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Kinetic Characterization of Swelling of Liquid Crystalline Phases of Glyceryl Monooleate

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Research in this paper focuses on the kinetic evaluation of swelling of the liquid crystalline phases of glyceryl monooleate (GMO). Swelling of the lamellar and cubic liquid crystalline phases of GMO was studied using two *in vitro* methods, a total immersion method and a Franz cell method. The swelling of the lamellar phase and GMO having 0 %w/w initial water content was temperature dependent. The swelling ratio was greater at 20°C than 37°C. The water uptake increased dramatically with decreasing initial water content of the liquid crystalline phases. The swelling rates obtained using the Franz cell method with a moist nylon membrane to mimic buccal drug delivery situation were slower than the total immersion method. The swelling was studied by employing first-order and second-order swelling kinetics. The swelling of the liquid crystalline phases of GMO could be described by second-order swelling kinetics. The initial stage of the swelling ($t < 4$ h) followed the square root of time relationship, indicating that this model is also suitable for describing the water uptake by the liquid crystalline matrices. These results obtained from the current study demonstrate that the swelling strongly depends on temperature, the initial water content of the liquid crystalline phases and the methodology employed for measuring the swelling of GMO.

Key words: Glyceryl monooleate, Liquid crystalline phases, Cubic phase, Lamellar phase, Swelling

INTRODUCTION

Glyceryl monooleate (monoolein, GMO) is a polar lipid that has been used commonly as a food or cosmetic additive. GMO is sparingly water-soluble (Sadhale and Shah, 1998) and its aqueous solubility was estimated as ~10^{-6} M (Engström, 1990). When GMO is in contact with aqueous media such as water and biological fluids, it swells to form several types of liquid crystalline phases such as cubic, lamellar and hexagonal phases (Engström *et al.*, 1992). The formation of the liquid crystalline phase is mainly dependent upon the water content of the system and temperature.

Recently, the liquid crystalline phases of GMO have been investigated as buccal peptide delivery systems (Lee and Kellaway, 2000). The important *in vitro* properties which reflect the actual *in vivo* behavior of the liquid crystalline phases of GMO are considered to be the swelling and drug release characteristics in aqueous media. In the current work, we focus our attention on the measurement of swelling of the liquid crystalline phases of GMO and kinetic characterization of the swelling process. The intrinsic water uptake properties were first evaluated using a total immersion method, allowing the liquid crystalline matrices to access unrestricted solvent availability. Alternatively, the water uptake across a pre-hydrated nylon membrane mounted in a Franz-type diffusion cell was measured to mimic the *in vivo* situation when applied to the buccal mucosa. In the Franz cell method, the solvent availability was restricted to the single side of the matrix so that the swelling data could be compared to those obtained under conditions where the solvent was freely available. All swelling experiments were performed in physiological pH using a phosphate buffered saline pH 7.4 as a swelling solvent (Panchagnula and Patel, 1997).

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MATERIALS AND METHODS

Materials

A commercial grade of GMO (RYLO™ MG19) was purchased from Danisco Ingredients (Copenhagen,

Denmark) and used as received. The total monoglyceride content of the GMO was about >98%. Phosphate buffered saline pH 7.4 (PBS) tablets were obtained from Sigma-Aldrich Company (Saint Louis, MO). One PBS tablet dissolved in 200 mL distilled water gives a 0.01 M sodium and potassium phosphate buffer, pH 7.4, containing 0.0027 M potassium chloride and 0.137 M sodium chloride. Distilled water was used throughout.

Preparation of liquid crystalline phases

Liquid crystalline samples were prepared in glass vials. The liquid crystalline phases containing 35 and 16 %w/w initial water content represented cubic and lamellar phases, respectively (Engström *et al.*, 1995). GMO itself (0 %w/w initial water content) was also used for the swelling experiment. To form liquid crystalline phases, GMO was warmed to 50°C in a water bath until it becomes a transparent and viscous fluid. Water was also warmed at the same temperature. The water was then added to the molten GMO and the mixture was homogenized by a Teflon-coated magnetic stirrer. The sample was incubated for 3 days and then allowed to equilibrate at room temperature for 5 days.

