Physicochemical effects of terpenes on organogel for transdermal drug delivery

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ARTICLE INFO

ABSTRACT

It is accepted that terpenes are effective penetration enhancers to promote the passage of drugs or chemicals through the human skin barrier. However the physical and chemical changes of a pharmaceutical vehicle induced by the incorporation of terpenes have not been explored. Thus, this study examines the effects of three terpenes (linalool, cineole, limonene) on the rheology and chemical stability of an organogel composed of dibutyllauroylglutamide (GP1) and propylene glycol (PG). At a given GP1 concentration, oxygen-containing linalool and cineole decreased gel moduli (elastic and viscous) and brittleness, and the reverse was obtained for hydrocarbon limonene. Probably, linalool and cineole interfered with hydrogen bonding between GP1 molecules while limonene could have initiated a phase separation-mediated gelation, changing the gel morphology. Microcalorimetry detected minute heat endotherms for gels (with and without terpenes) subjected to accelerated heat testing. These heat changes could arise from a small degree of structural disruption of the gel network. Heat endotherms normalized with respect to GP1 content were used to assess gel chemical stability. Although the terpenes altered rheology, they did not significantly affect the chemical stability of the gels. This is the first in the literature that reports the effect of penetration enhancers, such as terpenes, on the physical, rheological and chemical characteristics of a model pharmaceutical formulation for topical and transdermal drug delivery.

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1. Introduction

Gels are classified into hydrogels (Hoffman, 2002; Peppas et al., 2000) and organogels (Abdallah and Weiss, 2000; Terech and Weiss, 1997). Hydrogels have been extensively explored as a matrix for the release of protein- and peptide-based drugs or drugs with a high aqueous solubility (Bromberg and Ron, 1998; Wu et al., 2005). Organogels (organogelators dissolved in organic solvents) are potential drug delivery systems. An organogel could be used as a drug vehicle in a topical application or as a drug reservoir/matrix in a transdermal patch. Advantages of this gel vehicle include the capacity to accommodate polar and non-polar drugs, thermoreversibility, high degree of stability to moisture and temperature, and the ability to control drug release (Anand et al., 2001).

Organogels are usually prepared by heating the gelators in organic liquids and cooling the solutions to ambient temperature (Terech and Weiss, 1997). There are several types of organogelators which include steroid derivatives, anthryl derivatives, amino acid-type organogelators and organometallic compounds. Aggregation in organogels results from a different set of interactions. In non-aqueous liquids, the binding forces are primarily dipolar interactions, intermolecular hydrogen bonds or metal-coordination bonds. Dibutyllauroylglutamide (GP1) (Fig. 1) is an example of an amino acid-type gelator. Intermolecular hydrogen bonding between the amide groups and hydrophobic interaction between the long alkyl chains of GP1 molecules are the dominant attractive forces in GP1 organogels. The self-assembly of GP1 molecules into nano-sized fibres forms a heavily branched interlocking network (Li et al., 2005; Lim et al., 2006) which immobilizes the liquid component via surface tension (Lu and Weiss, 1995).

A biocompatible organogel composed of GP1 in propylene glycol (PG) incorporated with a terpene as a chemical enhancer has been used as a vehicle to deliver haloperidol, an anti-psychotic drug, across human skin in vitro (Kang et al., 2005; Lim et al., 2006). Terpenes, natural volatile oils extracted from plant sources, were reported to promote percutaneous absorption of several other drugs (Williams and Barry, 2004). However, the effects of terpenes on an organogel, to our knowledge, have not been examined. The terpene molecules in the gel could disrupt or weaken the intermolecular forces between the gelator molecules, and hence could change the characteristics of the gel.

