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In vitro peptide release from liquid crystalline buccal delivery systems☆

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Abstract

Swelling and [D-Ala^2, D-Leu^5]enkephalin (DADLE) release from the lamellar and cubic liquid crystalline phases of glyceryl monooleate (GMO) were studied using two in vitro methods, a total immersion method and a Franz cell method. The swelling of the lamellar phase and glyceryl monooleate (0% w/w water content) and DADLE release from the liquid crystalline phases were temperature dependent. The swelling ratio was greater at 20°C than 37°C while DADLE release increased at 37°C compared to 20°C for both the lamellar and cubic phases. The water uptake increased dramatically with decreasing initial water content of the liquid crystalline phases. However, DADLE release increased with increasing initial water content, which corresponded to increased viscosity. The swelling and DADLE release profiles obtained using a Franz cell method with a moist nylon membrane to mimic buccal drug release conditions were slower than the total immersion method. These results show that the swelling and DADLE release strongly depended on temperature, the initial water content of the liquid crystalline matrix and the methodology employed for determining the swelling and DADLE release.

Keywords: Buccal; Liquid crystalline phase; Glyceryl monooleate; Swelling; In vitro release

1. Introduction

Glyceryl monooleate (monoolein-GMO) is a common food additive and sparingly water-soluble lipid (Sadhale and Shah, 1998) that can form lyotropic liquid crystalline phases in the presence of water. The formation of liquid crystalline phases is dependent on the water content and temperature. Its lamellar and cubic liquid crystalline phases are bioadhesive although the mechanism of bioadhesion has not yet been identified (Engström et al., 1995; Nielsen et al., 1998). They can therefore be considered as mucoadhesive drug carriers for various mucosal routes including the buccal mucosa. To examine the potential of the glyceryl monooleate–water system as a buccal-ad-

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hesive peptide delivery carrier we have investigated the effect of liquid crystalline type (determined by the initial water content in the system) and temperature on swelling behaviour and peptide release profile using two in vitro methods i.e. a total immersion method and a Franz cell method. The latter attempted to mimic the in vivo situation when applied to the buccal mucosa. [D-Ala^2, D-Leu^5]enkephalin (DADLE, $M_w = 569.7$) was selected as a model peptide due to its relative stability to enzymatic hydrolysis (Dubocovich and Langer, 1980; Zlokovic et al., 1985; Schulteis et al., 1989) and the fact that DADLE is transported across biological barriers such as jejunal and colonic membranes without the addition of protease inhibitors (Uchiyama et al., 1998).

2. Materials and methods

2.1. Materials

A commercially available grade of distilled GMO was purchased from Danisco Ingredients (Copenhagen, Denmark) and used as received. DADLE and phosphate-buffered saline pH 7.4 (PBS) tablets were obtained from Sigma–Aldrich Company (Poole, UK). [*Tyrosyl*-3,5-^{3}H(N)]-DADLE ([^{3}H]DADLE, 1 mCi/ml in ethanol) was obtained from Du Pont (Hertfordshire, UK).

2.2. Preparation of liquid crystalline phases

The required amount of aqueous phase consisting of [^{3}H]DADLE, unlabelled DADLE and water was added to GMO at 50°C to form cubic (35% w/w water) and lamellar (16% w/w water) phases (Table 1). Samples were kept in an incubator maintained at 37°C for 3 days and then allowed to equilibrate at room temperature for 5 days. The liquid crystalline phases were examined by a light microscope equipped with a polarisation filter (Olympus BH2, Olympus Optical Co., Japan) and photomicrographs of the phases were taken by an Olympus C-35AD camera at room temperature.

2.3. Swelling behaviour

2.3.1. Immersion method

A polypropylene cylindrical mould (8.4 mm internal diameter, 3.0 mm thickness) was filled with liquid crystalline phases or molten GMO. The set sample was removed from the mould onto a glass slide, weighed and then allowed to equilibrate at room temperature. The sample was immersed in 300 ml of de-aerated PBS. The swelling ratio (SR) was calculated as $[W_t/W_0]$, where W_t indicates the weight of sample at time t and W_0 denotes the initial weight of the sample.

2.3.2. Franz cell method

Cylindrical (8.4 mm diameter, 3.0 mm thickness) samples of the liquid crystalline phases were placed onto pre-hydrated nylon membranes as a supporting mesh and weighed. These were

Table 1
Composition of liquid crystalline formulations used for swelling and in vitro DADLE release studies

	Swelling			Release	
Constituents	GMO	LM[a]	CB[b]	LM	CB
Glyceryl monooleate (g)	10.0	8.4	6.5	8.4	6.5
Water (g)	–	1.6	3.5	1.6	3.5
DADLE (mg)	–	–	–	2.0	2.0
[^{3}H]DADLE (μCi)	–	–	–	30.0	30.0

[a] Lamellar liquid crystalline phase.
[b] Cubic liquid crystalline phase.