Swelling studies

Immersion method. A polypropylene cylindrical mould (8.4 mm i.d., 3.0 mm thickness) filled with liquid crystalline phases or molten GMO was kept in a refrigerator for 0.5 h. The set sample was removed from the mould and transferred onto a slide glass, weighed using an analytical balance (1702 MP8, Sartorius Limited, Surrey, UK) and then allowed to equilibrate at room temperature. The sample was immersed in 300 mL of de-aerated PBS maintained at 20 or 37°C. At pre-determined time points up to 24 h, the samples were removed, carefully dried by blotting with a tissue paper and re-weighed to monitor weight changes. The swelling ratio (SR) was then calculated as $[W_t/W_0]$, where W_t indicates the weight of the sample at time t and W_0 denotes the initial weight of the sample. Size changes of swelling liquid crystalline matrix were also determined by placing the blot-dried matrix sample on a transparent millimeter-ruled glass plate (accuracy ± 0.25 mm). The average of the dimensional changes (diameter and thickness) was recorded.

Franz cell method. A nylon membrane (0.45 μm pore size Whatman International Limited, Maldstone, UK) was pre-treated by immersing it in PBS for a period of 24 h and blot-dried with a tissue paper prior to use. The membrane was chosen for its compatibility with the components of the liquid crystalline phases. The same formulations as those used in the immersion method were investigated. The cylindrical (8.4 mm diameter, 3.0 mm thickness) liquid crystalline phase was placed onto the pre-hydrated nylon membrane as a supporting mesh and weighed. This was mounted in a Franz diffusion cell. Teflon washers were placed around the outer circumferences of the receiver and donor compartments to ensure that the liquid crystalline phase remained in contact with the nylon membrane. The receiver compartment was filled with PBS (2.2 - 2.4 mL) to initiate the experiment and the donor compartment was sealed with a silicone-greased cover slip to maintain constant humidity. The assembled units were placed in a water bath at 20 or 37°C. At pre-determined time intervals, the Franz cells were disassembled and the liquid crystalline matrix on the membrane was taken, blot-dried with a tissue paper and re-weighed. The liquid crystalline sample on the nylon membrane was promptly returned to the Franz cell to continue the experiment. The swelling ratio was determined by comparing weight changes as mentioned above.

RESULTS AND DISCUSSION

Two representative types of liquid crystalline phases determined by initial water content *i.e.* 35 (cubic phase) and 16 (lamellar phase) %w/w, together with GMO itself (0 %w/w initial water) were examined. Dynamic swelling of the liquid crystalline phases and GMO was investigated gravimetrically and geometrically.

The swelling of GMO-water system was first characterized by determining the amount of water taken up by the swelling of the liquid crystalline phases. 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. 1). The water uptake increased dramatically with decreasing initial water content, indicating that the swelling ratio was inversely proportional to the initial water content of the sample. The cubic phase did not swell at both 20 and 37°C since it already contained the equilibrium amount of water (Fig. 1). When the swelling ratio was measured in the 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. 1).

During the 24 h period of the swelling experiment, the appearance of the liquid crystalline phases was also observed by visual inspection. The cubic and lamellar phases could easily be identified by their isotropic and anisotropic natures, respectively. As swelling progressed, the outer layer of GMO and the lamellar matrix formed an isotropic gel, the thickness of which increased with time. At the end of the experiment (24 h), all matrices tested by

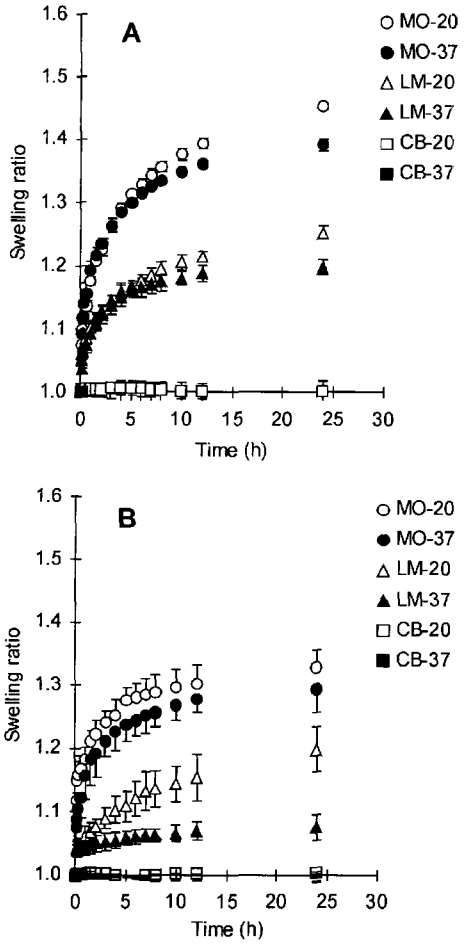

Fig. 1. Plots of swelling ratio measured using the immersion method (A) and Franz cell method (B) as a function of time: MO, glyceryl monooleate; LM, lamellar liquid crystalline phase; CB, cubic liquid crystalline phase; 20, measured at 20°C; 37, measured at 37°C. Each point represents mean ± SD, n = 3.