Basically, our study focuses on the physical stability, physical breakdown of the gel network under the influence of an external physical strain, and the chemical stability, degradation of the gel network into individual gelator molecules under storage at an accelerated temperature, in the presence of three
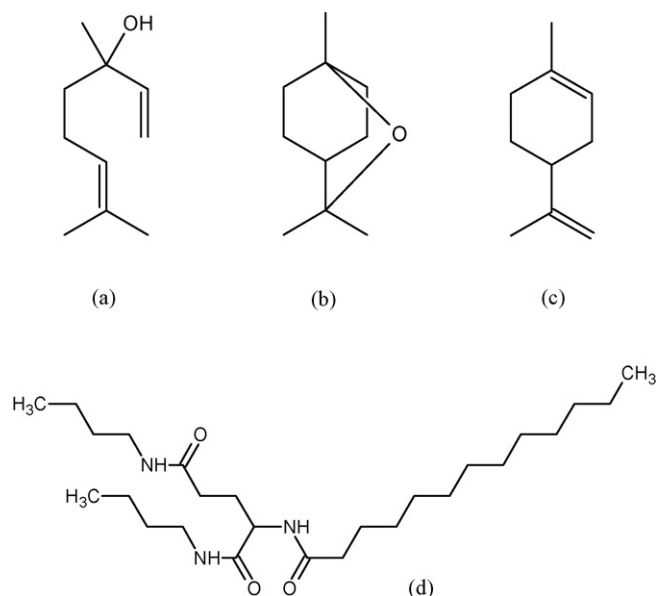
Fig. 1. Chemical structures of (a) $(\pm)$-linalool (MW = 154.3; $\log P$ = 2.97), (b) 1,8-cineole (MW = 154.3; $\log P$ = 2.50), (c) (+)-limonene (MW = 136.2; $\log P$ = 4.83) and (d) dibutyllauroylglutamide (GP1) (MW = 453.7; $\log P$ = 5.02) (Howard and Meylan, 1997; Kang et al., 2005).

terpenes—linalool, cineole and limonene (Fig. 1). These two aspects were investigated via rheometry and microcalorimetry. The information generated in this study could provide insights to the appropriate use of the gel (either topical or transdermal application) and the shelf-life of the gel.

2. Materials and methods

2.1. Materials

1,8-Cineole (99%), (+)-limonene (97%), $(\pm)$-linalool (97%), propylene glycol were obtained from Sigma–Aldrich (Steinheim, Germany). Dibutyllauroylglutamide (>85%) was obtained from Ajinomoto Co. (Japan). Chemicals were of at least reagent grade. All materials were used as received.

2.2. Gel preparation

Organogels containing GP1 and terpene in PG were prepared. Henceforth, organogels containing terpenes are collectively referred to as terpene gels, and organogels containing linalool, limonene and cineole are, respectively, referred to as linalool, limonene and cineole gels. Organogel without terpene is referred to as control gel. Measured amounts of GP1, terpene and PG in a covered glass container were mixed and placed in an oven at 120 °C for ca. 30 min for fast dissolution. A white gel was formed following cooling to room temperature (Liu and Sawant, 2002; Sawant and Liu, 2002).

2.3. Gel rheology and gel–sol transition

Gel rheology was determined with advanced rheometric expansion system (ARES) from Rheometric Scientific (New Jersey, USA). The gel sample was placed between an upper plate fixture of 25 mm diameter and a *Peltier* surface and subjected to sinusoidal oscillations. A gap of 1 mm was maintained between the two surfaces. In a dynamic strain sweep test conducted at 1 Hz and 32 °C, elastic modulus G' and viscous modulus G'' versus strain γ profiles were

Table 1
Rheological behaviour of organogels (with and without terpenes) expressed in the form of empirical correlations

Organogel	Correlation	r^2
Control[a]	$\log G' = 4.27 \log C_{GP1} + 1.74$	0.976
	$\log G'' = 4.42 \log C_{GP1} + 0.738$	0.970
	$\log G' = -14.0\gamma_o + 6.70$	0.984
	$\log G'' = -14.5\gamma_o + 5.88$	0.978
Linalool[b]	$\log G' = 4.03 \log C_{GP1} + 1.77$	0.968
	$\log G'' = 4.21 \log C_{GP1} + 0.731$	0.979
Limonene[c]	$\log G' = -3.98 \log \gamma_o + 0.855$	0.993
	$\log G'' = -4.24 \log \gamma_o - 0.117$	0.996
Cineole[d]	$\log G' = 3.87 \log C_{GP1} + 1.76$	0.980
	$\log G'' = 4.11 \log C_{GP1} + 0.681$	0.982
	$\log G' = -18.2\gamma_o + 7.18$	0.971
	$\log G'' = -19.3\gamma_o + 6.45$	0.973

