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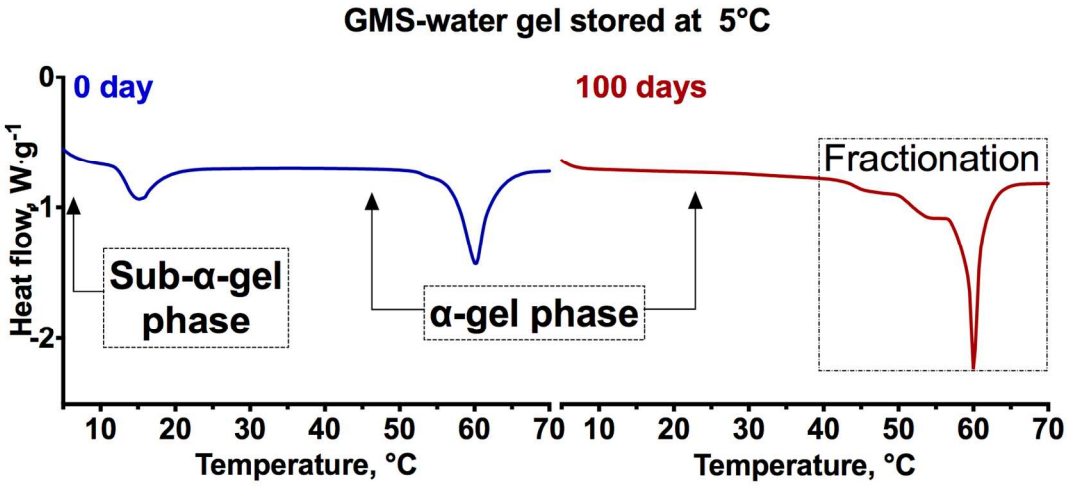
GMS-water system transformed to the sub-α-gel phase below 13°C. The α-gel is stable at 5°C but fractionation takes place.



Nature and dynamics of monostearin phase transitions in water: stability and the sub-α-gel phase

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Abstract

This work examines the polymorphic transition of a glycerol monostearin-water system (GMS-water) stored at 5ºC, 25ºC and 45ºC for 100 days. Upon addition of co-emulsifiers, such as sodium stearoyl lactylate (SSL), the GMS-water system forms a metastable α-gel structure, which transforms into a coagel structure over time. The GMS-water system loses its water swelling capacity during the polymorphic transition from the α-gel phase to the coagel phase. Therefore the α-gel phase is a more favorable structure for water structuring. Powder X-Ray diffraction (XRD), XRD with temperature control, differential scanning calorimetry (DSC), and microscopy were used to examine the nature and dynamics of the phase transition. Results suggest that the α-gel phase is stable for 100 days when stored at 5ºC. Increasing the storage temperature will increase the rate of transformation from the α-gel phase to the coagel phase. However, even though no phase transformation from the α-gel phase to the coagel phase took

place; seemingly, fractionation took place within the α-gel structure when stored at 5ºC, altering the melting profile of the gel and affecting the calculation of the Coagel Index. The GMS-water system forms a thermally reversible and metastable sub-α-gel phase when cooled below 13ºC. This work characterizes the XRD diffraction patterns and the DSC melting profiles of the sub-α-gel phase of GMS-water system for the first time. XRD patterns suggest that the water layer thickness in the sub-α-gel phase is ~140 Å. This thick layer of water may contribute to the high water swelling capacity and stability of the monoglyceride-water system at refrigeration temperatures.

1. Introduction

Monoglycerides (MGs) are surface active lipid molecules with a single fatty acid chain attached to a glycerol backbone. The amphiphilic properties of MGs make them commonly used emulsifiers in food, personal care and pharmaceutical products. MGs have polymorphic and mesomorphic properties.[1,2] The polymorphic and mesomorphic behaviors of MGs affect their application as emulsifiers. MG phase behavior has been characterized for dry MG crystals, MG-water mixtures (MG "gels"), MG-oil mixtures, and MG-water-oil emulsions.[1–5] Depending on temperature and age, MG-structured systems and MG crystals occur in the sub-α phase, α phase, β phase, liquid crystal lamellar phase and cubic phase depending on temperature and age.[1–7]

The phase behavior of saturated MG crystals has been studied extensively. [3,5,7–9] Crystals of glycerol monosteatate (GMS) and glycerol monopalmitate (GMP) have more than four different polymorphic forms as neat solids. Two sub-α forms of GMS have been observed, one with a melting temperature of 30°C (sub-α 2) and the other with a melting temperature of 45°C

(sub-α 1).[1,9] The MG crystal transforms into the α crystal form at 50°C, and can undergoes a thermally reversible transition to the sub-α phases when the temperature is lower than 50°C.[1,9] Increasing the temperature will melt the α phase at 75°C. The two sub-α phases and the α phase are thermodynamically unstable, and will all gradually transform to the β phase.[3,9] The XRD spacings of the sub-α phase, the α phase and the β phase of GMS crystals are summarized in Table 1.[3,7,9] The α phase has a small angle X-ray diffraction (SAXS) peak at 50.3Å, while the β crystal has a slightly smaller lamellar spacing at 50.17Å, indicating that the hydrocarbon chains of the MG molecules are more tilted in the triclinic structure of the β crystals than the hexagonal structure of the α crystals.[10]

Mixing MG crystals with oil can form a lamellar structure when heated above the Krafft temperature of the MG (the temperature at which the hydrocarbon chain melts) and crystallizes into the α-gel phase when cooled below the Krafft temperature without shear.[11] The α-gel network is metastable and the system transforms into the coagel phase upon aging.[11] Because of the amphiphilic nature of MG molecules, the gel phase is in an inverse lamellar arrangement in a MG-oil system, with the glycerol head groups in the middle of the MG bilayers and the hydrocarbon chains in contact with the oil.[12] Similarly to dry MG crystals, the inverse lamellar phase (α-gel phase) and the sub-α crystal can be formed upon cooling.[12] In MG-oil systems structured with GMP or GMS, the α-gel is formed when the system is cooled below 55°C, and the sub-α crystal is formed when the temperature is below 36°C[12]. The lattice parameters for the sub-α phase, the α-gel phase and the coagel phase of MG-oil systems are summarized in Table 1.