the immersion method changed to an isotropic gel due to the amount of water permeating into the GMO and the lamellar phase, leading to an equilibrium state of swelling. This finding clearly implied that when fully swollen, the GMO and lamellar phase were transformed to the cubic phase. Indeed, the lamellar phase can be transformed to the cubic phase either by temperature increase or by the further addition or absorption of water (Hyde *et al.*, 1984). At 37°C, the lamellar phase is transformed to the cubic phase without a change in water content. Water ingression into the GMO results in the formation of the lamellar phase as an intermediate step to the formation of the cubic phase.

The swelling profiles were evaluated kinetically to estimate the rate constants for water uptake. According to first order-swelling kinetics (Equation 1) (Schott, 1992), the rate of swelling at any given time, *t* is directly proportional to the uptake of swelling medium that has yet to occur before the maximum or equilibrium uptake (W_{max}) has

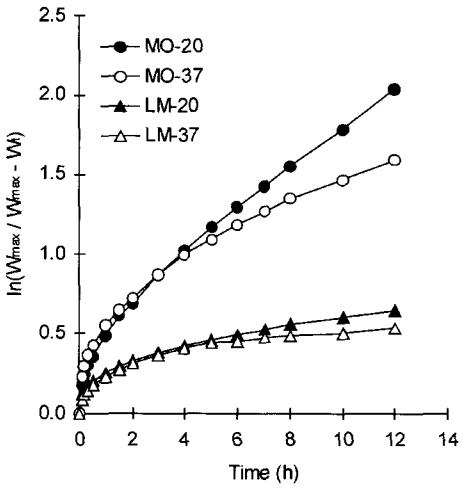

Fig. 2. Plots of swelling isotherms of lamellar phase (LM) and glyceryl monooleate (MO) according to first-order swelling kinetics. Swelling data were obtained from the immersion method. 37 and 20 denote 37 °C and 20°C respectively.

been reached:

$$\ln\frac{W_{max}}{W_{max}-W_t}=kt \qquad (1)$$

where, W_{max} is the maximum or equilibrium uptake. W_t is the uptake at time *t* and *k* is the proportionality constant. For most polymer matrices, the swelling rate constants are known to be determined by first-order swelling kinetics (Schott, 1992). However, the swelling data of GMO and lamellar phase obtained using the immersion method showed no linear relationship when plotted according to equation 1 (Fig. 2).

Second-order swelling kinetics (Equations 2 and 3) can also describe the rate and maximum uptake at equilibrium (Ofner and Schott, 1986):

$$\frac{t}{W_t}=A+Bt \qquad (2)$$

where, W_t is the amount of swelling medium taken up at time *t*, and A and B are constants. Rearranging and differentiating equation 2 results in equation 3:

$$\frac{dW_t}{d_t}=\frac{A}{(A+Bt)^2} \qquad (3)$$

When $t \rightarrow 0$, the initial rate of swelling is 1/A, *i.e.* the reciprocal of the *y*-intercept in the plot of t/W_t versus *t* according to equation 2. The reciprocal of the slope, 1/B indicates W_{max}, the maximum or equilibrium uptake since at long times, $W_t \rightarrow W_{max}$ and $Bt >> A$, therefore, from the equation 2, $B = 1/W_{max}$. The units of A and B are $h \cdot g_{matrix}/g_{buffer}$ and g_{matrix}/g_{buffer}, respectively (Ofner and Schott, 1986).

