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Combined effect of oleic acid and polyethylene glycol 200 on buccal permeation of [D-Ala^2, D-Leu^5]enkephalin from a cubic phase of glyceryl monooleate

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Abstract

The combined use of the lipophilic permeation enhancer, oleic acid together with polyethylene glycol 200 (PEG 200) as a co-enhancer and incorporated into the cubic liquid crystalline phase of glyceryl monooleate was investigated in the ex vivo buccal permeation of [D-Ala^2, D-Leu^5]enkephalin (DADLE) through porcine buccal mucosa mounted in a Franz cell. The addition of oleic acid (1%) and PEG 200 (1–10%) did not change the intact appearance of the cubic phase. PEG 200 increased the aqueous solubility of oleic acid incorporated into the cubic phase and hence promoted the transport of oleic acid into the porcine buccal mucosa. The solubilising effect of PEG 200 on oleic acid incorporated into the cubic phase was dependent on the PEG 200 concentration but was non-linear. The buccal permeation flux of DADLE significantly increased when 5% ($P < 0.01$) or 10% ($P < 0.001$) of PEG 200 was co-administered with 1% oleic acid compared with the cubic phase containing 1% oleic acid alone. The present results suggest that PEG 200 enhances the action of the lipophilic permeation enhancer oleic acid and that the combination of oleic acid and PEG 200 as a co-enhancer can be a useful tool to improve the membrane permeability in the buccal delivery of peptide drugs using a cubic liquid crystalline phase of glyceryl monooleate and water.

Keywords: Buccal delivery system; Peptide drugs; Permeation enhancement; Cubic phase of glyceryl monooleate; Oleic acid; Polyethylene glycol

1. Introduction

The oral mucosal route offers a number of advantages over parenteral and other non-invasive routes for the systemic delivery of biologically active peptides and proteins as well as conventional drugs (de Vries et al., 1991). However, most drugs absorbed via the mucosal membrane of the oral cavity have exhibited low bioavailabilities due largely to the low mucosal membrane permeability, relatively small surface area available for absorption and poor retention of the drug and/or delivery systems at the site of absorption (Rath-

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bone et al., 1994). These problems can be often overcome by utilising mucoadhesive dosage forms that provide increased residence time leading to an increased total permeability of macromolecular drugs such as peptides and proteins (Jiménez-Castellanos et al., 1993; Lehr, 1994; Ahuja et al., 1997). Cubic and lamellar liquid crystalline phases of glyceryl monooleate (GMO) are considered as attractive buccal delivery carriers for peptide and protein drugs since they have bioadhesive properties (Engström et al., 1995; Nielsen et al., 1998) and the ability to enhance the ex vivo buccal membrane permeability of a peptide drug (Lee and Kellaway, 2000b). Furthermore, the cubic phase has shown a protective action against enzymatic degradation of some peptide drugs which makes it an ideal candidate for the buccal delivery of peptide and protein drugs (Ericsson et al., 1991).

The permeation enhancement approach was considered in order to further improve mucosal membrane permeation rate (Ganem-Quintanar et al., 1997). Oleic acid was selected as a buccal permeation enhancer since it has been shown to effectively increase the percutaneous and transmucosal absorption rates (Lee et al., 1991; Niazy, 1991; Kararli et al., 1992) and can be easily incorporated into the cubic phase of GMO due to its lipophilic nature by solubilising it with molten GMO. Oleic acid alters the intercellular lipid fluidity within the stratum corneum (Walker and Hadgraft, 1991) and in buccal membranes, it also disrupts lipid bilayers and therefore similar penetration enhancing effects may occur (Turunen et al., 1994; Aungst, 1996).

In the present study, we investigated the effect of polyethylene glycol 200 (PEG 200) on increasing the release of a lipophilic permeation enhancer oleic acid incorporated into the cubic phase of glyceryl monooleate and water. The combined effect of oleic acid and PEG 200 as a permeation enhancer and so called co-enhancer, respectively, was also evaluated with respect to their ability to enhance ex vivo buccal permeability of a model peptide from the cubic phase. [D-Ala^2, D-Leu^5]enkephalin (DADLE, $Mw = 569.7$) was chosen as the model peptide due to its relative metabolic stability (Lee and Kellaway, 2000a).

