JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 629–636

Received October 30, 2000
Accepted January 12, 2001
ISSN 0022-3573

Water quantitatively induces the mucoadhesion of liquid crystalline phases of glyceryl monooleate

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Abstract

The possible role of water in the mucoadhesion phenomenon exhibited by the liquid crystalline phases of glyceryl monooleate was investigated using an in-vitro tensile strength technique. The mucoadhesion of the liquid crystalline phases of glyceryl monooleate was found to occur following uptake of water. The mucoadhesive force of the cubic phase was consistent since it is not capable of taking up additional water. An increase in pre-load period greatly facilitated the mucoadhesion of glyceryl monooleate (0% w/w initial water content), suggesting that the mucoadhesion is dependent upon the extent of the dehydration of the substrate. A good linear relationship between initial water content of the liquid crystalline phases and mucoadhesive force led to the conclusion that the mucoadhesive force increased with decreasing initial water concentration. Rheological properties of the liquid crystalline phases were also studied to allow a correlation between physical changes and mucoadhesion of the liquid crystalline phases, revealing that higher water concentrations in the liquid crystalline phases led to a more ordered structure that showed less mucoadhesion. The results of this study indicated that the mucoadhesive force of the liquid crystalline phases of glyceryl monooleate is determined by the capability to take up water from a water-rich environment. It may, therefore, be advantageous to use the lamellar phase as a buccal drug carrier as opposed to the relatively less mucoadhesive cubic phase.

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Introduction

Bioadhesive materials have attracted significant interest in attempts to retain the drug, or drug-delivery system, at a biological tissue surface to increase drug absorption and improve drug bioavailability (Mortazavi & Smart 1994; Ahuja et al 1997). Furthermore, their application has extended to achieve particular therapeutic aims. Examples include localisation in specified regions to modify tissue permeability, to inhibit metabolising enzymes and to modulate immunological expression and targeting to specific tissues or organs to minimise undesirable systemic effects (Robinson 1990) or to maximise a site-specific drug therapy (Martinelli et al 1999).

The cubic (35% w/w water content) and lamellar (5–20% w/w water content) liquid crystalline phases of glyceryl monooleate have previously been demonstrated to be bioadhesive (Engström et al 1995; Nielsen et al 1998). In these investigations, in-vitro methodologies revealed that both the cubic and lamellar phases possess mucoadhesive properties and that the lamellar phase has a greater mucoadhesive force than the cubic phase on mucous tissues including porcine lingual, sublingual

and intestinal mucosae and rabbit jejunum. Further, it was demonstrated that solvents such as water, excipients that influence the formation of liquid crystalline phases and incorporated drug concentration can affect the mucoadhesive behaviour of glyceryl monooleate as measured by the in-vitro flushing bioadhesive test system and tensiometer (Nielsen et al 1998). Although it is evident that the cubic and lamellar phases of glyceryl monooleate require water to exhibit mucoadhesion (Dash et al 1999), little information is available on the mechanism of glyceryl monooleate mucoadhesion.

The aim of this study was to understand the role(s) of water in in-vitro mucoadhesion exhibited by the liquid crystalline phases of glyceryl monooleate. Among the test methods to study in-vitro mucoadhesion (Kamath & Park 1994), the measurement of tensile strength was employed since this method is readily applicable for the liquid crystalline phases of glyceryl monooleate (Kellaway 1990; Pritchard et al 1996). Rheological characterisation was also carried out to establish the effect of water concentration on the viscoelastic properties of liquid crystalline phases.

Materials and Methods

Materials

A commercially available grade of distilled glyceryl monooleate was purchased from Danisco Ingredients (Copenhagen, Denmark) and used as received. Mucin (Type III; partially purified from porcine stomach) was obtained from Sigma-Aldrich Company Ltd (Poole, UK). Freshly prepared distilled water was used throughout. All other chemicals were of analytical grade. Phosphate buffer pH 7.4 was prepared according to the relevant monograph of the *British Pharmacopoeia* 1998. A simulated saliva solution was prepared by dissolving disodium hydrogen orthophosphate (2.38 g), potassium dihydrogen orthophosphate (0.19 g) and sodium chloride (8.0 g) in 1000 mL of water at pH 6.75 (Wong et al 1999). Fresh porcine buccal mucosa was obtained from pig heads supplied by a local abattoir on the day of slaughtering.