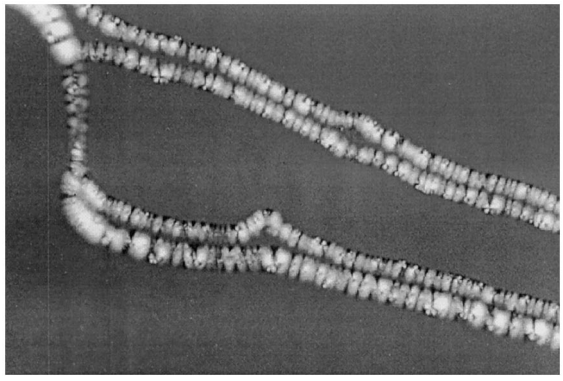

Fig. 1. Photomicrograph of the lamellar liquid crystalline phase of glyceryl monooleate at room temperature. Magnification: × 400.

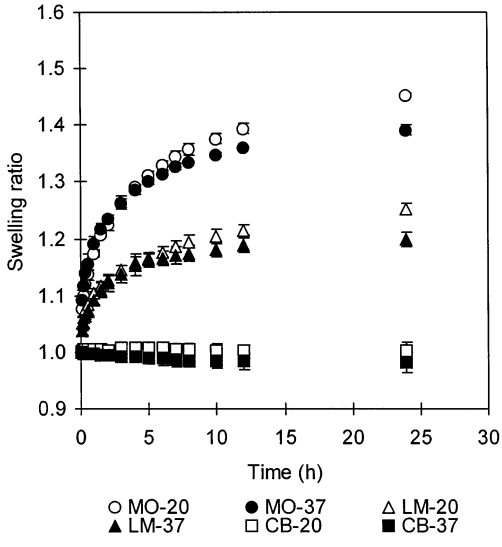

Fig. 2. Plots of swelling ratio measured using immersion method as a function of time: MO, glyceryl monooleate; LM, lamellar liquid crystalline phase; CB, cubic liquid crystalline phase; 20, measured at 20°C; 37, measured at 37°C. Each point represents mean ± S.D., $n = 3$.

mounted in the receiver compartment of Franz cells. The receiver compartment was filled with PBS and the assembled Franz cells were placed in a water bath at 37°C. At pre-determined time intervals the Franz cells were disassembled and the membranes with samples were taken, blot-dried and re-weighed. The membranes with samples were returned to the Franz cell.

2.4. In vitro drug release

2.4.1. Immersion method

Cylindrical (8.4 mm diameter, 3.0 mm thickness) samples of the liquid crystalline phases were placed on a platform composed of sieve and supporter. The platform was immersed in 15 ml de-aerated PBS. 0.2 ml aliquots were taken periodically and replaced with the same volume of fresh PBS. A volume of 3 ml of liquid scintillation cocktail were directly added to the sample and assayed by liquid scintillation counting.

2.4.2. Franz cell method

Cylindrical (8.4 mm diameter, 3.0 mm thickness) samples of the liquid crystalline phases were placed on a pre-hydrated nylon membrane located in Franz cells. Each Franz cell was clamped and PBS pipetted into the receiver compartment to initiate the experiment. The assembled Franz cells were placed on a magnetic stirring block in a water bath. A volume of 0.1 ml of receptor solution was withdrawn at pre-determined time intervals and replaced with the same volume of fresh PBS. Liquid scintillation counting was used to determine levels of [^{3}H]DADLE released.

3. Results and discussion

The cubic liquid crystalline phase of GMO was transparent at room temperature while the lamellar phase was opaque. Polarised microscopy revealed the cubic phase as a dark background because of its optically isotropic nature. In contrast, the lamellar phase being anisotropic appeared as planar bilayers of the lipid on a dark background (Fig. 1).

GMO and lamellar liquid crystalline phases containing initial water contents of 0 and 16% w/w, respectively, exhibited initial rapid water uptake when measured using the immersion method (Fig. 2). The initial rate of swelling was calculated by second-order swelling kinetics (Schott, 1992) and values of 0.19 (lamellar, 37°C), 0.15 (lamellar, 20°C), 0.35 (GMO, 37°C) and 0.27 (GMO 20°C) g/h were obtained, demonstrating a higher initial rate of swelling at 37°C. However,

the maximum water uptake measured at 24 h was achieved at 20°C rather than 37°C (Fig. 2). Since lyotropic liquid crystalline phases are known to be sensitive to temperature change (Chang and Bodmeier, 1997), the maximum water uptake will vary with temperature. The cubic phase did not swell at both 20 and 37°C since it already contained the equilibrium amount of water. When the swelling ratio was measured in a Franz cell with a moist nylon membrane, the water uptake profiles were similar but the rates of swelling were much slower (decreased by approximately 50%) than those measured using the immersion method due to the restriction of media access to the swelling matrix by the nylon membrane.

