-cineole	482	7	142	2
	4-terpineol	2496	4	550	1
	α-terpineol	275	3	46	1
30% ointment	1,8-cineole	3642	32	214	2
	4-terpineol	10047	10	663	1
	α-terpineol	1120	8	58	0.4

Figure 1

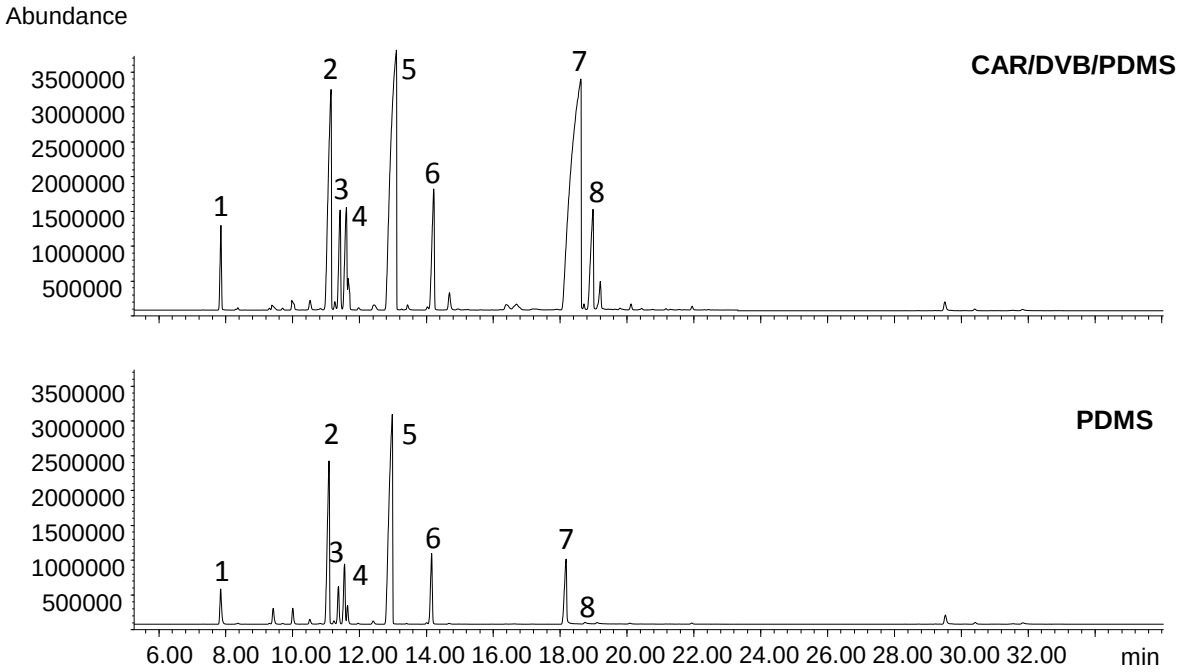

Abundance
CAR/DVB/PDMS
PDMS
3500000
3000000
2500000
2000000
1500000
1000000
500000
1
2
3
4
5
6
7
8
6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00
min