Preparation of liquid crystalline phases

The required amount of water maintained at 50°C was added to molten glyceryl monooleate at 50°C in a water bath. The mixture in a glass vial was sealed tightly and kept in an incubator maintained at 37°C for 3 days to form liquid crystalline phases and then allowed to equilibrate at room temperature ($\sim$ 22°C) for 5 days. The liquid crystalline phases were examined by visual inspection and a light microscope equipped with a polarisation filter (Olympus BH2, Olympus Optical Co., Japan).

Differential scanning calorimetry (DSC)

The thermal properties of the liquid crystalline phases of glyceryl monooleate having various water concentrations (8–35 % w/w) were explored using a Perkin Elmer DSC7 differential scanning calorimeter (Perkin Elmer Corporation, Norwalk, CT). The differential scanning calorimeter was calibrated for temperature and enthalpy using indium. Samples were weighed (10 ± 5 mg) directly into aluminium pans and crimp sealed. The calorimetric traces were obtained by scanning the samples against a blank reference aluminium pan at 5°C min^{-1} under a constant flow of pure nitrogen gas. The onset and peak of transition were monitored using a Perkin Elmer 7 series software.

Rheological behaviour

Rheological examination was carried out using a CSL^2 100 Carri-Med Rheometer (TA Instruments Ltd, Surrey, UK) fitted with a $2° \times 2$ cm stainless steel cone and plate geometry and utilising a 50-μm gap. The rheometer was equipped with a Peltier plate as a temperature control system and the Peltier plate served as the sample plate of the cone and plate geometry. Oscillatory analysis was performed over a frequency range of 0.1–10 Hz since this serves to characterise the rigidity and elasticity of the test materials simultaneously and to determine structural changes occurring in the sample (Madsen et al 1998). The measurements were carried out at both 20 and 37°C. The sample equilibrated at the appropriate temperature was loaded on the rheometer plate and further equilibrated for 3 min without stress. Initially, a torque sweep at 1.0 Hz was carried out to determine the linear viscoelastic region of the liquid crystalline phases and 100 Pa was chosen as a suitable oscillatory shear stress for all systems investigated. A temperature sweep for 5–50°C was conducted using a stress of 100 Pa. The sample stored at room temperature was loaded on the rheometer plate, cooled to 5°C and equilibrated for 3 min. The temperature of the plate was then raised at a rate of 2°C min^{-1}. Three viscoelastic parameters (i.e. storage or elastic modulus (G'), loss or viscous modulus (G'') and dynamic viscosity (η')) were measured concurrently as functions of temperature and frequency.

In-vitro mucoadhesion property

The mucoadhesion test apparatus described by Durrani et al (1995) was used with a slight modification. Two different biological substrates were employed – reconstituted glycoprotein (mucus gel) and porcine buccal mucosa. The porcine buccal mucosa was chosen to mimic actual buccal application conditions. All measurements were made at room temperature.

Mucus substrate

Mucin (20 % w/w) was mixed with pH 7.4 phosphate buffer to produce the mucus gel and allowed to hydrate for 4 h. The mucus gel (0.3 g) was weighed on a Whatman cellulose nitrate membrane (0.45 μm pore size, 25 mm diameter) and evenly spread to form a thin, continuous gel layer. The membrane was carefully positioned on the central perforated region of the lower part of a mucoadhesion cell fixed on a balance pan. Cylindrically set liquid crystalline phase (8.4 mm diameter × 5.0 mm thickness) was placed over the Whatman filter paper disc (18 mm diameter, No 1, Qualitative) lying across the end piece of the tubular probe. The probe was firmly attached to the syringe pump. Once the substrate and liquid crystalline phases were correctly positioned the probe was lowered onto the mucus gel. A pre-load (0.25 N) was applied for 30 s and at the end of the pre-load period the probe was raised (16 mm min^{-1}). A personal computer running a data capture programme (WinWedge version 1.2, TAL Technologies Inc., Philadelphia, PA) connected to the balance was used to record the weight changes in grams.