Table 1. XRD patterns of different polymorphic forms of GMS crystal, GMS-oil system, and GMS-water system.[3,9,13–16,20,25–27]

		MG crystal	MG-oil	MG-water
Sub-α	SAXS	50 Å	50 Å	
	WAXS	4.3 Å and 3.9-3.7 Å	4.27 Å and 4.17 Å-4.11 Å	
α or α-gel	SAXS	50 Å	52 Å	55Å
	WAXS	4.2 Å	4.17-4.11 Å	4.18Å
ß or coagel	SAXS	49 Å		
	WAXS	Several peaks at 3.6 Å-4.6 Å		

The phase behavior of MG-water systems was first described by Krog and Larsson.[13] When heating the MG-Water mixture (MG-gel) above its Krafft temperature, MGs form a highly hydrated lamellar phase.[13] Upon cooling below the Krafft temperature, the hydrocarbon chains lose some mobility and transform into a structured Lβ phase (α-gel), which can retain thick layer of water between the MG bilayers. However, the α-gel is only in a metastable state, and is thermodynamically unfavorable; the α-gel will gradually crystallize into the more organized and densely packed Lβ' phase, also called a β-gel or coagel.[13–15] The coagel phase is the thermodynamically favorable phase. During the α-gel to coagel phase transition, MG bilayers undergo a structural reorganization, in which they expel the water between the bilayers. The system cannot revert back to the α-gel phase without heating above the Krafft temperature and forming the lamellar phase again.[6,13,16,17] Cooling the MG-gel under shear or shearing after cooling accelerates the α-gel to coagel phase transition and enhances the release of water.[4,18,19] The α-gel phase or the coagel phases are accessible at 10-60% (w/w) MG in water concentrations below 55°C.[6] Further heating of the MG-gel above 80°C may cause the formation of cubic phases.[6,17] Only the XRD peak positions of the α-gel form and the coagel form have been

identified in literature, while the diffraction peak positions of the sub-α phase had not been identified in MG-water system (Table 1).[13,14,16]

The extent of the phase transition from the α-gel phase to the coagel phase can be characterized using a Coagel Index (CI).[15] CI can be determined by heating the MG-gel above its Krafft transition temperature, cooling the sample down, and heating it up again above the Krafft temperature. The two heating cycles yielded two enthalpies of melting. The first heating melts both the α-gel and the coagel phases, while the cooling cycle and second heating cycle forms and melts only the freshly formed α-gel phase.[15] The CI can be calculated by taking the ratio of the enthalpy change of the first melting (ΔH1) and the second melting (ΔH2), therefore CI = ΔH1/ ΔH2. The coagel phase has a more ordered structure than the α-gel phase, thus the enthalpy change associated with melting the coagel is always higher than that for melting the α-gel. A CI of 1 means that the MG-gel is in the α-gel form, while a higher CI means that relatively more coagel phase is present.[15] Over time, as the phase transition from the α-gel to coagel takes place, the CI will increase.

This work focuses on developing an understanding of the nature and dynamics of the phase behavior of the GMS-water system. Morley & Tiddy studied the phase behavior of C16-18 saturated MGs in water, where a melting peak was observed at ~10°C.[20] They briefly indicated that this melting peak corresponded to the sub-α phase, however they did not characterize the structure of the phase.[20] Studies also showed that the melting temperature of the sub-α phase was ~36°C in a GMS-oil system.[12] Introducing 10% (w/w) water into this MG-oil system depressed the melting temperature of the sub-α phase to ~18°C.[12,21] The proposed mechanism was that water disturbed the inverse lamellar packing, was trapped at the center of the MG bilayers, and stayed in contact with the glycerol head group of the MG molecule [21]. Based on Chen &

Terentjev's work, the DSC melting peak at ~18°C represents the Krafft point of MGs demixing from the MG-oil network, which is accompanied by the formation of the sub-α phase.[21] The WAXS diffraction patterns of the system at 15°C had characteristic powder XRD reflections at 4.17 and 4.11 Å, corresponding to the spacing between in-plane glycerol head groups and the distance between the two glycerol layers within the inverse lamellar structure.[21] Systems with higher water content lack the diffraction pattern at 4.11 Å, because the presence of water increases the distance between the two layers inside the inversed bilayer structure.[21] Even though the literature mentions the sub-α phase of MG-structured systems contains water, the characteristics of the sub-α phase of MG-water systems are still unclear.

MG-based emulsions are widely used in food and cosmetics products.[4] The polymorphic transition of the MG emulsifier limits the shelf life of these products because of the loss of nanostructured water. In order to increase the water holding capacity of the MG-water system, slowing down the α-gel to coagel phase transition is crucial. Cassin *et al* found that the α-gel is stable when stored at above 45°C because the driving force for coagel formation was larger at lower storage temperatures.[15] Comparison of the stability of the α-gel phase of MG-water system at various storage temperatures helps further understand the dynamics of polymorphic transformations of MGs.

When making a MG-structured system, impurities such as free fatty acids and diglycerides assist in the structuring of water, while distilled MGs alone cannot gel water.[14,22] As a result, an anionic co-emulsifier is required to form the gel in distilled MG-water systems.[23]. Neutralized stearic acid and sodium stearoyl lactylate (SSL) have been used as effective anionic co-emulsifiers in monoglyceride-based emulsions in previous studies.[4,23] Similar to the triclinic (α)

form of crystalline MGs, SSL crystals are stable in the α form, displaying a single powder X-ray diffraction wide angle reflection at 4.1 Å.[5]

The aim of the research presented here is to develop a better understanding of the dynamics of saturated MG phase transitions in water over long periods of time at various storage temperatures. Here we also discuss the phase behavior of MG-water systems (MG-gel) below room temperature. An increased understanding of the temperature dependence of mesomorphic phase transitions in saturated MGs will ultimately help in efforts geared towards the stabilization of the α-gel phase of monoglyceride structured oil and water continuous systems.

2. Experimental details

2.1 Materials

The MG-gel system has of 20% (w/w) solid in water. The solid phase consists of glycerol monostearate (GMS) and SSL (1:19 SSL: GMS w/w); the water phase has 0.1% w/w potassium sorbate as an antifungal agent. The GMS used was Alphadim 90 SBK monoglyceride (Caravan Ingredients, Lenexa, KS, USA). The GMS sample contains 83.5% monostearin, 12.4% monopalmitin, with the trace amount of diglycerides and triglycerides. The SSL used was provided by Caravan Ingredients (Lenexa, Kansas, USA), and the potassium sorbate was from SigmaAldrich Canada Co. (Oakville, ON).

The MG-gel was prepared by mixing GMS powder and SSL powder with water. The mixture was heated above 57°C, the Krafft transition temperature of the GMS, and kept in a hot water bath until the powders were fully dissolved / melted. The gel was then cooled on the bench top with no shear. Gel samples were stored in capped glass sample vials at 5ºC, 25ºC and 45ºC for 100 days. Gel samples were made in duplicates, and each gel was sampled twice.

2.2 Powder X-Ray diffraction (XRD)

The lamellar spacing and polymorphic forms of the gel were characterized with a Rigaku Multiflex x-ray Diffractometer (RigakuMSC Inc., The Woodlands, TX, USA). This unit has a copper source (l=1.54Å) set at 44 kV and 40 mA, and the divergence slit, receiving slit, and scattering slit were set at 0.3 mm, 0.5°, and 0.5°, respectively. Gel samples were placed onto a glass sample holder with an area of 20×20 mm and depth of 1 mm. Diffraction angles in the range $1° < 2\theta < 35°$ were studied. The scanning rate was 1° per minute. Experiments were conducted at room temperature. The XRD results were analyzed with Jade 6.0 (Jade Software Corporation Limited).

2.3 X-Ray diffraction with temperature control

In order to obtain the X-Ray diffraction (XRD) patterns of the MG-gels below room temperature, samples were measured with an X-Ray unit with temperature control system (Hecus S3 MICRO diffractometer system, Hecus X-ray Systems GmbH, Reininghausstrasse 13a, 8020 Graz, Austria). The sample was placed in capillary tubes and heated at 2°C per minute from 1 to 75°C and cooled from 75 to 1 °C at 0.5°C per minute. Diffraction peaks were collected with SAXS and WAXS detectors every minute.