Units: G' (Pa); G'' (Pa); γ_o (%); C_{GP1} (%, w/v).
  [a] Gel composition: GP1, PG.
  [b] Gel composition: GP1, 5% (v/v) linalool in PG.
  [c] Gel composition: GP1, 5% (v/v) limonene in PG.
  [d] Gel composition: GP1, 5% (v/v) cineole in PG.

generated as γ increased from 0.01 to 10%. G' and G'' were obtained from the initial linear viscoelastic region. Critical strain γ_o, the onset of gel fibre rupture, was taken as the strain level where G' began to drop (Lim et al., 2006).

The determination of gel–sol transition temperature T_{GS} of an organogel (Terech et al., 2000) was also performed on ARES. The gel was heated progressively from 30 to 100 °C at a rate of 1.5 °C/min and G' at 1 Hz and 0.01% strain was measured. The intersection of two distinct linear regions at low and high temperatures of a G' versus temperature T plot gave T_{GS}.

2.4. Microcalorimetry

Gel chemical stability was determined with thermal activity monitor (TAM) from Thermometric AB (Jarfalla, Sweden). One millilitre of control or terpene gel in the sol phase was pipetted into a 4 mL glass ampoule. Once the solution had cooled and solidified, the ampoule was capped and then placed in the measuring cylinder of TAM at 40 °C for 4 days. Heat changes or endotherms were integrated with Digitam®.

2.5. Statistical analysis

Minitab 13.32 was used in statistical analysis. Comparisons between multiple groups of data were performed using one-way ANOVA with Tukey post-hoc test. Results were considered significant if $P < 0.05$.

3. Results and discussion

3.1. Rheology of organogel

Fig. 2a and b illustrates the elastic G' and viscous G'' moduli of control and terpene gels against GP1 concentration C_{GP1}. Terpene concentration C_{Terp} in terpene gel was fixed at 5% (v/v). G' and G'' of control, linalool and cineole gels increased with C_{GP1}. Increased gel stiffness was due to a denser fibrous network (Sawant and Liu, 2002). These upward curves were adequately described by a power law (Table 1) (Brinksma et al., 2000). The moduli (G', G'') of limonene gel, which exhibited a marked increase followed by a plateau with C_{GP1}, did not conform to a power law. It was demonstrated in our earlier work (Lim et al., 2006) that varying C_{GP1} modulates the fibre density and gel stiffness, and presents different degrees of resis-
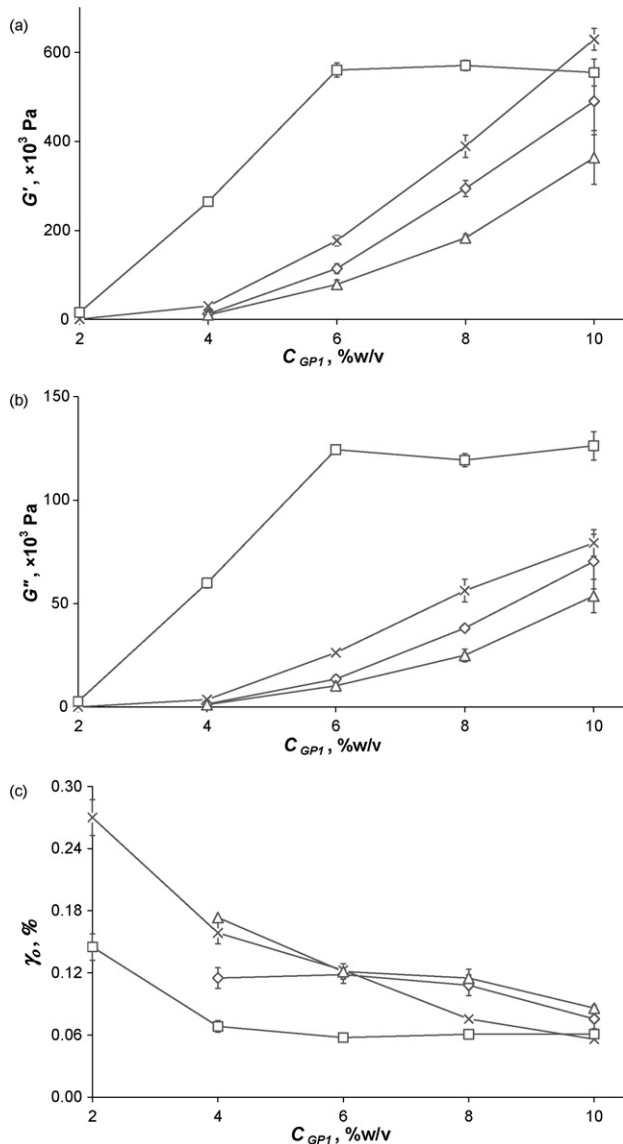
Fig. 2. (a) Elastic modulus G', (b) viscous modulus G'' and (c) critical strain γ_o of control and terpene gels against GP1 concentration C_{GP1}. Terpene concentration C_{Terp} in terpene gel was fixed at 5% (v/v). Organogel: control gel ($\times$); linalool gel ($\diamond$); limonene gel ($\square$); cineole gel ($\triangle$). Each point represents mean ± S.D. ($n = 3$). At $C_{GP1} = 2\%$ (w/v), rheology of linalool and cineole gels could not be determined as moduli were too low.