Porcine buccal mucosa substrate

Porcine buccal tissue was freshly excised and stored in pH 7.4 phosphate buffer at 4°C. The mucosal membrane was separated by removing the underlying connective tissue with tweezers and surgical scissors. The porcine buccal mucosa was then washed with cold phosphate buffer and blot-dried with tissue paper to remove surface-associated water. The porcine buccal mucosa was mounted in the mucoadhesion cell and before measurement, simulated saliva solution (0.2 mL) was evenly sprinkled on the mucosal surface of the tissue. The measuring procedure was as described above.

Calculation of detachment force

The peak detachment force was considered as a mucoadhesive force. The force per cm^2 required to separate the liquid crystalline phases from the biological substrates was calculated from the recorded readings according to equation 1.

$$\text{Detachment force (N cm}^{-2}) = [\text{maximum detachment weight (kg)} \times \text{acceleration due to gravity (m s}^{-2})]/\text{contact area (cm}^2) \quad (1)$$

Statistical analysis

Statistical comparisons between groups were performed using analysis of variance. $P < 0.05$ was considered significant.

Results

The cubic liquid crystalline phases (25–35 % w/w water content) of glyceryl monooleate were transparent at room temperature while the lamellar phases (8–20 % w/w water content) were opaque. Polarised microscopy revealed the cubic phase as a dark background due to its optically isotropic nature. In contrast, the lamellar phase, being anisotropic, appeared as planar bilayers of the lipid on a dark background.

Differential scanning calorimetry

DSC measurements of the liquid crystalline phases containing various water concentrations (0–35 % w/w)

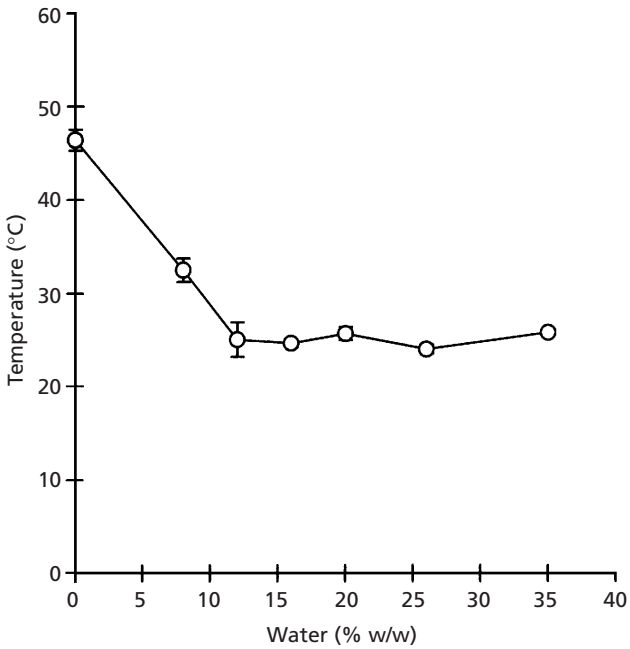

Figure 1 Influence of water concentration on melting temperature of the acyl chain of glyceryl monooleate determined by differential scanning calorimetry. Mean ± s.d., n = 3.

exhibited a single endothermic peak. This peak is probably due to the melting of the acyl chain of glyceryl monooleate since there was no significant difference between the sample exhibiting phase transition (i.e. 16 and 20% w/w water contents) and those showing no phase transition (i.e. 25 and 35% w/w water contents) within the examined temperature range and based on the phase diagram between glyceryl monooleate and water (Engström 1990; Czeslik et al 1995). Generally, lipid hydration lowers the temperature of chain fluidisation (Cevc 1991) as seen in Figure 1. However, saturation with respect to chain fluidisation was found in the samples containing higher water concentration than 10% w/w (i.e. limited hydration). The additional water is structurally incorporated but without appreciably affecting the thermodynamic behaviour of the glyceryl monooleate (Seddon et al 1983).