Due to limitations of the equipment, it was not possible to match the heating and cooling rate of the XRD experiments with those conducted with the TA DSC analyzer. MG-gel samples were then measured with the DSC using the same heating and cooling rates as the XRD experiments as a control. The melting temperatures of the MG-gel measured using the two thermal programs were similar.

2.4 Differential scanning calorimetry (DSC)

The heating and melting profiles of the MG-gels were measured with a TA DSC analyzer (Q-2000 calorimeter, TA Instruments, New Castle, DE). Two heating cycles were applied to the gel samples in order to obtain the Coagel Indices. They were heated from 1°C to 75°C, held at 75°C for 5 minutes, cooled to 1°C, held for 5 minutes, and heated to 75°C for the second time. The applied heating and cooling rate was 10°C per minute, which is sensitive enough to detect the phase transitions, while providing relatively high signal/noise ratio (Chen & Terentjev, 2009). The peak integrations were preformed with Universal Analysis 2000 (TA Instruments, New Castle, DE), where the enthalpy and peak temperature of melting / crystallization were computed.

The Coagel Index was calculated by taking the ratio of the melting enthalpy at the first heating cycle and the melting enthalpy at the second heating cycle, based on Cassin et al (1998). The first melting curve of samples stored at 5°C were fitted into three Vogit area peaks against a linear baseline with PeakFit v4.12 (Systat Software Inc, San Jose, CA, USA) for further result presentation. The melting peak with shoulder was fitted

In order to study the thermal reversibility of the MG-gel, another set of DSC experiments was conducted. Freshly made MG-gel samples were heated from 1°C to 25°C, held at 25°C for 5 minutes, cooled to 1°C, held for 5 minutes, and heated to 25°C for a second time.

2.5 Microscopy

The MG gel morphology over 100 days was observed with bright filed transmitted light microscopy at room temperature. Samples were observed with a Leica DMRXA2 microscope

(Leica Micro- systems Canada Inc., Richmond Hill, Canada), and images were taken with a CCD camera (RETIGA 1300i, Burnaby, BC) and Openlab software (Improvision, Lexington, MA, USA).

Additionally, fluorescent microscopy was used to examine the gel structure. A lipid soluble fluorescent dye, Coumarin 6 (Sigma-Aldrich Canada Co, Oakville, ON) was used to tag the MG crystals. Coumarin 6 has the maximum absorption wavelength at 444 nm, with fluorescence emission at 505 nm in ethanol and 515-558 nm in DMSO under XeCl light source. The fluorescent dye was dissolved in 95% ethanol to make a saturated coumarin-ethanol solution. 4 mL of this filtered solution was added into 2 grams of dry GMS and SSL (19:1 w/w) powder mixture. This powder was heated to 75ºC, the melting temperature of GMS, in order to dissolve the fluorescent dye. This mixture was then cooled and flushed under compressed air to evaporate the ethanol. After evaporation, the dry powder was made into a 20% solid gel following the procedure mentioned previously. A XeCl lamp source was installed onto the microscope as the light source. A Hamamatsu C11400 digital camera (Japan) was used to capture the images. The L5 cube, with the excitation wavelength of 480±50 nm and suppression wavelength of 527±30 nm, was used to identify the Coumarin fluorescent dye. Images were processed with Volocity 6.2.1 software (PerkinElmer, Woodbridge, ON, Canada) for presentation.

3. Results and discussion

3.1 Powder X-Ray diffraction

Small angle diffraction patterns of the gel are presented in Figures 1a, 1b and 1c. The gels displayed broad diffraction peaks when fresh. Diffraction peaks were detected at 52, 26, 17, 13 and 8.5 Å, indicating a lamellar packing structure of the gel MGs. The peak corresponding to the

(001) plane was at approximately 52 Å for samples stored at all three temperatures, and the higher order peaks could be observed up to the (006) plane with a diffraction peak at 8.5 Å.

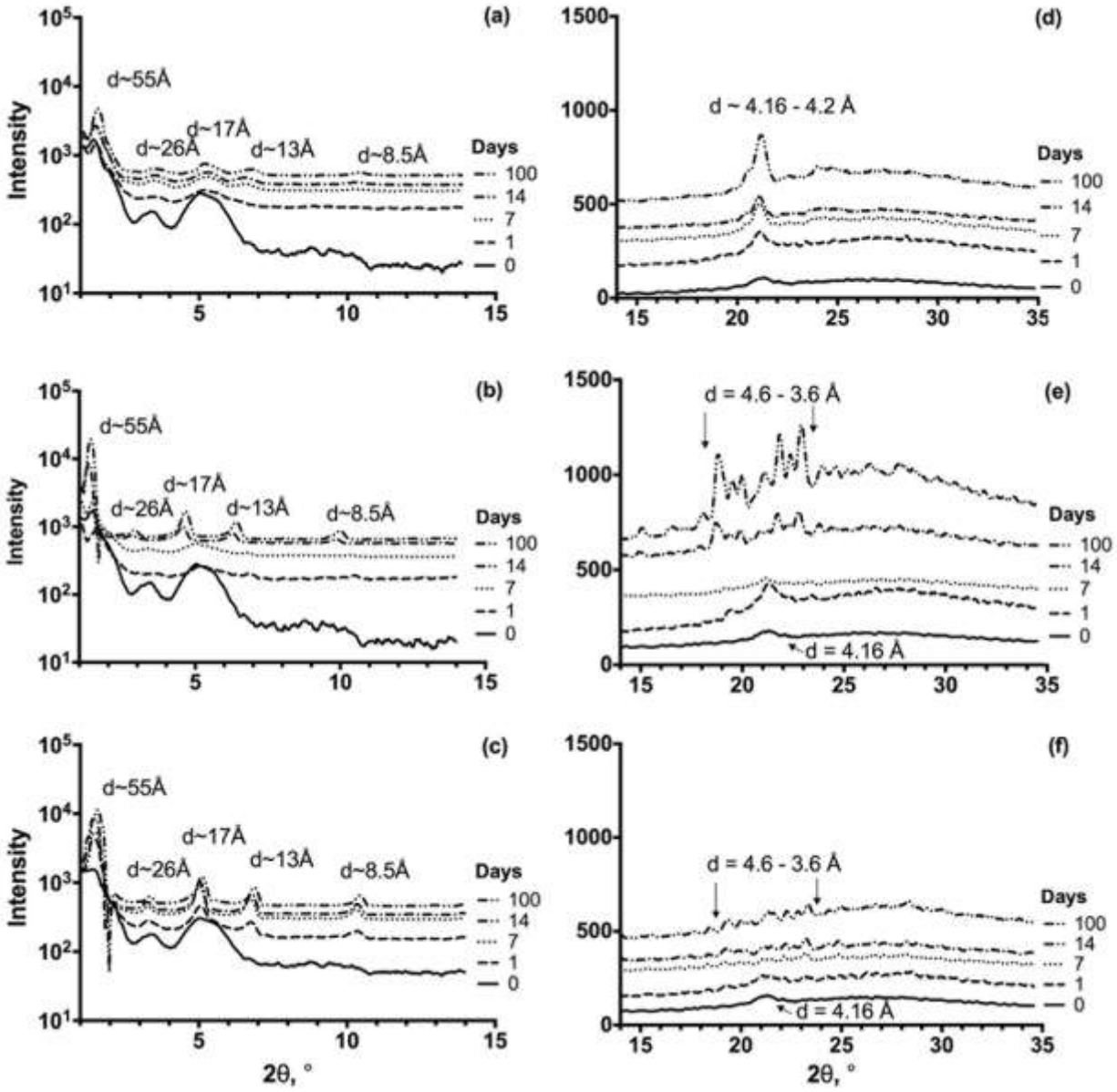

Figure 1. XRD patterns of gel samples. SAXS diffraction patterns of gel samples stored at (a) 5°C, (b) 25°C and (c) 45°C over 100 days and WAXS pattern of gel samples stored at (a) 5°C, (b) 25°C and (c) 45°C over 100 days.