2. Materials and methods

2.1. Materials

A commercial grade of GMO (RYLO MG 19) was purchased from Danisco Ingredients (Copenhagen, Denmark) and used as received. Oleic acid was a BP grade and obtained from Thornton & Ross Ltd. (Huddersfield, UK). DADLE and phosphate buffered saline pH 7.4 (PBS) tablets were obtained from Sigma–Aldrich Company (Poole, UK). PEG 200, ethanol and acetic acid were provided by BDH Chemical Ltd. (Poole, UK). [Tyrosyl-3,5-^{3}H(N)]DADLE ([^{3}H]DADLE, specific activity: 33.50 Ci/mmol) and [1-^{14}C]oleic acid ([^{14}C]oleic acid, specific activity: 55 mCi/mmol) were obtained from Du Pont UK Limited (Hertfordshire, UK) and Amersham Pharmacia Biotech UK Limited (Bucks, UK) respectively. NCS-II Tissue Solubilizer (Amersham Corporation, Arlington Heights, IL) and OptiPhase 'HiSafe' 3 liquid scintillation cocktail (Fisher Chemicals, Loughborough, UK) were also used. Distilled water was used throughout.

2.2. Preparation of cubic liquid crystalline phase

The compositions of the cubic phases used in this study are reported in Table 1. GMO was added to a glass vial containing oleic acid and PEG 200 and warmed at 50°C in a water bath. Radiolabelled compounds were dispensed into a different vial and storage solvent was evaporated under a nitrogen stream at room temperature. The required amount of DADLE stock solution (5 mg/ml in distilled water) and water was added to the vial containing the labelled compounds (aqueous phase). This aqueous phase was vortex-mixed, warmed and added to the vial containing GMO, oleic acid and PEG 200. The mixture was incubated for 3 days and then allowed to equilibrate at room temperature for 5 days. The cubic phase was examined by visual inspection and a light microscope equipped with a polarisation filter (Olympus BH2, Olympus Optical Co., Japan).

2.3. In vitro release of oleic acid from cubic phase

Cylindrically (5.0 mm diameter × 3.0 mm thickness) set cubic phase was placed on a platform composed of sieve and supporter. The platform was immersed in 15 ml de-aerated PBS stirred by a magnetic bar at 37°C. Aliquots (0.2 ml) were taken at pre-determined time points up to 24 h and replaced with the same volume of fresh PBS. Liquid scintillation cocktail (3 ml) was directly added to each sample to determine levels of radioactivity released into the medium by liquid scintillation counting (Wallac 1409 DSA liquid scintillation counter, EG&G Wallac, Turku, Finland).

2.4. Ex vivo buccal permeation study

To produce a thin film of the cubic phase in direct contact with the buccal mucosa, the following procedure was employed. Porcine buccal tissue was freshly excised and stored in PBS at 4°C upon removal. The mucosal membrane was separated by removing the underlying connective tissue with tweezers and surgical scissors. The porcine buccal mucosa was then hydrated in PBS for 0.5 h at 37°C, blot dried and placed on a bottom washer (1.6 mm thick, 5.1 mm id, 30.1 mm od) on a piece of Parafilm (9 cm^2). A top washer (1.6 mm thick, 5.1 mm id, 30.1 mm od) was placed on the buccal tissue and weighed. The cubic phase was applied to the surface of the buccal mucosa and re-weighed to determine the mass of cubic phase applied. After removing the Parafilm and mounting in a Franz cell, PBS was pipetted into the receiver compartment (2.2–2.4 ml) to initiate the diffusion experiment. The Franz cell was located on a magnetic stirring block (Model: HP15 S/ST, Camlab Limited, Cambridge, UK) in a water bath at 37°C and its donor compartment was sealed with a silicone-greased cover slip to prevent moisture loss. At pre-determined time intervals over an 8 h period, samples (0.2 ml) were withdrawn with a microsyringe from the sampling arm of the receiver compartment and replaced with an equivalent volume of fresh PBS. The samples were assayed by liquid scintillation counting.