Rheological behaviour

The rheological profiles obtained from temperature sweep demonstrated two obvious peaks with respect to viscoelastic parameters (region A and region B; Figure 2). Peaks in region A are considered to represent changes in the viscoelastic parameter due to the formation of a gel that exists as a solid at 5°C (start temperature). The changes were strongly affected by the lipid concentration in the sample (i.e. a higher lipid concentration caused greater change in viscoelastic parameter). At around 20–35°C there was no significant change in the viscoelastic parameters for samples containing 20% w/w and 35% w/w water since no phase changes are observed in the phase diagram (Engström 1990). In region B, the lamellar phase containing 10% w/w water alone exhibited a change in rheological properties which is probably due to the formation of a more ordered lamellar structure above the acyl-chain fluidisation temperature (i.e. around 25°C) of glyceryl monooleate. Liquid crystalline phases containing 20 and 35% w/w water, since no phase transitions occur in region B (35–50°C) based on the phase diagram constructed by Engström (1990), did not show changes in rheological parameters in region B. They existed as cubic phases in region B.

The frequency sweep demonstrated a significant increase in viscoelastic parameters for the lamellar phase containing 10% w/w water as the temperature changed from 20°C to 37°C (Figure 3). The increases observed at 37°C were also evident in the temperature sweep (Figure 2 region B).

Loss tangent values (tan $\delta = G''/G'$) of all samples

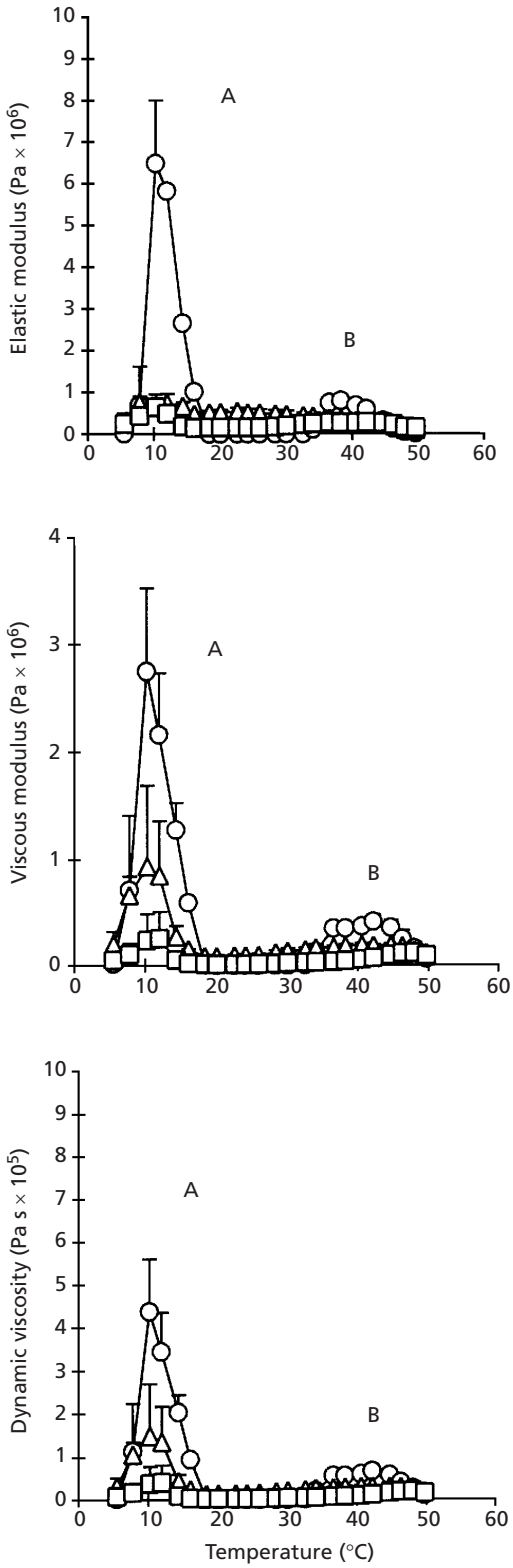

Figure 2 Effect of temperature on the rheological behaviour of the liquid crystalline phases of glyceryl monooleate. ○, 10% water; □, 20% water; △, 35% water. Mean ± s.d., n = 3.