Gel samples stored at 5°C preserved the original broad peak morphology over the storage period (Figure 1a), while well-defined, sharper peaks developed upon aging for samples stored at 25 and 45°C (Figure 1b and 1c). Broad peaks indicate a swollen structure of well hydrated gels, as is the case for fresh gels and gels stored at refrigeration temperatures.[24] Changes to sharper, well-defined peaks indicated that the sample transformed from a well-hydrated swollen lamellar structure to a dehydrated and more crystalline lamellar packing structure over time. This transition was observed together with the phase transition from the α-gel phase to the coagel phase in the WAXS region. The development of the crystalline lamellar structure and the phase transition indicates that water is released from the swollen structure when the phase transition takes place.

Samples stored at 5°C show a single WAXS peak at ~4.15Å from day 1 to day 100 (Figure 1d). Samples stored at 25°C show a peak at 4.15Å for the first three days which then developed into multiple peaks between 3.6 and 4.6Å (Figure 1e). Samples stored at 45°C showed similar peaks to the 25°C gel samples, with multiple peaks developing faster than the 25°C samples (Figure 1e and 1f). The diffraction peaks of gels stored at 45°C were less distinctive and more blended into the baseline, compared with samples stored at lower temperatures.

This indicates that gel samples stored at 5°C remained in the α-gel phase for 100 days. Samples stored at 25°C and 45°C showed a phase transition from the α-gel phase to the coagel phase over time. The corresponding peaks for the coagel phase (3.6 - 4.6Å) started to appear at day 3 at 25°C, in combination with the α-gel phase (4.16Å). Phase transitions took place faster at 45°C, as the peaks associated with the coagel phase were observed after one day. Therefore, increasing the storage temperature will accelerate the phase transition to the coagel phase, contrary to previous reports [15]. Comparing the WAXS pattern of samples stored at 25°C and

45°C, the less distinctive peaks observed in the 45°C samples indicate a less organized structure even though both samples were in the coagel form. At this temperature, the system is close to the gel to liquid crystalline phase transition and molecules would have a greater mobility (energy), thus affecting long-range order in the crystalline lattice. This greater disorder and mobility would be evident as a less organized coagel crystalline structure.

3.2 Differential scanning calorimetry (DSC)

DSC results for the gel samples are summarized in Figure 2. The first melting curves of the gel samples were compared with the second melting curves, where the first melting curves represent the melting behavior of samples over time, and the second melting curve represents the melting behavior of a fresh sample. The MG-gels had two melting peaks between 1 and 75°C when freshly made. The melting temperature of the first peak is ~13°C and the melting temperature of the second peaks is ~60°C. The two observed melting temperatures were similar to those reported by Chen & Terentjev for a MG-oil-water ternary system [21].

The area under the first melting peak at ~13°C decreased over storage and the peak eventually disappeared at all three temperature conditions. The melting peak at ~13°C disappeared after three weeks when samples were stored at 5°C, two weeks when stored at 25°C and one week when stored at 45°C.

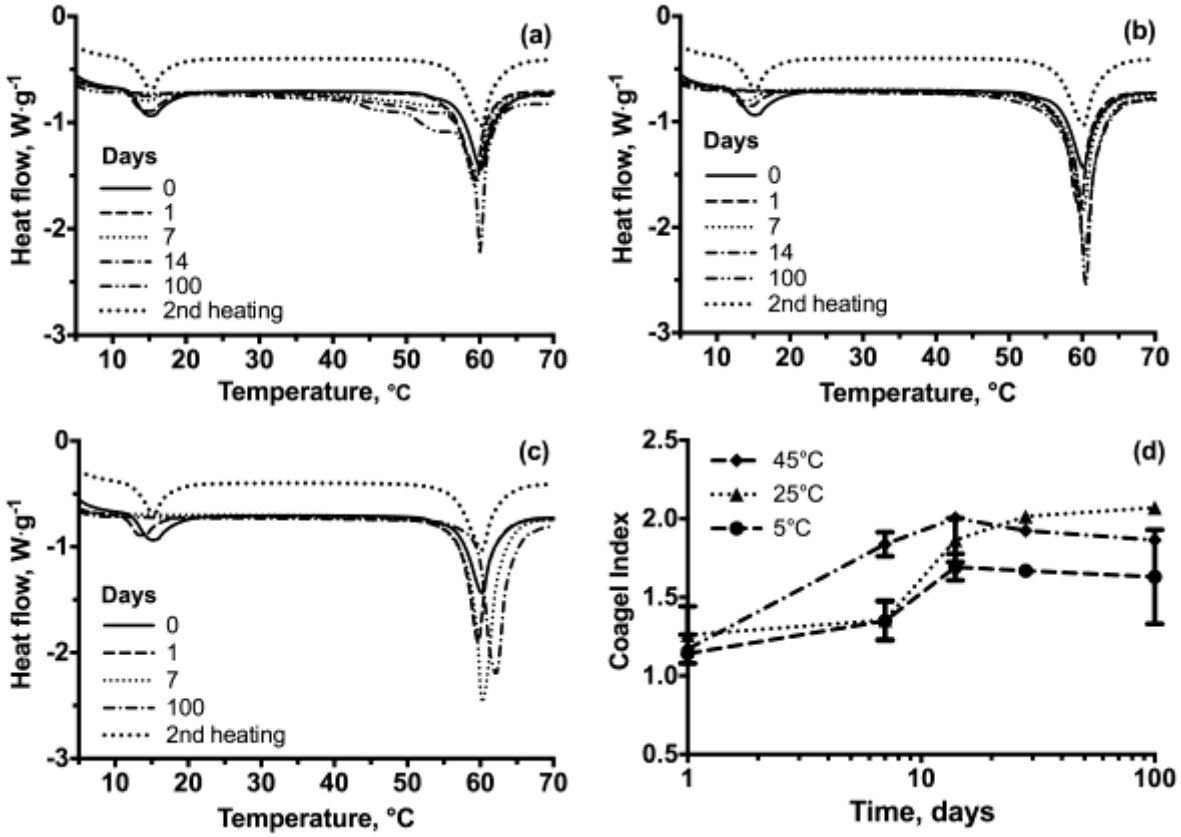

Figure 2. DSC melting profiles of the MG-gel samples stored at (a) 5°C, (b) 25°C and (c) 45°C over 100 days and (d) calculated Coagel Indexes. The melting curves of the second heating were plotted 0.3 W/g higher for presentation.