At the end of the experiment, the buccal tissue was thoroughly washed with 50% ethanol to remove the residual cubic phase and weighed. The tissue was then digested with 1 ml of NCS-II Tissue Solubilizer at 37°C for 3 days. Acetic acid (30 μl) was added to neutralise the buccal tissue solution. Assay for the determination of radioactivity found in the buccal tissue was carried out by liquid scintillation counting after the addition of 3 ml of liquid scintillation cocktail.

2.5. Statistical analysis

The ex vivo buccal permeation results were statistically analysed using ANOVA and P values of 0.05 or less were considered significant except where stated otherwise.

Table 1
Compositions of the cubic phases[a]

Constituents	Formulation code							
	A	B	C	D	E	F	G	H
GMO	65.0	65.0	65.0	65.0	65.0	65.0	65.0	65.0
Water	34.9	33.9	32.9	28.9	23.9	33.9	29.9	24.9
Oleic acid[b]	–	1.0	1.0	1.0	1.0	–	–	–
PEG 200	–	–	1.0	5.0	10.0	1.0	5.0	10.0
DADLE[c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[a] The compositions are expressed as percent (w/w).
[b] [^{14}C]Oleic acid was included to give a radioactivity of 0.1 μCi/mg of cubic phase.
[c] [^{3}H]DADLE was included to give a radioactivity of 0.2 μCi/mg of cubic phase.

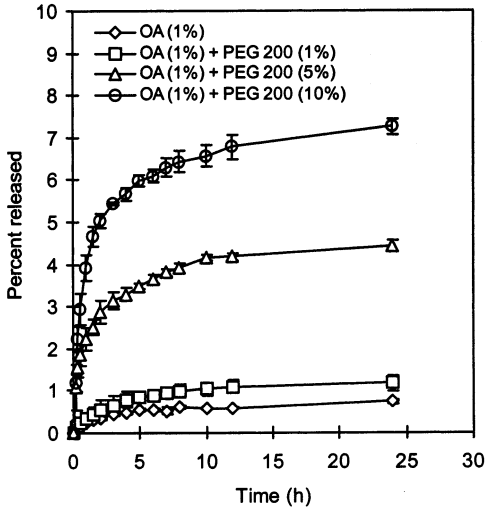

Fig. 1. Effect of PEG 200 on the release of oleic acid (OA) from GMO cubic phase as a function of time at 37°C. Mean ± SD, $n = 3$.

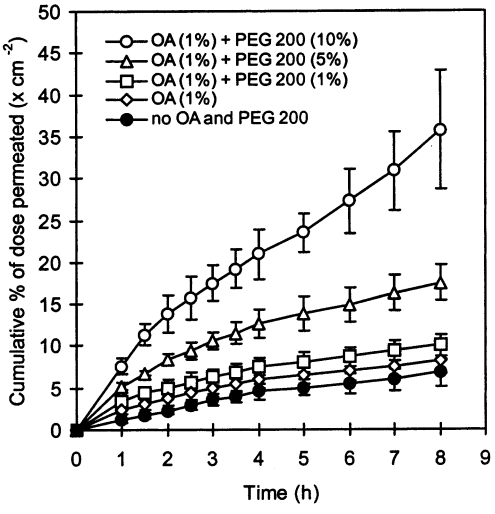

Fig. 2. Permeation profiles of DADLE across porcine buccal mucosa from a cubic phase of GMO containing oleic acid (OA) and PEG 200. Mean ± SD, $n = 5$.

3. Results and discussion

Our preliminary experiments concerning the in vitro release of oleic acid from the cubic phase of GMO (Fig. 1) and the permeation enhancing effect of oleic acid on the ex vivo buccal transport of DADLE from the cubic phase (Fig. 2) have revealed that most oleic acid incorporated was retained within the cubic phase resulting in a poor permeability enhancing effect. We hypothesised that increased release of oleic acid from the cubic phase would cause a greater permeation enhancing effect. In order to increase the amount of oleic acid released from the cubic phase, we co-administered PEG 200 with oleic acid since PEGs have the ability to solubilise lipophilic compounds (Veiga et al., 1993; Chattaraj et al., 1998; Khidr et al., 1998). Moreover, PEG 200 is miscible with oleic acid and did not affect the intact structure of the cubic phase at the concentration employed when examined by visual observation and polarised microscopy.