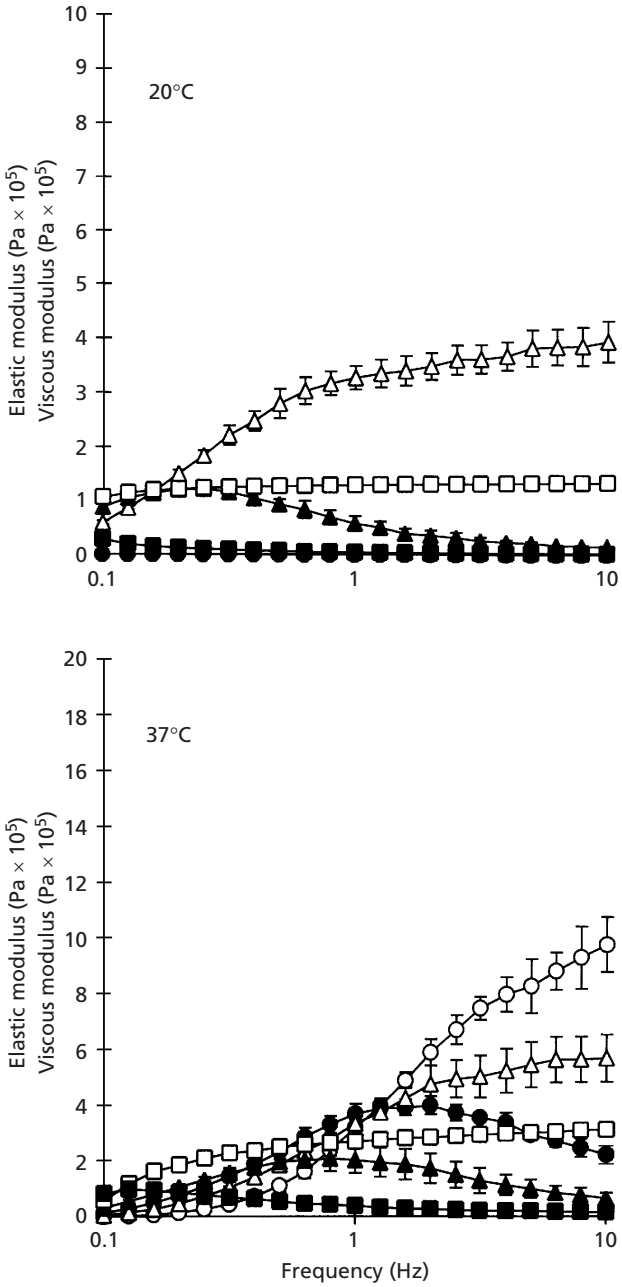

Figure 3 Frequency sweep of the liquid crystalline phases of glyceryl monooleate at 20°C and 37°C. ○, 10% water (elastic modulus); ●, 10% water (viscous modulus); △, 20% water (elastic modulus); ▲, 20% water (viscous modulus); □, 35% water (elastic modulus); ■, 35% water (viscous modulus). Mean ± s.d., n = 3.

declined with frequency (Figure 4), implying that the elasticity (solid-like component) of the liquid crystalline phases increased relative to the viscous (liquid-like) component with increasing frequency. At 20°C, the