Figure 2

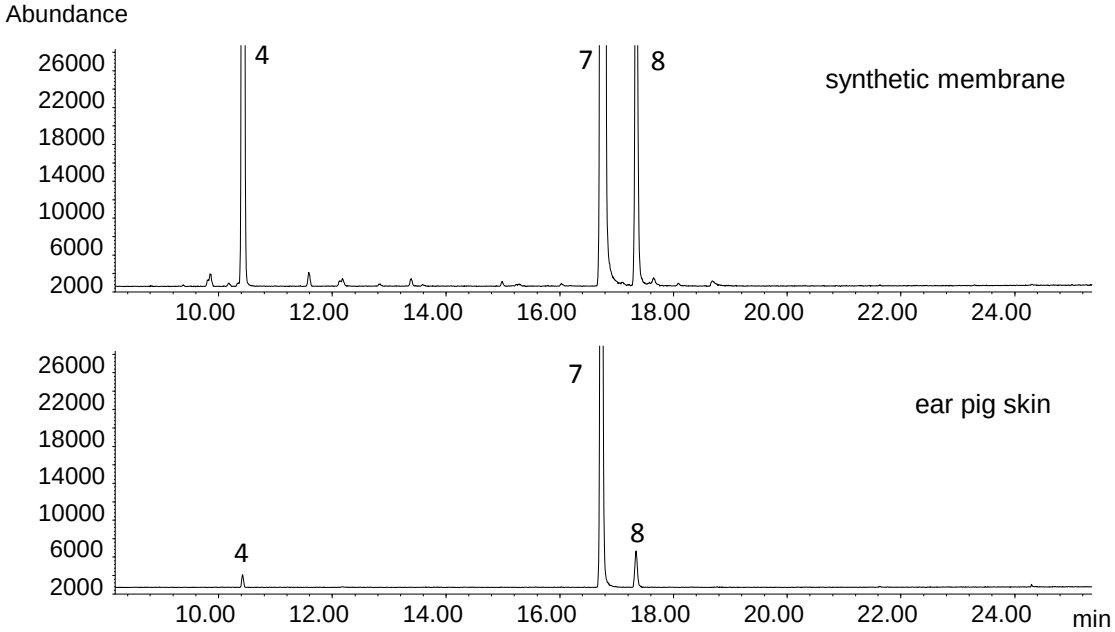

Abundance
26000
22000
18000
14000
10000
6000
2000
4
7
8
synthetic membrane
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
ear pig skin
min