All the cubic liquid crystalline phases tested were transparent at room temperature. Polarised microscopy also confirmed that the addition of oleic acid and PEG 200 did not affect the appearance of the cubic phase being seen as a dark background at the concentration employed. The permeation enhancer, oleic acid was incorporated into the cubic phase consisting of GMO and water. The concentration of oleic acid chosen was 1.0% w/w as at this concentration no changes in the appearance of the cubic phase were observed. The molecular packing of the monoglyceride can be affected by the increase in the hydrocarbon chain space obtained upon solubilisation of oleic acid (Chang and Bodmeier, 1997). For example, the addition of 5 and 10% w/w oleic acid caused a phase change from the transparent cubic phase to a milky gel.

As a first step to investigate the enhancing effect of oleic acid on the ex vivo buccal permeability of DADLE from the GMO cubic phase, the in vitro release of DADLE and oleic acid from the cubic phase was examined by employing radiolabelled tracers. The addition of oleic acid and PEG 200 did not modify the DADLE release profile (Fig. 3) probably because oleic acid (1% w/w) and PEG 200 (1–10% w/w) did not alter the essential structure of the cubic phase. Another possible explanation for the consistent release pattern of DADLE is that DADLE is sufficiently soluble in the release medium employed, therefore the presence of the pharmaceutical solubiliser PEG 200 does not make a significant contribution.

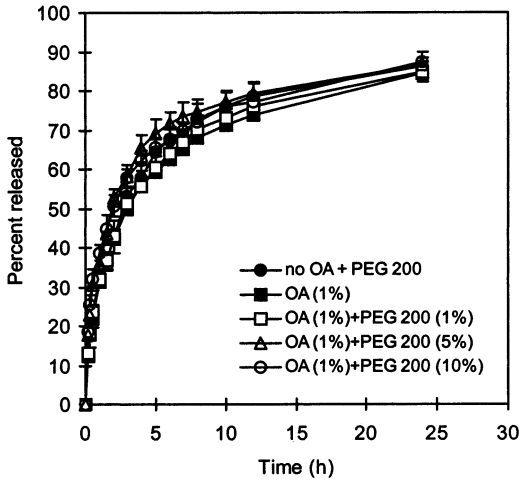

Fig. 3. Effect of oleic acid (OA) and PEG 200 on the release of DADLE from GMO cubic phase as a function of time at 37°C. Mean ± SD, $n = 3$.

The in vitro release of oleic acid from the cubic phase increased as the PEG 200 content increased (Fig. 1). This could be explained by the impact of PEG 200 on solubilising oleic acid. In fact, since the oleic acid is lipophilic and is therefore incorporated into the lipid domain of the cubic phase (Chang and Bodmeier, 1997), its release from the cubic phase into aqueous PBS medium is not readily achieved. The solubilising effect of PEG 200 on oleic acid incorporated into the cubic phase was not linear i.e. enhancement ratios, calculated from cumulative amount of oleic acid released with PEG 200 per cumulative amount of oleic acid released without PEG 200, were 1.60 (1% PEG 200), 6.05 (5% PEG 200) and 9.93 (10% PEG 200). However, the partitioning of oleic acid between the lipid domains and the aqueous regions of the cubic phase of glyceryl monooleate can be controlled by the PEG 200 concentration. The alternative reason for the increased release of oleic acid by PEG 200 might be that PEG 200 enhances the penetration of water into the cubic phase matrix thereby leading to phase changes which effect the release of oleic acid.

The addition of PEG 200 to the cubic phase containing 1.0% oleic acid as a permeation enhancer facilitated the ex vivo permeability of DADLE across porcine buccal mucosa (Fig. 2). The steady-state flux was obtained from the linear part of the cumulative amount permeated versus time plot. The fluxes of DADLE were significantly increased at the PEG 200 concentrations of 5 ($P < 0.01$) and 10% ($P < 0.001$) compared with the cubic phase formulation A (i.e. no OA and PEG 200 added) (Table 2). On the other hand, 1% oleic acid alone and the addition of 1% PEG 200 to 1% oleic acid did not show a statistically significant enhancing effect even though their mean fluxes slightly increased (Table 2). Thus, the

Table 2
Ex vivo buccal permeation parameters of DADLE from the cubic phase of glyceryl monooleate