tance to drug (haloperidol) permeation through human skin on the vehicle side. Based on rheological and permeation studies performed then showed that an increase in C_{GP1} increased gel moduli and decreased haloperidol permeation simultaneously.

It was observed that the solubility of limonene in PG is ca. 3% (v/v). Limonene gel ($C_{Terp} = 5\%$, v/v) appeared to be uniform (Fig. 4), even though excess limonene was used. The fast gelation process prevented phase separation into two distinct layers. An excess amount of the terpene was nevertheless incorporated into the transdermal gel formulation so as to ensure a maximum enhancing activity (Lim et al., 2006).

At a given C_{GP1}, the moduli of limonene gel were generally greater than those of control gel ($P < 0.05$), indicating a change in the gel morphology. The reverse was observed for linalool and cineole gels. Hydroxyl and ether groups in linalool and cineole may participate in competitive hydrogen bonding which disrupted the self-assembly of GP1 molecules and lowered gel moduli. Gel con-
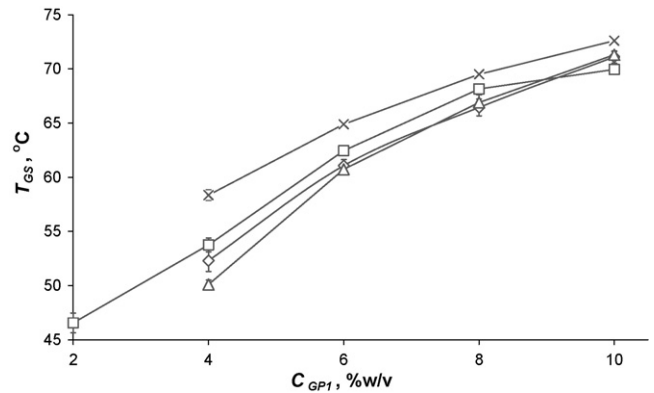

Fig. 3. Gel–sol transition temperature T_{GS} of control and terpene gels against GP1 concentration C_{GP1}. Terpene concentration C_{Terp} in terpene gel was fixed at 5% (v/v). Organogel: control gel ($\times$); linalool gel ($\diamond$); limonene gel ($\square$); cineole gel ($\triangle$). Each point represents mean ± S.D. ($n = 3$). At $C_{GP1} = 2\%$ (w/v), T_{GS} of control, linalool and cineole gels could not be determined as there were no defined G' versus T plots.

taining linalool or cineole could be used as a topical formulation where the softer consistency of the gel renders an easy application on the skin.