The area under the second melting peak at about 60°C of the first melting curve represents the enthalpy of melting of the aged gel (ΔH1), while the area under the peak in the second curve represents the enthalpy of melting of the fresh gel (ΔH2). Δ H1 increased over time for samples at all the three storage temperatures, where higher storage temperature led to faster increase of Δ H1. This increase in enthalpy is associated with the α-gel to coagel phase transition, because it requires higher amount of energy to melt the coagel phase. Similar to the XRD results, DSC

results suggest that the phase transition from the α-gel phase to the coagel phase was faster at higher storage temperatures, as the areas under the peak increased faster for samples stored at 45°C (Figure 2b and 2c).

The melting peak of the 5°C (Figure 2a) stored samples displayed a different shape, where a shoulder started to appear at around 30°C, before the melting peak at about 60°C appeared. The area under the shoulder increased in relative proportion upon aging.

After computing the enthalpy of melting (ΔH1 and ΔH2) of the gel samples, the CI was calculated following Cassin et al's method, where CI= ΔH1 /ΔH2.[15] For samples stored at 5°C, the entire area under the melting peak, including the shoulder, was integrated to obtain the enthalpy of melting.[15] CI values obtained are summarized in Figure 2(d). CI trends were in agreement with the XRD results, showing a faster increase in CI at higher storage temperature. Within two weeks, the sample stored at 45°C reached a CI of 2, while the sample stored at 25°C had a CI of 1.86. The CI increased to 2 for both temperatures after one month of storage.

The CI of MG-gel stored at 5°C gradually increased to ~1.6 after two weeks based on this calculation, suggesting that the gel also underwent the phase transition to the coagel phase at this temperature. However, WAXS results suggested that the α-gel phase was stable over the entire storage period. The shoulder observed in the main melting profile may then correspond to phases other than the α-gel phase. Therefore, an alternative method for calculating the CI of the samples stored at refrigeration temperature is necessary in order to obtain an accurate estimate of the CI.

3.3 Modified method for the determination of the Coagel Index of MG-gels

In order to calculate the CIs of gels stored at 5°C, melting curves with a shoulder were deconvolved into three peaks, as illustrated in Figure 3(a). The melting peak temperatures

derived were ~47°C, ~55°C, and ~60°C depending on the age of the sample. The enthalpy of melting of each species was then calculated as an area percentage of the original peak. Among the three deconvolved peaks, the peak at ~60°C has the similar melting temperature than samples stored at 25°C and 45°C (Figure 2). Therefore, only the enthalpy of the melting peak at ~60°C was thus used to calculate the Coagel Index.

Figure 3(b) compares the CI calculated using the deconvolved peak at 60°C to the CI calculated from the entire melting peak including the shoulder. The fitted data gave a CI between 0.9 and 1, indicating that the gel was in α-gel phase over storage, now in agreement with XRD results.

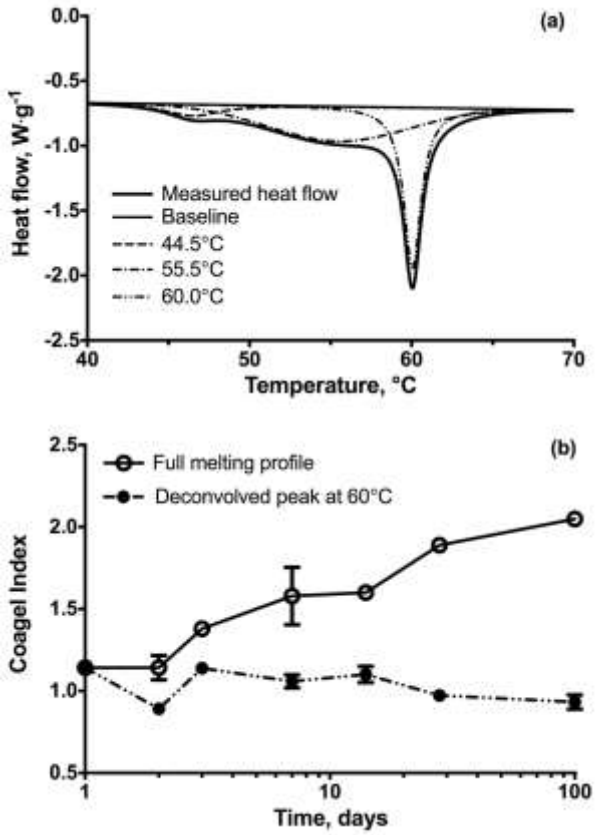

Figure 3. Coagel Index calculation using the original and deconvolved DSC melting profiles. (a) the DSC melting profile of a sample stored at 5°C for 100 days (R^2 = 0.9876). (b) comparison of

the CI calculated from the full melting profile (solid line in Figure 3a) and the CI calculated from the fitted melting peak at 60.0°C.

One possible reason for the presence of multiple melting peaks over storage is that when stored at 5°C, the α-gel may undergo nanoscale phase separation, or fractionation, leading to the formation of multiple DSC melting peaks. Even though the nature of this fractionation is still unknown, it interferes with the determination of the enthalpy of melting of the α-gel phase, which is then affecting the determination of the Coagel Indexes. Therefore only the melting enthalpy of the main transition at 60°C should be used for the CI calculation when shoulders begin to appear in the DSC thermal traces.

3.4 The sub-α phase of MG-water system

The structures of the MG-gel below and above the phase transition at ~13°C were characterized by simultaneous XRD-DSC. WAXS and SAXS patterns obtained from the melting and crystallization of freshly made MG-gels are presented in Figure 4. Reflections obtained in the wide angle region upon melting showed three distinct regions (Figure 4a). When the temperature is under 12°C, the gel displayed two diffraction peaks at 4.3Å and 4.16Å. Only one peak at 4.2Å was detected above 16°C. No peaks appeared in the WAXS range when the temperature was higher than 60°C. Research already suggested that the MG-gel is in the α-gel phase with a single reflection at 4.2 Å (Krog, 1997; Chen et al, 2009). Our results are in agreement with the literature. No diffraction peaks were detected when then temperature was above the Krafft temperature of 57°C, indicating that the MG-gel was in a liquid crystalline state. The positions of the two peaks below 12°C were similar to the peaks corresponding to the sub-α

phase of GMS-oil system (Table 1), therefore the MG-water system was in the sub-α phase when the temperature is at 12°C or lower.

The sub-α gel phase and the α-gel phase of the MG-gel also displayed different SAXS patterns (Figure 4b). When the MG-water system was in the sub-α-gel phase below 12°C, the gel showed one well-defined diffraction peak at ~190 Å, and another broad peak at ~54Å. Only one broad peak at ~50Å was detected when the sample was in the α-gel phase when the temperature was above 16°C. The two spacings at ~190 Å and ~54Å suggest that the sub-α-gel could structure water layers as thick as ~140Å.