Formulation codes of cubic phase	Flux[a] (% dose/cm^2 per h)	Total DADLE permeated [a,b] (% dose/cm^2)	ER[c]	Significance of difference[d]
A: no OA[e] and PEG 200	1.21 ± 0.32	6.74 ± 1.64	1.00	–
B: OA (1%)	1.31 ± 0.22	8.13 ± 1.48	1.08	$P > 0.5$
C: OA (1%) + PEG 200 (1%)	1.39 ± 0.24	9.99 ± 1.25	1.15	$P > 0.1$
D: OA (1%) + PEG 200 (5%)	2.67 ± 0.35	17.43 ± 2.14	2.21	$P < 0.01$
E: OA (1%) + PEG 200 (10%)	4.83 ± 0.92	35.75 ± 7.15	3.99	$P < 0.001$
F: PEG 200 (1%)	1.20 ± 0.26	6.88 ± 1.36	0.99	$P > 0.5$
G: PEG 200 (5%)	1.50 ± 0.46	7.71 ± 1.34	1.24	$P > 0.1$
H: PEG 200 (10%)	1.26 ± 0.45	7.17 ± 1.34	1.04	$P > 0.5$

[a] Each value represents the mean ± SD of five determinations.
[b] Values were obtained at the end of the permeation experiment (8 h).
[c] Enhancement ratio, ER = permeation flux with OA and/or PEG 200/permeation flux of the formulation A (no OA and PEG 200).
[d] The flux of Formulation A was used as a control.
[e] Oleic acid.

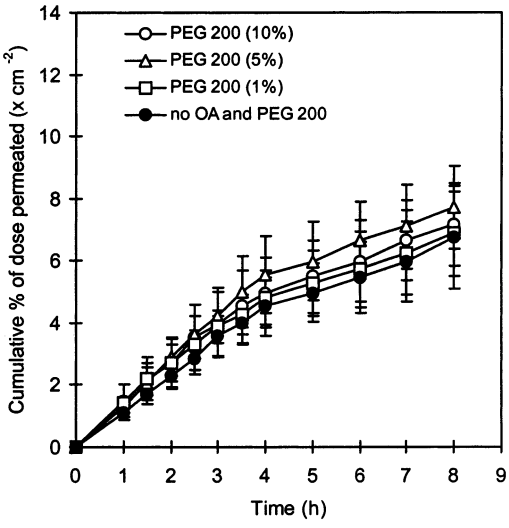

Fig. 4. Permeation profiles of DADLE across porcine buccal mucosa from a cubic phase of GMO containing PEG 200. Mean ± SD, $n = 5$.

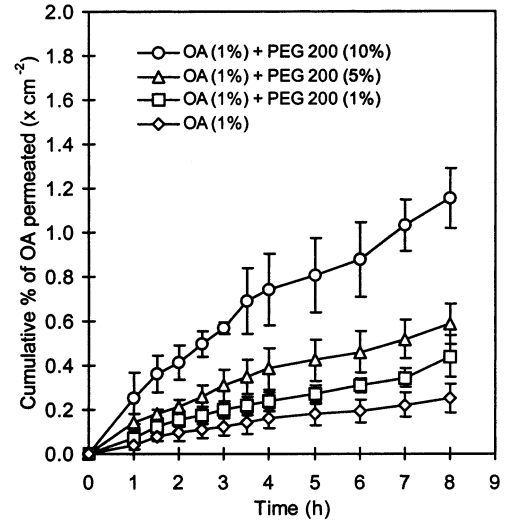

Fig. 5. Effect of PEG 200 on the permeation of 1% w/w oleic acid (OA) across porcine buccal mucosa from a cubic phase of GMO. Mean ± SD, $n = 5$.

steady-state flux improvement may be due to increased oleic acid solubility brought about by the PEG 200 and the concomitant increase in oleic acid concentration in the buccal tissue (Fig. 6). It is worth noting that PEG 200 alone in varying concentrations (1–10% w/w) did not increase the flux of DADLE (Fig. 4, Table 2). In this study, it is evident that the appreciable enhancement effect of buccal permeation of DADLE from the cubic phase containing 1% oleic acid and PEG 200 (5 and 10%) can be clearly attributed to the solubilisation of the lipophilic permeation enhancer by PEG 200. Accordingly, the combination of lipophilic enhancer together with a co-solvent in the drug carrier may lead to optimisation of the enhancing capability. These results are also in accordance with the findings published by Adachi et al., (1993) and Abe et al., (1995) where cyclodextrin derivatives improved the release of a lipophilic penetration enhancer from an ointment base or served as a biocompatible solubiliser for a lipophilic absorption enhancer. It is interesting that, in the case of the formulation E containing 1% oleic acid and 10% PEG 200, DADLE permeability progressively increased after 5 h, suggesting a time-dependent modification of the integrity of the barrier (Fig. 2).