Critical strain γ_o, the onset of gel fibre rupture, is inversely related to gel brittleness. Fig. 2c illustrates the contrasting effects of hydrocarbon and oxygen-containing terpenes on γ_o. With respect to control gel, limonene gel had a lower γ_o at $C_{GP1} \leq 6\%$ (w/v) ($P < 0.05$), whereas linalool and cineole gels had a greater γ_o at $C_{GP1} \geq 8\%$ (w/v) ($P < 0.05$). At a given C_{GP1}, limonene gel tends to be more brittle than linalool and cineole gels. There was an apparent trade-off in mechanical properties of the gel where greater G' and G'' were accompanied by a lower γ_o and vice versa (Table 1).

Critical stress σ_o (product of G' and γ_o) is defined as the minimum stress required to initiate gel fibre rupture. Based on Fig. 2, the calculated σ_o of limonene gel was greater in comparison to those of control, linalool and cineole gels at $C_{GP1} \leq 6\%$ (w/v) ($P < 0.05$). The σ_o of control and terpene gels were similar at $C_{GP1} > 6\%$ (w/v). As such, limonene gel is physically more stable, and if utilized as a rate-controlling matrix in a transdermal patch (Lim et al., 2006), it is able to withstand a higher stress loading and maintain an intact gel network at a lower C_{GP1}, and hence giving rise to a consistent and reproducible drug release profile from the gel.

3.2. Gel–sol transition of organogel

Fig. 3 shows the gel–sol transition temperature T_{GS} of control and terpene gels against C_{GP1}. C_{Terp} in terpene gel was fixed at 5% (v/v). Gel–sol transition has often been analyzed using van't Hoff relation (Eq. (1)), where ΔH_{GS} is the enthalpy of gel–sol transition and R is the gas constant (Brinksma et al., 2000). By plotting the natural logarithm of C_{GP1} versus the reciprocal of T_{GS}, ΔH_{GS} is easily determined from the slope of the plot.

$$\ln C_{GP1} = -\frac{\Delta H_{GS}}{R T_{GS}} + \text{constant} \qquad (1)$$

The respective ΔH_{GS} for control, limonene, linalool and cineole gels were found to be 60.7, 58.0, 45.5 and 39.6 kJ/mol. The relatively high enthalpies are common for gels assembled or stabilized via intermolecular hydrogen bonding (Brinksma et al., 2000; Terech and Weiss, 1997). ΔH_{GS} of linalool and cineole gels were much lower than that of control gel, justifying the postulation that linalool and cineole could indeed weaken hydrogen bonding between GP1 molecules. ΔH_{GS} of limonene gel was slightly lower than that of control gel, probably due to the high lipophilicity of limonene and its inability to participate in hydrogen bonding.
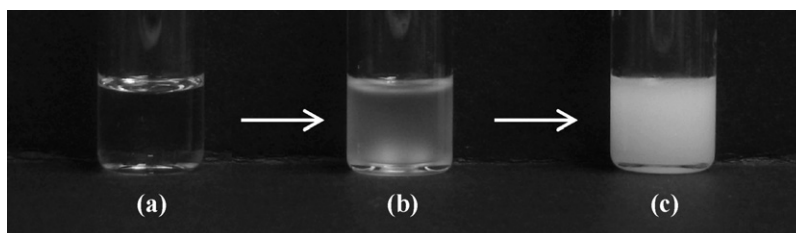
Fig. 4. Gelation process of organogel containing 2% (w/v) GP1 and 5% (v/v) limonene in PG: (a) clear solution after heating; (b) uniform, cloudy suspension upon cooling and standing; (c) white gel upon further standing.