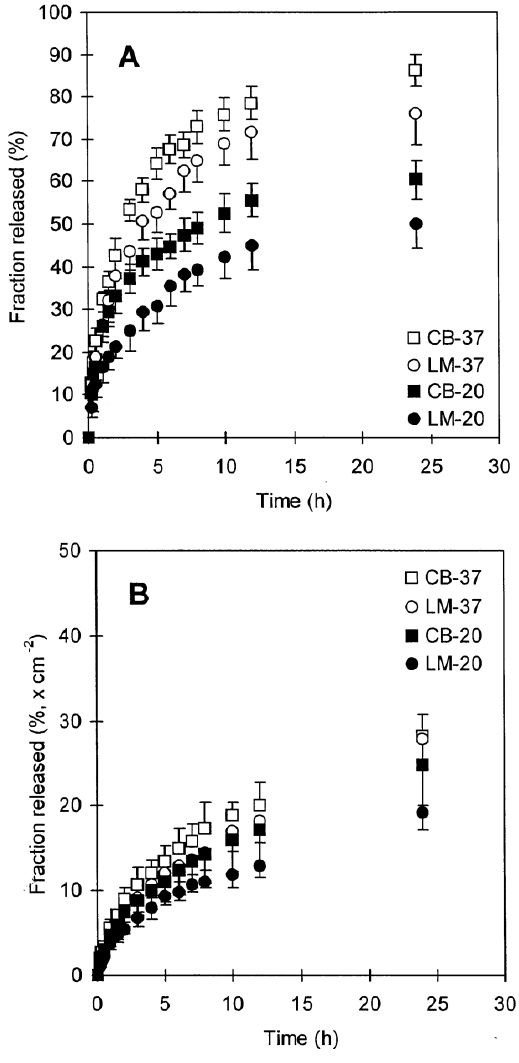

Fig. 3. Cumulative amount of DADLE released obtained from immersion method (A) and Franz cell method (B) as a function of time: CB, cubic liquid crystalline phase; LM, lamellar liquid crystalline phase; 20, measured at 20°C; 37, measured at 37°C. Each point represents mean ± S.D., $n = 3$.

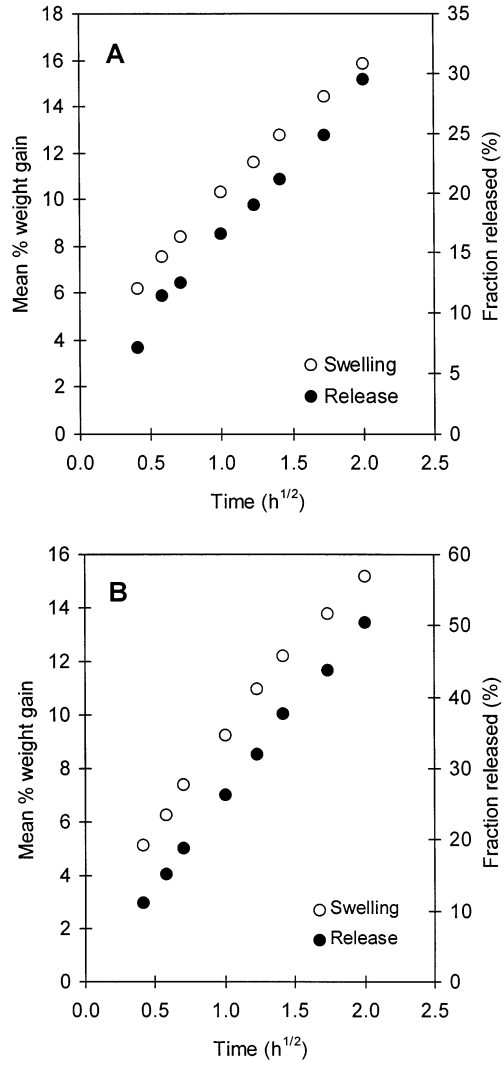

Fig. 4. Comparison of swelling profile and DADLE release of lamellar liquid crystalline phase at 20 (A) and 37 (B) °C. The data obtained by the total immersion method were plotted.

[^{3}H]DADLE release increased with increasing initial water content of the liquid crystalline phases (Fig. 3(A and B)) which also corresponded to increased viscosity. This is probably because

hydrophilic channels available during the release of [^{3}H]DADLE increased with increasing initial water content. [^{3}H]DADLE release increased at 37°C compared to 20°C for both the lamellar and cubic phases. This is attributed to an increase in [^{3}H]DADLE diffusivity within the lyotropic matrix at 37°C. The Franz cell method with a moist nylon membrane was intended to simulate the in vivo environment i.e. when applied to the buccal mucosa. The release profiles obtained by the Franz cell method were much slower than the immersion method due to the nylon membrane restricting access of media to the matrix. The lag times from the Franz cell method were negligible (1.13–2.14 min) due to the use of pre-hydrated membranes, implying that this membrane does not constitute a rate-limiting barrier for release process. The swelling and DADLE release of lamellar phase during the initial 4 h at 20 and 37°C showed a linearity against square root of time indicating that both the sorption of swelling medium by the system and DADLE release are diffusion processes (Fig. 4).

In conclusion, the swelling and DADLE release strongly depended on temperature, the initial water content of the liquid crystalline matrix and the methodology employed for determining the swelling and DADLE release.

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