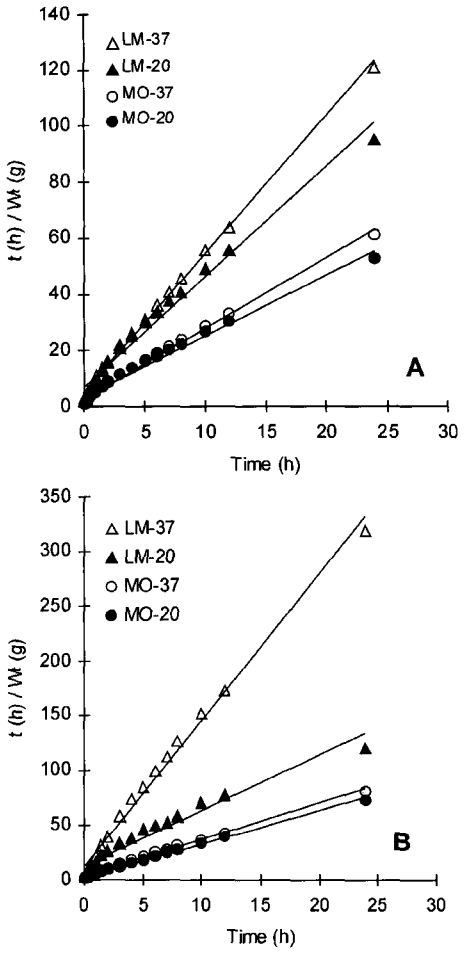

Fig. 3. Plots of swelling isotherms of lamellar phase (LM) and glyceryl monooleate (MO) according to second-order swelling kinetics. Swelling data were obtained from the immersion method (A) and Franz cell method (B). 37 and 20 denote 37°C and 20°C respectively.

Linear relationships were achieved between swelling data of the lamellar phase and GMO and time according to second-order swelling kinetics (Fig. 3). Linear correlation coefficients ranged from 0.9727 to 0.9979 (Table I).

The initial rate of swelling could be calculated by second-order swelling kinetics and values of 0.19 ± 0.06 (lamellar, 37°C), 0.15 ± 0.03 (lamellar, 20°C), 0.35 ± 0.05 (GMO 37°C) and 0.27 ± 0.01 (GMO, 20°C) g/h were obtained using the immersion method, demonstrating a higher initial rate of swelling at 37°C. The maximum water uptake for the immersion method was calculated from B values of the second-order swelling equation *i.e.* 0.20 ± 0.01 (lamellar, 37°C), 0.25 ± 0.01 (lamellar, 20°C), 0.40 ± 0.01 (GMO, 37°C) and 0.46 ± 0.01 (GMO, 20°C) g/g. Unlike the initial rate of swelling, the maximum water uptake was achieved at 20°C rather than 37°C. 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. Both the initial rate of swelling and the maximum water uptake increased with decreasing initial water content of the liquid crystalline phases.

Table I. Second-order swelling kinetic parameters for lamellar phase and glyceryl monooleate at 20 and 37°C. Mean ± SD, n=3.

	A ± SD ($h \cdot g_{matrix}/g_{buffer}$)	B ± SD (g_{matrix}/g_{buffer})	R^2 (mean)
LM[a]-IM[b]-37[c]	5.42 ± 1.72	4.92 ± 0.31	0.9978
LM-IM-20[d]	6.81 ± 1.12	3.94 ± 0.15	0.9899
MO[e]-IM-37	2.87 ± 0.41	2.51 ± 0.06	0.9969
MO-IM-20	3.77 ± 0.21	2.17 ± 0.03	0.9928
LM-FC[f]-37	11.82 ± 4.72	13.99 ± 3.87	0.9939
LM-FC-20	13.89 ± 3.72	5.14 ± 1.09	0.9727
MO-FC-37	3.31 ± 0.56	3.36 ± 0.33	0.9979
MO-FC-20	2.43 ± 0.26	3.05 ± 0.27	0.9979

[a]Lamellar liquid crystalline phase
[b]Immersion method
[c]Measured at 37°C
[d]Measured at 20°C
[e]Glyceryl monooleate
[f]Franz cell method

Swelling has also been characterized by determining diffusion rates of swelling medium (PBS) into the liquid crystalline phases. The diffusion of the swelling medium showed the square root of time relationship during the initial stage of the experiment (t < 4 h) as judged by correlation coefficients (Higuchi, 1963), demonstrating that this model is also suitable for describing the swelling of liquid crystalline matrices (Fig. 4). The correlation coefficients obtained from the immersion method were higher than