3.3. Gelation mechanism of limonene gel

To account for the rheological behaviour induced by limonene (Fig. 2), a phase separation-mediated gelation was proposed as limonene has a limited solubility of ca. 3% (v/v) in PG. The following describes the gelation process. At a high temperature ($T > T_{GS}$), limonene gel (C_{Terp} = 5%, v/v) is a clear solution (Fig. 4a). Upon cooling and prior to gelation, the solution becomes cloudy (Fig. 4b) as the excess limonene distributes uniformly as fine oil droplets in a continuous PG-rich phase. GP1, having a $\log P$ of 5.02 which is comparable to that of limonene (Fig. 1), would partition favourably into the dispersed limonene phase. Gelation begins with precipitation followed by aggregation ($T < T_{GS}$), and upon further standing, a white gel (Fig. 4c) is formed. The microscopic framework of the gel is a fibrous network which immobilizes the limonene droplets in PG. The fibre density in the dispersed phase is likely to be higher than that in the continuous phase. The marked increases in the moduli (Fig. 2) of limonene gel could have resulted from the formation of numerous dense domains. This gel morphology was however not observed under SEM which could be due to the high operating pressure and high solvating power of the sample pretreatment process, supercritical fluid extraction (Lim et al., 2006).

To verify the aforementioned gelation mechanism, the moduli (G', G'') of limonene gel with C_{GP1} fixed at 6% (w/v) was measured as a function of limonene concentration C_{Limo} (Fig. 5). There were small changes in the moduli at $C_{Limo} \leq 3\%$ (v/v). However, moduli at C_{Limo} = 5% (v/v) were three to five times higher than those at $C_{Limo} \leq 3\%$ (v/v) ($P < 0.05$). This somewhat supports the argument that the high moduli of limonene gel were due to an excess amount of the hydrocarbon terpene leading to a phase separation-mediated gelation. Fig. 5 also shows gradual, significant decreases in the moduli of linalool and cineole gels with C_{Terp} ($P < 0.05$). The more hydrophilic terpenes were able to further disrupt the self-assembly of GP1 molecules at a greater C_{Terp}.

3.4. Chemical stability of organogel

The gel samples were subjected to accelerated testing at 40 °C over 4 days in TAM. According to the International Conference on Harmonization (ICH) Tripartitie guidelines, the predictive factor for chemical stability at 25 °C is 5 (Grimm, 1998), i.e. 4 days at 40 °C corresponds to 20 days at 25 °C.

Chemical instability of GP1-based organogels could be associated with the breakage of intermolecular hydrogen bonds between GP1 molecules or the degradation of the gel network into individual GP1 molecules. This would inevitably affect the shelf-life of the gel formulations. Hence ΔH_n, heat change normalized with respect to GP1 content, was used to assess gel chemical stability. A chemically stable gel would have a low ΔH_n.

Fig. 6 shows the static TAM run of control gel containing 2% (w/v) GP1. Two prominent peaks designated as endotherms 1 and 2 were detected at 20.7 and 64.7 h, respectively. Fig. 7 shows the normalized enthalpies ΔH_n of endotherms 1 and 2 for control gel against

C_{GP1}. Endotherms 1 and 2 were detected between 15 and 30 h, and between 60 and 80 h, respectively. As no endotherms were found for GP1 alone and PG alone, the heat changes obtained for control gel could have resulted from some sort of gel destabilization. ΔH_n of endotherms 1 and 2 decreased significantly with C_{GP1} ($P < 0.05$), implying an enhanced chemical stability at a higher C_{GP1}. However at $C_{GP1} \geq 4\%$ (w/v), ΔH_n of both endotherms remained unchanged ($P > 0.05$).

Fig. 8 shows ΔH_n of endotherms 1 and 2 for control and terpene gels against C_{GP1}. C_{Terp} in terpene gel was fixed at 5% (v/v). Similarly, two distinct endotherms were detected for limonene, linalool and cineole gels. As in the case for control gel, the same trends in heat changes were also observed for terpene gels. Microcalorimetry is useful in determining the relative chemical stability of terpene-incorporated gels to its parent gel (control gel). Fig. 8 also illustrates that at a given C_{GP1}, hydrocarbon or oxygen-containing terpenes had no effect ($P > 0.05$) on gel chemical stability.