Figure 3

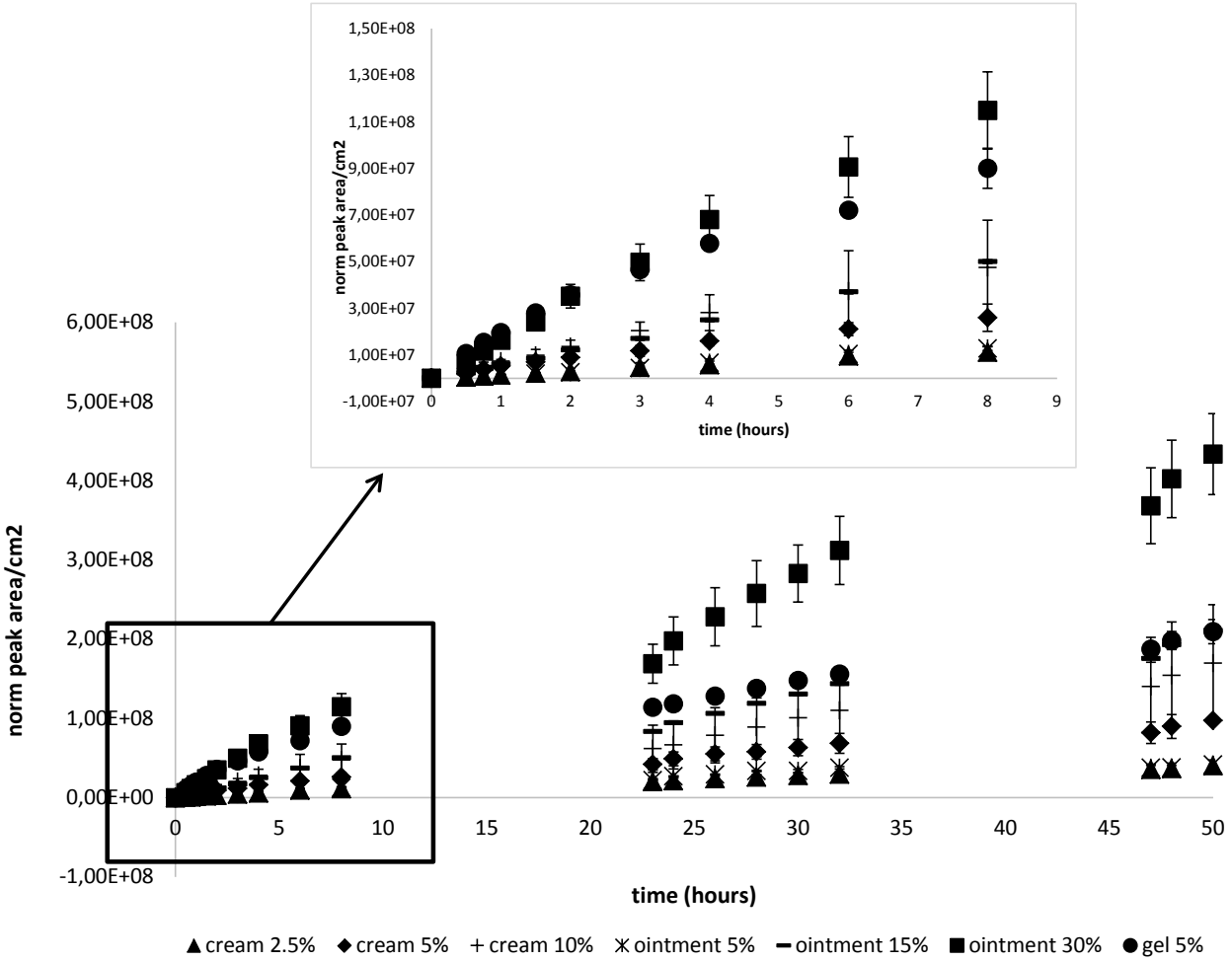

norm peak area/cm2
6,00E+08
5,00E+08
4,00E+08
3,00E+08
2,00E+08
1,00E+08
0,00E+00
-1,00E+08
0
5
10
15
20
25
30
35
40
45
50
time (hours)
1,50E+08
1,30E+08
1,10E+08
9,00E+07
7,00E+07
5,00E+07
3,00E+07
1,00E+07
-1,00E+07
0
1
2
3
4
5
6
7
8
9
norm peak area/cm2
time (hours)
cream 2.5%
cream 5%
cream 10%
ointment 5%
ointment 15%
ointment 30%
gel 5%