Oleic acid released from the cubic phase permeated the porcine buccal mucosa (Fig. 5) with the percentage of oleic acid transported increasing with increasing PEG 200 concentration. However, a significant difference in flux (% dose/cm^2 per h) calculated for $t < 4$ h was seen only between the cubic phases containing 1% oleic acid with 10% PEG 200 (0.15 ± 0.05) and 1% oleic acid alone (0.04 ± 0.03) ($P < 0.01$).

Both oleic acid and DADLE were found in the porcine buccal mucosa. The quantities of DADLE present in the buccal tissue at the end of the experiment expressed as % of the applied dose were approximately 5–6% (formulations A, B, C, F, G, H), 9.0% (formulation D: 1% oleic acid and 5% PEG 200) and 12.0% (formulation E: 1% oleic acid and 10% PEG 200). As a result of the increased release and permeation of oleic acid from the cubic phase by the addition of 5% and 10% PEG 200 the accumulation of oleic acid in the buccal mucosa was considerably increased 3- and 5-fold, respectively, compared with that from the cubic phase containing 1% oleic acid alone (Fig. 6). Consequently, PEG 200 increased the aqueous solubility of oleic acid within the cubic phase and hence promoted the transport of oleic acid into the porcine buccal mucosa.

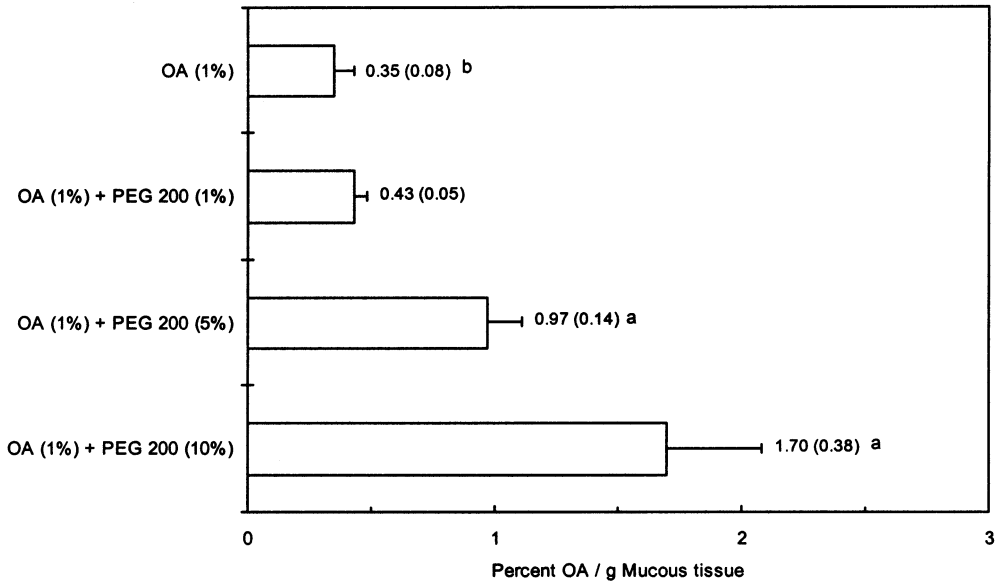

Fig. 6. Amount of oleic acid (OA) accumulated in the porcine buccal mucosa after 8 h. Mean ± SD, $n = 5$. Numbers in parentheses are standard deviations: a is significantly different from b ($P < 0.01$).

In conclusion, PEG 200 augments the action of the lipophilic permeation enhancer oleic acid and the combination of oleic acid and PEG 200 as a co-enhancer can be a useful tool to improve the membrane permeability in the buccal delivery of peptide drugs using a cubic liquid crystalline phase of glyceryl monooleate and water.

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