One important point to note is that ΔH_n values of control and terpene gels were only a minute fraction (<0.01%) of
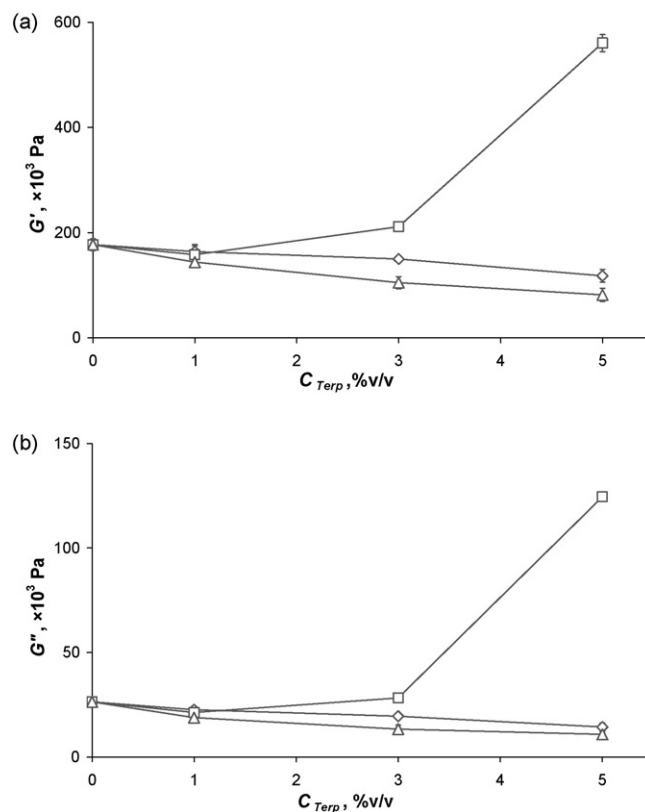

Fig. 5. (a) Elastic modulus G' and (b) viscous modulus G'' of terpene gel as a function of terpene concentration C_{Terp} at a fixed GP1 concentration C_{GP1} of 6% (w/v). Terpene gel: linalool gel (◊); limonene gel (□); cineole gel (△). Each point represents mean ± S.D. (n = 3).
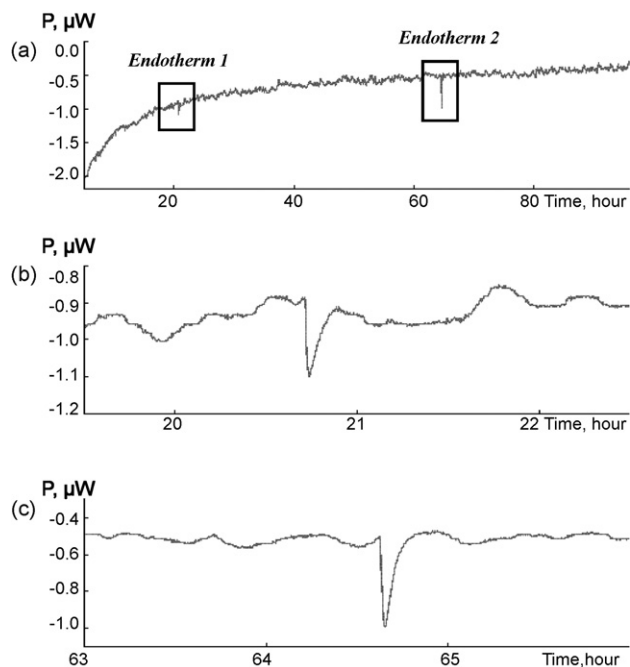
Fig. 6. Static TAM run for control gel containing 2% (w/v) GP1 at 40 °C over 4 days. (a) Full scan; (b) close-up of endotherm 1; (c) close-up of endotherm 2.