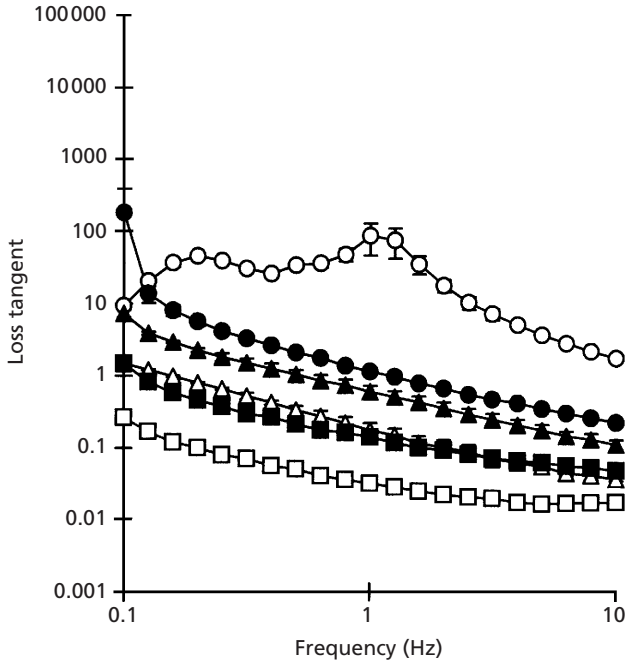

Figure 4 Plots of loss tangent (tan δ) values as a function of frequency. ○, 10% water at 20°C; △, 20% water at 20°C; □, 35% water at 20°C; ●, 10% water at 37°C; ▲, 20% water at 37°C; ■, 35% water at 37 °C. Mean ± s.d., n = 3.

rank order of tan δ was 10% w/w > 20% w/w > 35% w/w water content. Thus, while the overall viscous resistance to flow (η') was higher in the case of 20% w/w and 35% w/w water-content systems, the relative contribution of the loss modulus to the overall resistance to deformation was lower. This is clear evidence that at 20°C a higher water content (> ca 20%) leads to a far more ordered gel matrix. At 37°C, such a trend in tan δ was not observed; rather the three structures elicited loss tangent values < 1.0 at frequencies > 1.0 Hz, implying a well-structured gel (Bonferoni et al 1999).

Mucoadhesion

The lamellar phase (16% w/w water content) showed the greatest mucoadhesion both with mucus and porcine buccal mucosa substrates whereas the cubic phase and glyceryl monooleate displayed similar mucoadhesion under the conditions employed (0.25 N contact force and 30 s contact time) (Figure 5). When measured with porcine buccal mucosa, the mucoadhesive force decreased significantly ($P < 0.01$) compared with mucus substrate. This decrease is probably due to less moisture being available on the surface.

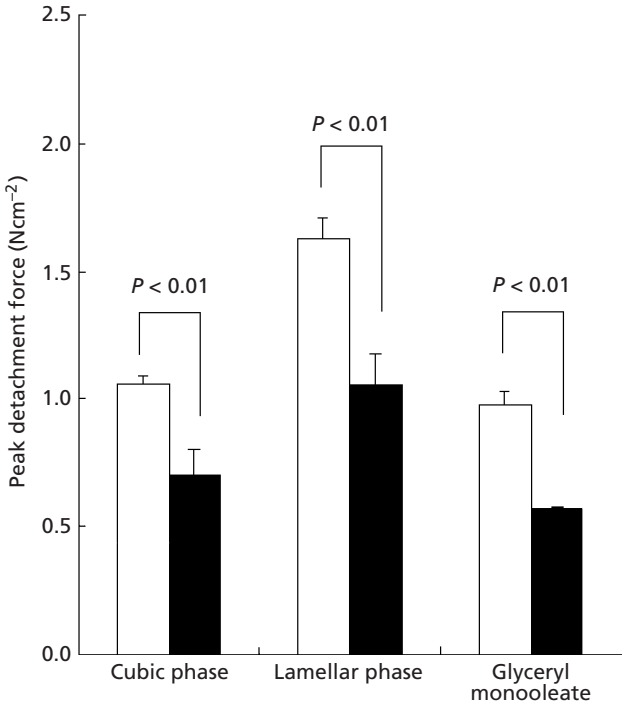

Figure 5 Mucoadhesion forces of cubic (35% w/w initial water content) and lamellar (16% w/w initial water content) liquid crystalline phases of glyceryl monooleate and glyceryl monooleate (0% w/w initial water content) measured with mucus (white columns) and porcine buccal mucosa substrates (black columns). Mean ± s.d., n = 4.