Figure 4

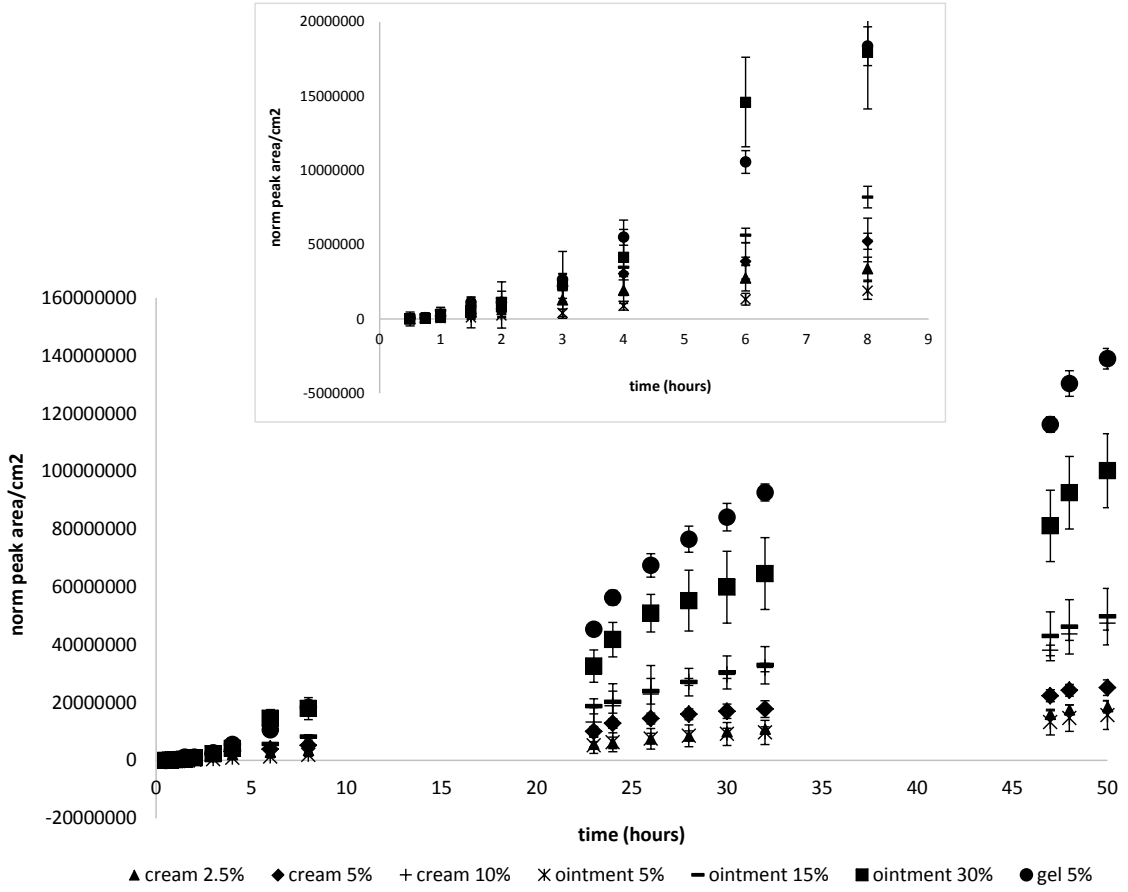
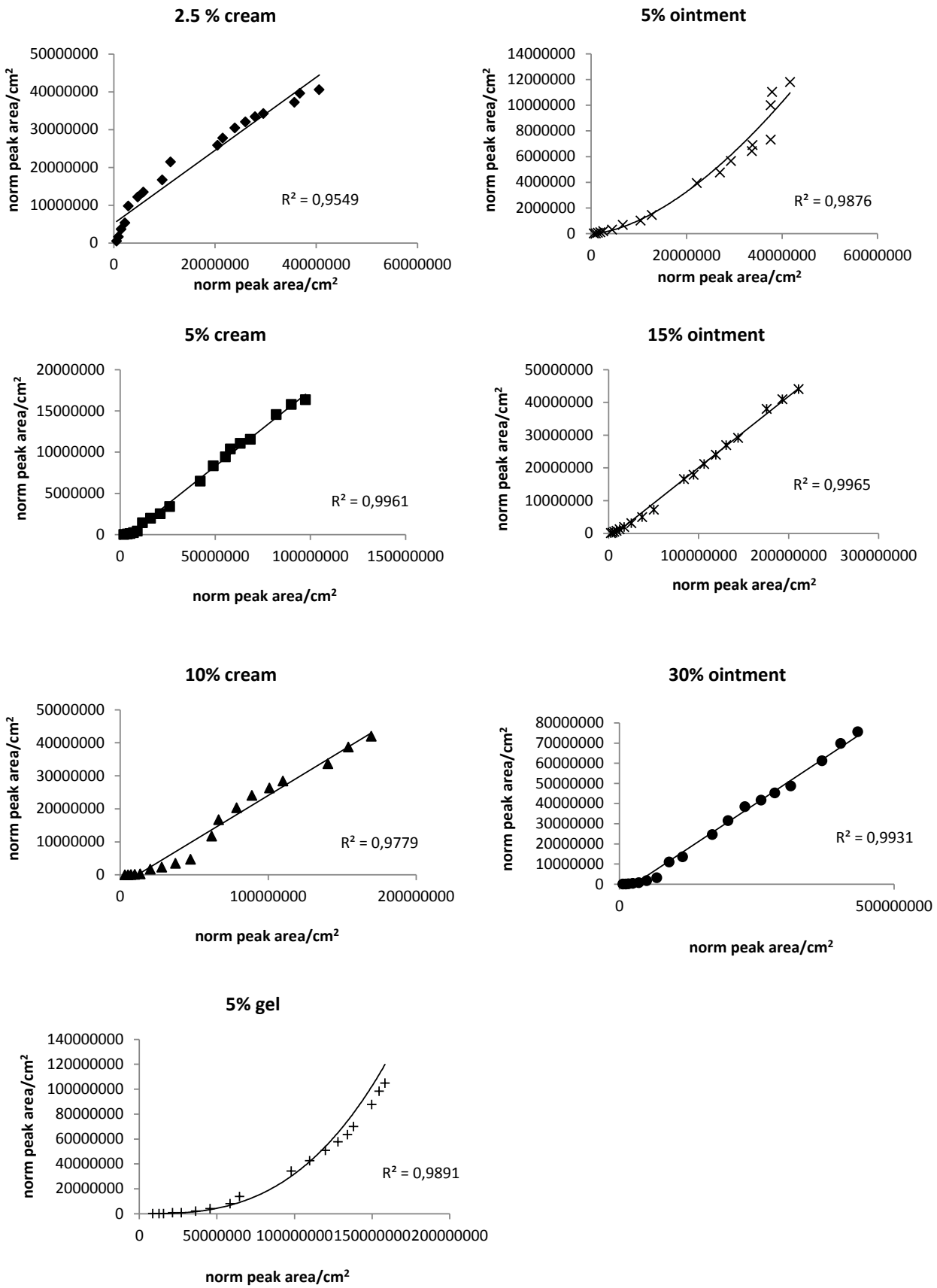

norm peak area/cm2
time (hours)
▲ cream 2.5%
◆ cream 5%
+ cream 10%
✕ ointment 5%
– ointment 15%
■ ointment 30%
● gel 5%

Figure 5


2.5 % cream
norm peak area/cm²
R² = 0,9549
5% ointment
R² = 0,9876
5% cream
R² = 0,9961
15% ointment
R² = 0,9965
10% cream
R² = 0,9779
30% ointment
R² = 0,9931
5% gel
R² = 0,9891