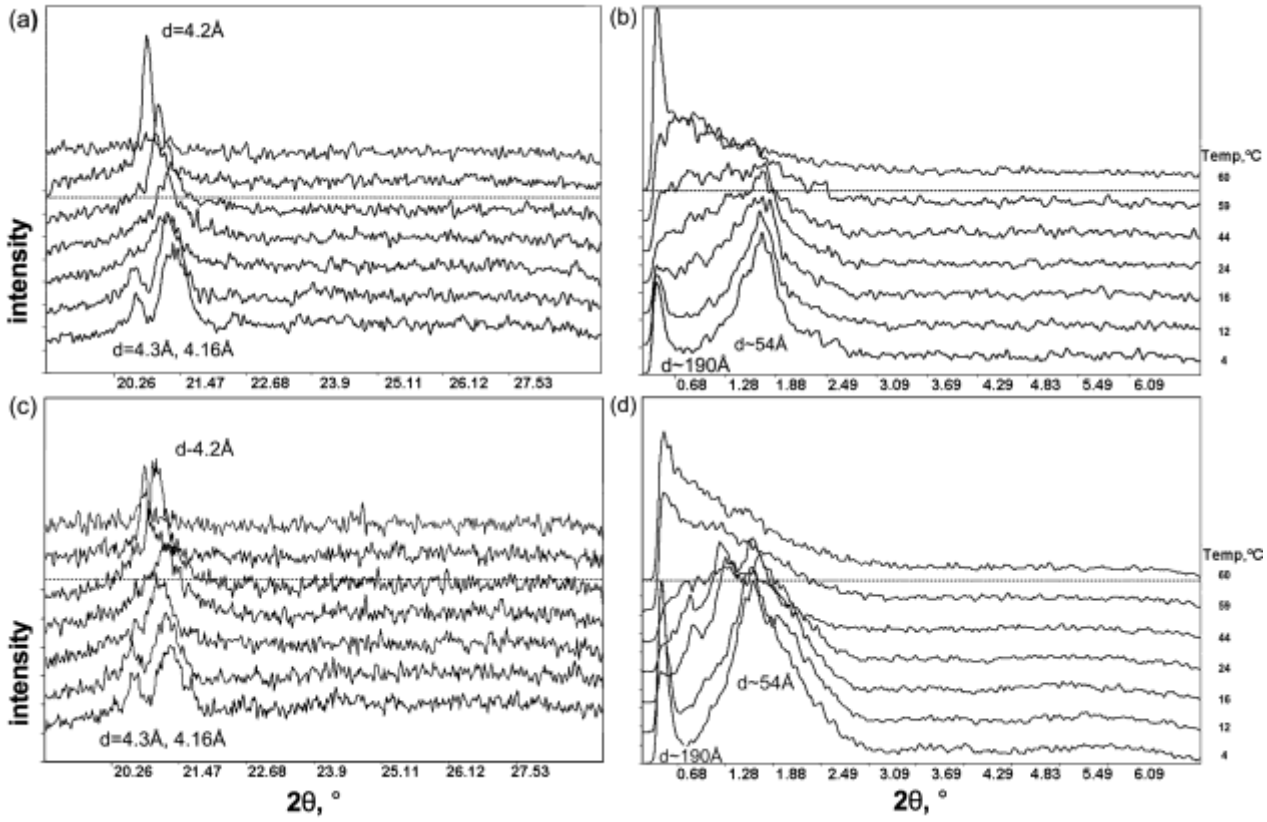

Figure 4. Melting and crystallization of fresh GMS-water system. (a) WAXS spectra taken during heating; (b) SAXS spectra taken during heating; (c) WAXS spectra taken during crystallization; (d) SAXS spectra taken during crystallization.

Figure 4c and 4d summarize the XRD patterns obtained from the crystallization of MG-water systems. The position of the reflections and peak morphologies are similar to the diffraction patterns obtained during the melting of MG-crystals.

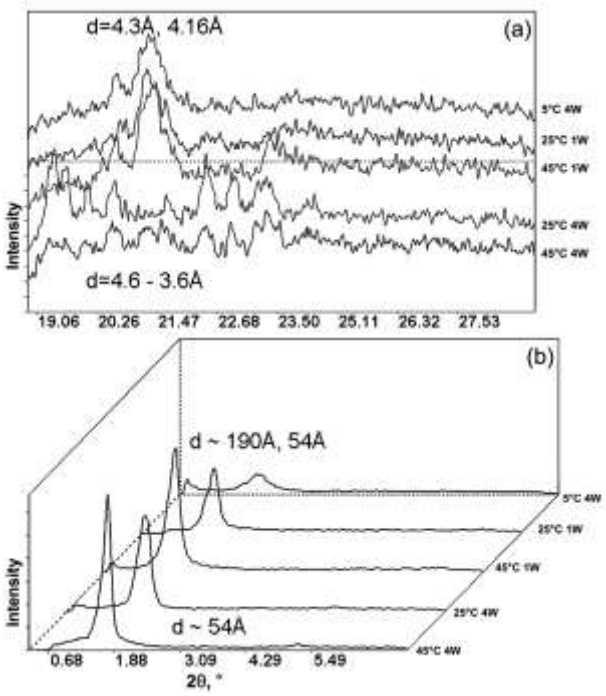

Figure 5. XRD patterns of the GMS-water system at 4°C (a) WAXS and (b) SAXS. Samples were stored at 5°C, 25°C and 45°C over one to four week.

XRD patterns of aged MG-gel samples stored at 5, 25 and 45°C were measured at 4°C. WAXS patterns and SAXS patterns are presented in Figure 5a and Figure 5b, respectively. Samples stored at 5°C for four weeks, 25°C for one week and 45°C for one week had two spacings at 4.3 Å and 4.16 Å (Figure 5a). Samples stored at 25°C and 45°C for four weeks had several spacings between 3.6 and 4.6 Å. These WAXS patterns indicate that the sub-α-gel phase of the MG-water system is stable at refrigeration temperature for four weeks, and it transfers into

the coagel phase when stored at higher temperatures for the same period of time. Therefore, XRD results are in agreement with the DSC melting profiles, that the sub-α-gel phase has higher stability at refrigeration temperatures.

When the MG-gel was in the sub-α-gel phase, as indicated by WAXS patterns, reflection positions in the SAXS region were different between samples stored at 5°C and samples stored at 25°C and 45°C (Figure 5b). Gel samples stored at 5°C for four weeks had two peaks at ~190 Å and ~54 Å, in agreement with freshly made samples. However, the diffraction peak at ~190Å was detected with reduced intensity or was not detected in samples stored at 25°C and 45°C for one week. Samples stored at 25 and 45°C for four weeks only showed one peak at ~54Å.The loss of the diffraction peak at ~190Å indicated that a polymorphic transition was taking place in the MG-water system, and the gel was in a transitional state between the sub-α-gel phase to the coagel phase. When the system was completely in the coagel phase (storage at 25°C for four weeks and 45°C for four weeks), the peak at~190Å was no longer detectable at 4°C, only a peak at ~50Å was detected.

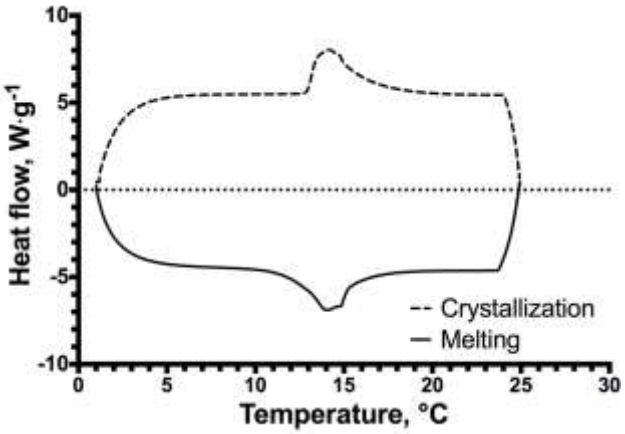

Figure 6. DSC thermogram of the MG-gel between 1 and 25°C when applying two heating cycles.