ΔH_{GS} of the respective gels. As the degree of gel destabilization was negligible, control and terpene gels were stable chemically on a short-term basis (4 days at 40 °C or 20 days at 25° C).
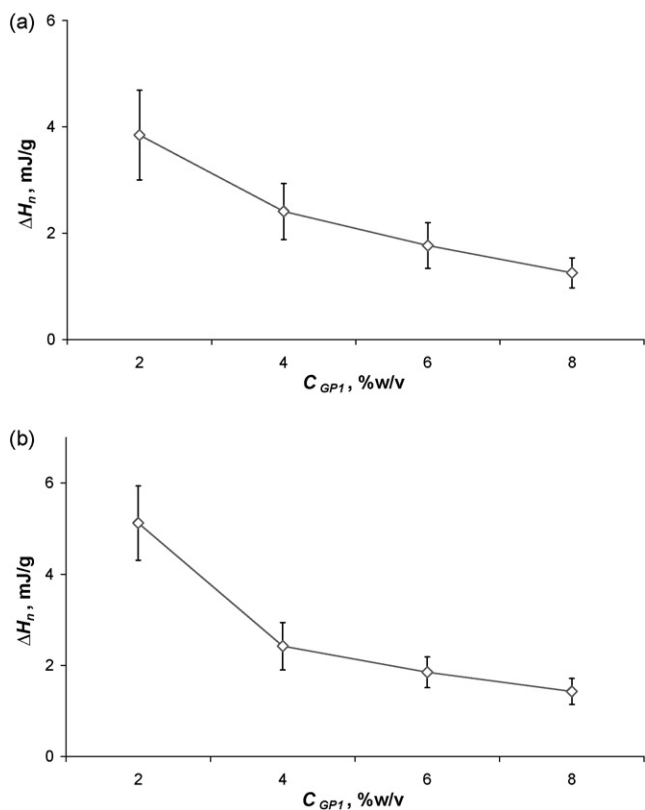

Fig. 7. Normalized enthalpy ΔH_n of (a) endotherm 1 and (b) endotherm 2 for control gel against GP1 concentration C_{GP1}. Endotherms 1 and 2 were detected between 15 and 30 h, and between 60 and 80 h, respectively. Each point represents mean ± S.D. ($n = 3$).
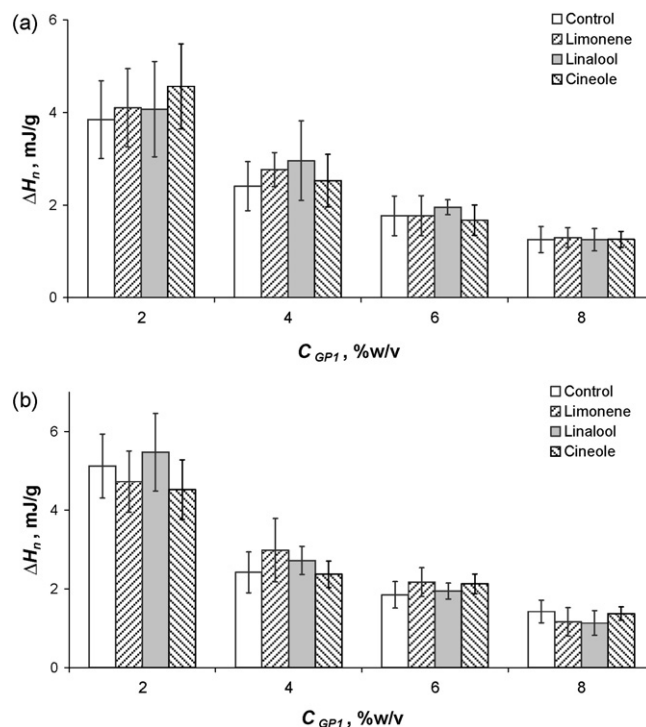

Fig. 8. Normalized enthalpy ΔH_n of (a) endotherm 1 and (b) endotherm 2 for control and terpene gels against GP1 concentration C_{GP1}. Terpene concentration C_{Terp} in terpene gel was fixed at 5% (v/v). Endotherms 1 and 2 were detected between 15 and 30 h, and between 60 and 80 h, respectively. Each bar represents mean ± S.D. ($n = 3$).

4. Conclusion

This study demonstrates that terpenes when incorporated in pharmaceutical gel formulations, not only act as chemical penetration enhancers, but are also rheology modifiers which change the flow and deformation characteristics of a gel vehicle for topical and transdermal drug delivery. In addition, changes in gel rheology induced by terpenes may not affect the chemical stability of the resulting gel formulations.

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