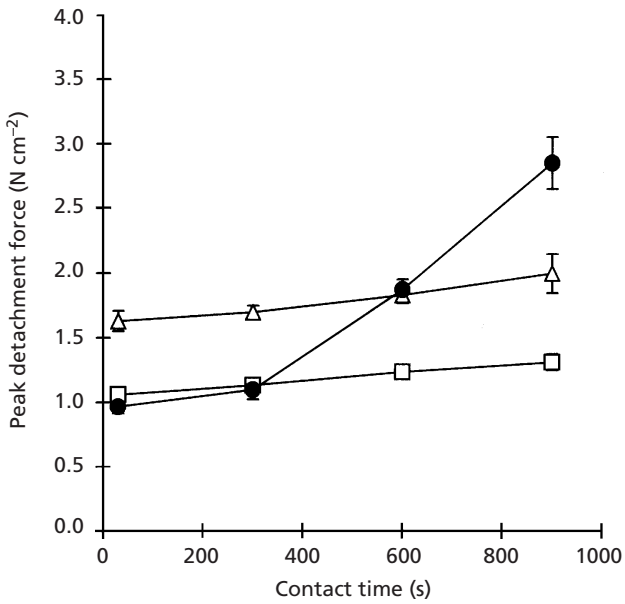

Figure 6 Effect of contact time on the mucoadhesion force of cubic (□, 35% w/w initial water content) and lamellar (△, 16% w/w initial water content) liquid crystalline phases of glyceryl monooleate and glyceryl monooleate (●, 0% w/w initial water content) measured with mucus substrate. Mean ± s.d., n = 4.

The mucoadhesion force of glyceryl monooleate increased greatly as the contact time increased (Figure 6). This is probably because as the contact time increases the water uptake by glyceryl monooleate increases. The reason why glyceryl monooleate showed less mucoadhesion measured at a contact time of 30 s is that the pre-load period of 30 s is comparatively short for glyceryl monooleate devoid of water channels at t = 0, to take up water from the substrate, although glyceryl monooleate displays initial rapid water uptake (Lee & Kellaway 2000). For contact times exceeding 600 s, the glyceryl monooleate exhibited the greatest mucoadhesion. The rank order of mucoadhesion force obtained at 900 s (i.e. glyceryl monooleate lamellar phase > cubic phase) is in agreement with the results published by Nielsen et al (1998), where they measured the work of adhesion (mJ cm^{-2}) using porcine intestinal tissue with a contact force of 0.2 N and a contact time of 30 min.

The cubic (35% w/w initial water content) and lamellar (16% w/w initial water content) liquid crystalline phases and glyceryl monooleate (0% w/w initial water content) completely swollen in PBS for 48 h at 20°C provided identical mucoadhesive forces (N cm^{-2}) (cubic phase, 1.01 ± 0.07; lamellar phase, 1.01 ± 0.06; glyceryl monooleate, 1.03 ± 0.02). Since glyceryl monooleate and its cubic and lamellar liquid crystalline phases have equilibrium water uptake (Geraghty et al 1996; Lee & Kellaway 2000), the fully swollen liquid crystalline phases were not capable of taking up additional water.

The mucoadhesive force of the liquid crystalline phases of glyceryl monooleate containing 8, 12, 16, 20, 26 and 35% w/w water was measured (Figure 7). However, the lamellar liquid crystalline phases containing 8 and 12% w/w water could not be placed in the mucoadhesion apparatus because of their fluidity at room temperature (~ 22°C). Plotting the peak detachment forces versus initial water content of the liquid crystalline phases of glyceryl monooleate demonstrated an inverse relationship with $r^2 = 0.9275$ (correlation coefficient) between the range 16% w/w and 35% w/w water concentration.