When applying temperature cycles between 1 and 25°C in the DSC, the sub-α-gel phase showed a thermally reversible solid-state polymorphic transitions, as illustrated in Figure 6. However if the MG-gel was already in the coagel phase, the sub-α-gel phase could only be obtained by cooling the MG-gel from above the Krafft transition temperature, when the system was in the lamellar phase.

3.5 Microscopy

Transmitted light microscopy images of the MG-gel observed after 100 days of storage are shown in Figure 7. Evenly distributed clusters of about 100 μm diameter were characteristic of the microstructure of samples stored at 5 and 25ºC (Figure 7a and 7b). The microstructures of samples stored at 45ºC showed larger clusters of 300-400 μm (Figure 7c). At higher magnifications, layered structures were identified in samples stored at 5 and 25ºC (Figure 7d and 7e); however the layered structure was not observed in samples stored at 45ºC (Figure 7f). Comparing samples stored at 5 ºC to those stored at 25ºC, the 25ºC samples had better-defined black and white regions between the layered structures, while gels stored at 5ºC displayed uniform grey regions between the layered structures. The loss of layered structures and the formation of bigger clusters observed in samples stored at 45ºC may be a consequence of the phase transition from the α-gel phase to the coagel phase, where the MG lamellae become dehydrated.

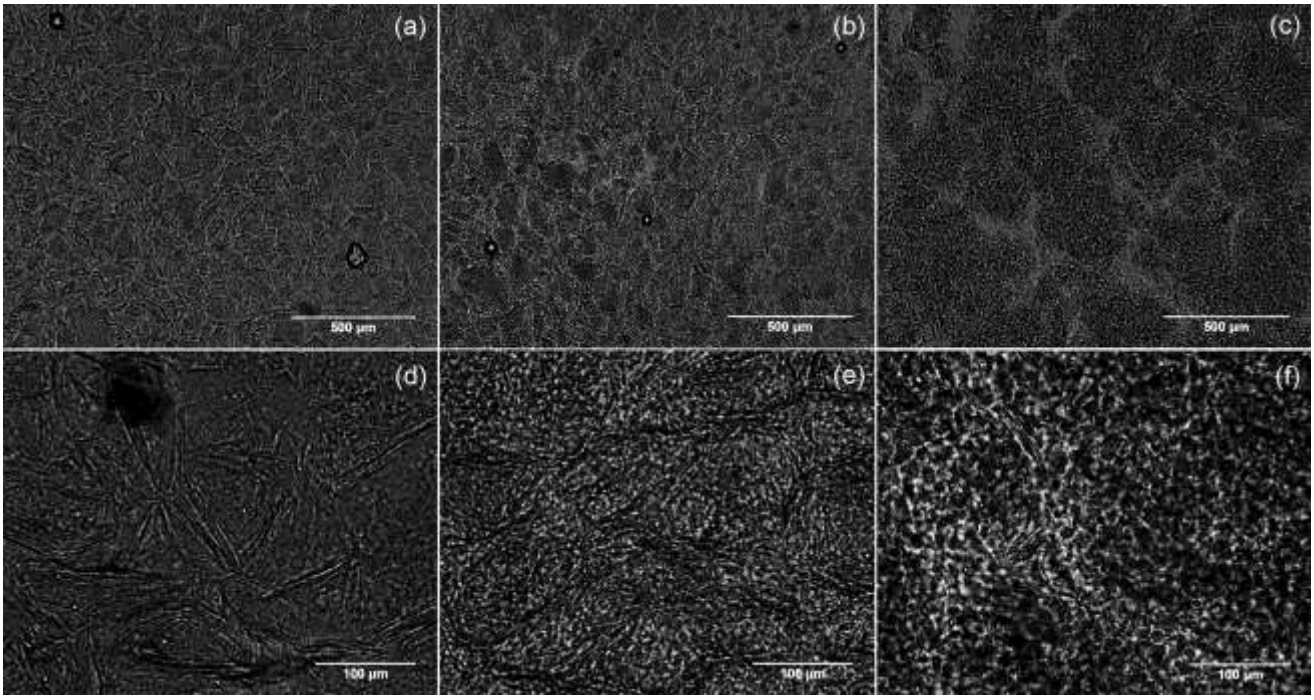

Figure 7. MG-gel morphology observed by transmitted light microscopy. Gels were stored at 5, 25 and 45°C for 100 days.

Figure 8 shows the gel microstructure observed by fluorescence microscopy. In fresh gel samples, the dye was evenly distributed within the gel structure and gave the sample an even "hazy" fluorescent appearance (Figure 8a). Gel samples stored at 25°C for two weeks started to display distinct spots of concentrated dye (Figure 8b), where the original even "hazy" appearance had become disrupted. When stored at 45°C for two weeks, samples displayed larger fluorescent spots, while the original hazy appearance was completely absent (Figure 8c). XRD and DSC results suggest that the MG-gel system is in the α-gel phase when freshly made. The uniform dye distribution in the fresh gel samples is associated with the hydrated and swollen α-gel structure when observed at room temperature. Stored at 25°C, the gel started to transform into the coagel

phase in time. Losses of water upon this transformation would have lead to the formation of dry, concentrated MG clusters, which appear as bright spots in the aged gel samples.

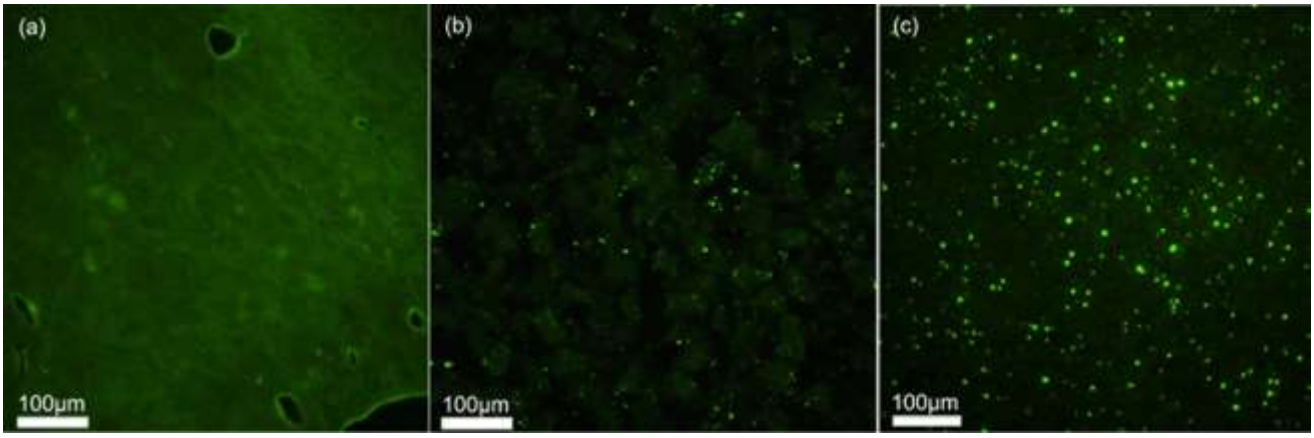

Figure 8. Gel microstructure observed by fluorescence microscopy. Coumarin 6 was used as the fluorescent dye. Samples were (a) fresh, (b) stored at 25°C for two weeks, and (c) stored at 45°C for two weeks.