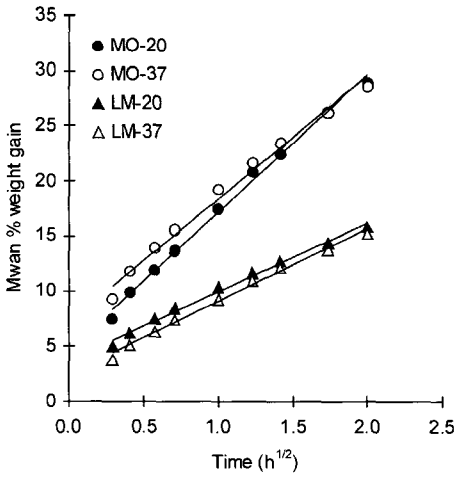

Fig. 4. Early stages (t < 4 h) of lamellar phase (LM) and glyceryl monooleate (MO) swelling (% weight gain) show a linear relationship with square root of time. Swelling data were obtained from the immersion method. 37 and 20 denote 37°C and 20°C respectively.

Table II. Diffusion rates of swelling medium obtained from the linear relationship between the amount of swelling medium (phosphate buffered saline, pH 7.4) diffused into the liquid crystalline phases and square root of time. Mean ± SD, n=3.

	Rate of swelling (%/h$^{0.5}$)	R^2 (mean)
LM[a] -IM[b]-37[c]	6.60 ± 0.58	0.9941
LM -IM-20[d]	6.24 ± 0.03	0.9950
MO[e]-IM-37	11.07 ± 0.77	0.9938
MO -IM-20	12.50 ± 1.07	0.9969
LM -FC[f]-37	0.97 ± 0.51	0.9624
LM -FC-20	3.62 ± 0.44	0.9842
MO -FC-37	9.11 ± 0.40	0.9832
MO -FC-20	7.45 ± 0.62	0.9780

[a]Lamellar liquid crystalline phase
[b]Immersion method
[c]Measured at 37°C
[d]Measured at 20°C
[e]Glyceryl monooleate
[f]Franz cell method

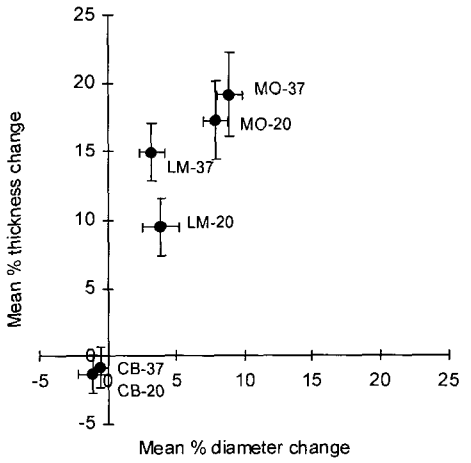

Fig. 5. Dimensional changes, measured using the immersion method at 37 and 20°C for 24 h, of swelling glyceryl monooleate and its lamellar and cubic phases. Mean ± SD, n=3: MO, glyceryl monooleate; LM, lamellar phase; CB, cubic phase; 37, measured at 37°C; 20, measured at 20°C.

those observed in the Franz cell method (Table II). The slower diffusion rates of PBS in the Franz cell method may indicate that the swelling was influenced by the methodology employed.

A significant increase occurred in the diameter and thickness of the swelling lamellar phase and GMO. Typical experimental results are presented in Fig. 5 where the swollen dimensions were determined at the end of the experiments (24 h).

It is noteworthy that the thickness increase was distinct as compared to the diameter increase due largely to the fact that the sample matrix was placed directly on the surface of a glass slide acting as an obstacle to radial expansion. As water uptake did not occur in the cubic phase, the dimensions of the cubic phase did not significantly change. The dimensional increase may result in an increase in the diffusional pathlength which will influence drug release rates.

CONCLUSION

Swelling of the lamellar and cubic liquid crystalline phases of GMO, together with GMO itself was studied using two *in vitro* methods, a total immersion method and a Franz cell method. The swelling of the lamellar phase and GMO having 0 %w/w initial water content was temperature dependent. The swelling ratio was greater at 20°C than 37°C. The water uptake increased dramatically with decreasing initial water content of the liquid crystalline phases. The swelling rates obtained using the Franz cell method with a moist nylon membrane to mimic buccal drug delivery situation were slower than the total immersion method. The swelling of the liquid crystalline phases of GMO could be described by second-order swelling kinetics. The initial stage of the swelling ($t < 4$ h) followed the square root of time relationship, indicating that this model is also suitable for describing the swelling of liquid crystalline matrices. These results obtained from the current study demonstrate that the swelling strongly depends on temperature, the initial water content of the liquid crystalline phases and the methodology employed for measuring the swelling of GMO.

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