Discussion

Salivary glands in the oral cavity provide a considerable amount of water in the form of saliva (0.5–2 L daily) that can be used for swelling or drug release from buccal drug-delivery systems (Nagai & Machida 1993). This

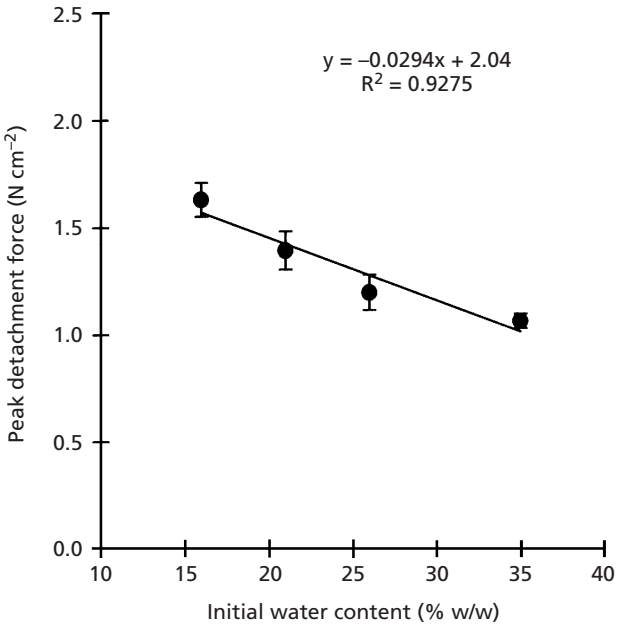

Figure 7 Plot of the relationship between peak detachment force and initial water concentration of glyceryl monooleate–water liquid crystalline phases measured with mucus substrate. Mean ± s.d., n = 4.

feature is useful for the bioadhesion of liquid crystalline phases of glyceryl monooleate, since the mucoadhesion of the liquid crystalline phases occurs following uptake of water.

The measurement of tensile strength as a parameter for mucoadhesion supports the hypothesis that the possible mechanism of the mucoadhesion phenomenon exhibited by the liquid crystalline phases of glyceryl monooleate is the dehydration of the substrate (i.e. water uptake by the mucoadhesive material). A quantitative relationship was established between the capability of taking up water and the mucoadhesive force determined as maximum force of detachment. The amount of water taken up by the liquid crystalline phases governed the mucoadhesive force and among the liquid crystalline phases tested, the phase containing the least amount of water showed the greatest mucoadhesion. Therefore, the dependence of mucoadhesion on water uptake suggests that mucoadhesion occurs through the dehydration of the substrate. However, since there is an equilibrium level of swelling for the liquid crystalline phases of glyceryl monooleate, the fully swollen liquid crystalline phases exhibited a similar mucoadhesive force to the cubic phase that already contains the equilibrium amount of water (Geraghty et al 1996; Lee & Kellaway 2000). Glyceryl monooleate requires sufficient time (e.g. > 10 min) to exhibit better mucoadhesion than the lamellar and cubic phases. Therefore the use of the lamellar phase as a buccal drug carrier will undoubtedly be preferred.

The two lamellar phases, having different water concentrations, displayed different rheological behaviour; a higher water concentration resulted in a more ordered structure. In practice, the cubic phase (35% w/w water content) and more ordered lamellar phase (20% w/w water content) were difficult to handle due to their rigidity at ambient temperature. However, the less ordered lamellar phase containing less water (10% w/w) was free to flow at ambient temperature, which can lead to the development of various dosage forms, including dental injection, intramuscular depot or liquid-filled soft or hard gelatin capsules. Hence, the lamellar phase containing smaller water contents (5–10% w/w) is expected to show a versatile applicability as well as good mucoadhesion. The rheological experiments also revealed that at body temperature the low-viscous lamellar liquid crystalline phase at 20°C transfers to the highly ordered gel structure that may be able to resist salivary washout and mechanical movement, resulting in prolongation of the residence time. It has been suggested that systems with a low tan δ generally show greater mucoadhesion (Tamburic & Craig 1995). However, the glyceryl monooleate system demonstrated an opposite trend, whereby systems with a higher tan δ showed greater mucoadhesion.

In conclusion, the tensile mucoadhesive force of detachment of liquid crystalline phases of glyceryl monooleate is determined by the capability to take up water from a water-rich environment such as a mucus gel or a mucosal surface. For strong mucoadhesion to occur, liquid crystalline phases should possess minimal water content. The low-viscosity lamellar liquid crystalline phase that can be transformed to the more ordered cubic phase in the oral cavity is the preferred candidate to be used as a mucoadhesive drug carrier.

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