3.6 Updated phase behavior of the GMS-water system

A schematic diagram demonstrates the phase behavior of GMS-water system is therefore proposed after characterizing the sub-α-gel phase (Figure 9). The phase diagram now includes the phase behavior of MG-gel below room temperature.

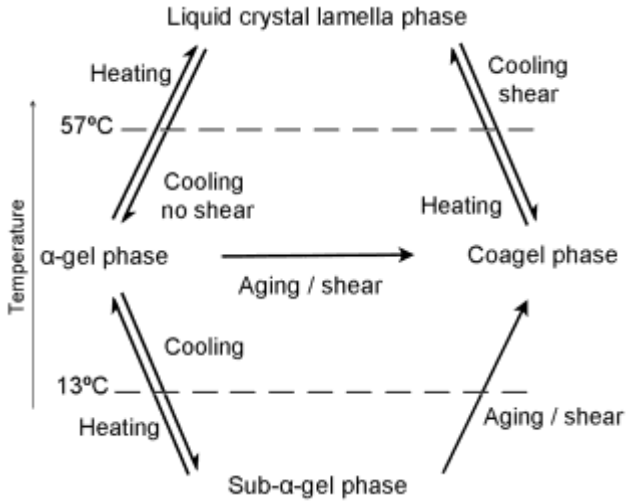

Figure 9. Schematic diagram of the phase behavior of the GMS-water system.

The sub-α-gel phase of GMS in water system showed similar XRD patterns, thermal reversibility, and metastability to that of neat GMS crystals.[1,9] However, as Krog suggested, the presence of water depressed the phase transition temperature of the sub-α-gel phase and the α-gel phase of the GMS-water system compared to pure GMS crystals.[5]

4. Conclusion

This work examined the dynamics of the phase transition of an MG-water system at various storage temperatures over time. It suggested that the α-gel phase was stable at 5°C for 100 days. Increasing the storage temperature will increase the rate of transformation from the α-gel phase to the coagel phase. During the phase transition from the α-gel phase to the coagel phase, MG bilayers become dehydrated, while MG molecules organize into dehydrated crystals.

When stored at refrigeration temperatures, fractionation took place within the α-gel structure. The fractionation altered the melting profile of the α-gel phase, which in turn interfered with the determination of the enthalpy of melting and the calculation of the Coagel Index.

This work characterized the nature of the sub-α-gel phase of MG-water system for the first time. The phase transition temperature of the sub-α-gel phase is below room temperature at ~13°C. Distinct WAXS and SAXS patterns of the sub-α-gel phase and the α-gel phase were identified at below and above 13°C. SAXS patterns suggested that the water layer thickness in the sub-α-gel phase may be as thick as ~140 Å, which contributes to the water binding capacity and stability of MG-water system at refrigeration temperatures.

The sub-α-gel phase is metastable, and it undergoes a polymorphic transformation to the coagel phase in time. The polymorphic transition of the sub-α-gel phase is thermally reversible until the system has completely transformed to the coagel phase.

The GMS-water system showed similar polymorphic behavior to that of GMS crystals; however, water depressed the phase transition temperature of the sub-α-gel phase and the α-gel phase in the GMS-water systems.

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References

1. E. S. Lutton, *J AOCS*, 1950, **27**, 276–281.

2. K. Larsson, *Zeitschrift für Phys. Chemie Neue Folge*, 1967, **56**, 173–198.

3. E. S. Lutton, *J. AOCS*, 1971, **48**, 778–781.

4. H. D. Batte, A. J. Wright, J. W. Rush, S. H. J. Idziak, and A. G. Marangoni, *Food Biophys.*, 2007, **2**, 29–37.

5. N. J. Krog, in *Food Emulsions*, ed. F. Emulsions, Marcel Dekker, New York, 1997.

6. A. Zetzl, M. Ollivon, and A. Marangoni, *Cryst. Growth Des.*, 2009, **9**, 3928–3933.

7. J. W. Hagemann, in *Crystallization and Polymorphism of Fats and Fatty Acids*, eds. N. Garti and K. Sato, Marcel Dekker, New York, 1988.

8. E. S. Lutton and F. L. Jackson, *J. AOCS*, 1948, **70**, 2245–2249.

9. J. Vereecken, W. Meeussen, I. Foubert, A. Lesaffer, J. Wouters, and K. Dewettinck, *Food Res. Int.*, 2009, **42**, 1415–1425.

10. J. Vereecken, W. Meeussen, A. Lesaffer, and K. Dewettinck, *Food Res. Int.*, 2010, **43**, 872–881.

11. N. K. Ojijo, I. Neeman, S. Eger, and E. Shimoni, *J. Sci. Food Agric.*, 2004, **84**, 1585–1593.

12. C. H. Chen, I. Van Damme, and E. M. Terentjev, *Soft Matter*, 2009, **6**, 432–439.

13. N. Krog and L. Kare, *Chem. Phys. Lipids*, 1968, **2**, 129–143.

14. N. Krog and A. P. Borup, *J. Sci. Fd Argic*, 1973, **24**, 691–701.

15. G. Cassin, C. de Costa, J. P. M. van Duynhoven, and W. G. M. Agterof, *Langmuir*, 1998, **14**, 5757–5763.

16. A. Sein, J. a Verheij, and W. G. M. Agterof, *J. Colloid Interface Sci.*, 2002, **249**, 412–22.

17. K. Larsson, K. Gabrielsson, and B. Lundberga, *J. Sci. Fd Agric*, 1978, 909–914.

18. S. Da Pieve, S. Calligaris, E. Co, M. C. Nicoli, and A. G. Marangoni, *Food Biophys.*, 2010, **5**, 211–217.

19. A. Goldstein, A. Marangoni, and K. Seetharaman, *Food Biophys.*, 2012, **7**, 227–235.

20. W. G. Morley and G. J. T. Tiddy, *J. Chem. Soc. Faraday Trans.*, 1993, **89**, 2823–2831.

21. C. H. Chen and E. M. Terentjev, *Langmuir*, 2010, **26**, 3095–105.

22. G. Y. Brokaw, W. C. Lyman, and D. P. Industries, *J. AOCS*, 1958, **35**, 5–8.

23. A. G. Marangoni, S. H. J. Idziak, C. Vega, H. Batte, M. Ollivon, P. S. Jantzi, and J. W. E. Rush, *Soft Matter*, 2007, **3**, 183.

24. F. Peyronel and R. Campos, in *Structure-Function Analysis of Edible Fats*, ed. A. G. Marangoni, AOCS Press, Urbana, 2012, pp. 235–294.

25. K. Larsson and N. Krog, *Chem. Phys. Lipids*, 1973, **10**, 177–180.

26. K. Larsson, K. Fontell, and N. Krog, *Chem. Phys. Lipids*, 1980, **27**, 321–328.

27. J. P. M. van Duynhoven, I. Broekmann, A. Sein, G. M. P. van Kempen, G.-J. W. Goudappel, and W. S. Veeman, *J. Colloid Interface Sci.*, 2005, **285**, 703–10.