Phase Transitions and Microstructure of Emulsion Systems Prepared with Acylglycerols/Zinc Stearate Emulsifier

Adam Macierzanka,* Halina Szelań,† Thomas Moschakis,‡ and Brent S. Murray‡

Department of the Technology of Fats and Detergents, Chemical Faculty, Gdańsk University of Technology, Narutowicza 11/12, 80-952 Gdańsk, Poland, and Procter Department of Food Science, University of Leeds, Leeds LS2 9JT, United Kingdom

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The emulsification processes, during which acylglycerols/zinc stearate emulsifier, water, and oil phase formed ternary systems, such as water-in-oil (W/O) emulsions, oil-in-water (O/W) dispersions, and unstable oil-water mixtures, were investigated in order to characterize the progressive transformations of the dispersed systems. The type, structure, and phase transitions of the systems were found to be determined by temperature and water phase content. Crystallization of the emulsifier caused the destabilization and subsequent phase inversion of the emulsions studied, at a temperature of 60–61 °C. The observed destabilization was temporary and led, at lower temperature, to W/O emulsions, “O/W + O” systems, or O/W dispersions, depending on the water content. Simultaneous emulsification and cooling of 20–50 wt % water systems resulted in the formation of stable W/O emulsions that contained a number of large water droplets with dispersed oil globules inside them (“W/O + O/W/O”). In water-rich systems (60–80 wt % of water), crystallization of the emulsifier was found to influence the formation of crystalline vesicle structures that coexisted, in the external water phase, with globules of crystallized oil phase. Results of calorimetric, rheological, and light scattering experiments, for the O/W dispersions obtained, indicate the possible transition of a monostearoylglycerol-based α-crystalline gel phase to a coagel state, in these multicomponent systems.

I. Introduction

One of the most common emulsifiers for the stabilization of W/O emulsions is monoesters of glycerol and fatty acids (monoacylglycerols; MAGs). Usually, transesterification of hydrogenated natural oils/fats with glycerol (glycerolysis), or direct esterification of glycerol with fatty acids, are used to synthesize the mixture of mono- and diacylglycerols (typically 40–55% of MAG). The mixture contains also triacylglycerols. Upon further purification by molecular distillation, 90–95% MAG product can be prepared.1,2 Both, mono/diacylglycerols and distilled MAGs are generally recognized as safe and can be used in many pharmaceuticals, food products, and cosmetic formulations.

One very important feature of MAGs is their ability to form lyotropic liquid crystalline phases in the presence of water.3–6 When distilled, saturated MAGs are mixed with water and heated to the Krafft temperature (Tk; defined as the melting point of the hydrocarbon chains5), the so-called lamellar mesophase will be formed. In this phase, bimolecular lipid layers are separated by water, which penetrates into the planes of polar headgroups of the MAG molecules. Below Tk, the mesophase transforms to the α-gel phase, in which MAG molecules are still aligned in individual bilayers, separated by water. However, the hydrocarbon chains are not still in a liquidlike state. Since the α-gel is not thermodynamically stable, it converts into the coagel phase (a network of platelike β-crystals in water). A possible mechanism of the α-gel→coagel conversion has been reported by Sein et al.7 and van Duynhoven and co-workers.8

In most emulsion systems, the concentration of emulsifier is usually not high enough to allow for the formation of multimellar liquid-crystalline structures at the water–oil interface. As explained in the literature,4,9 very hydrophobic emulsifiers (e.g., diacetyl tartric acid ester of MAG, polysorbates or sodium dodecyl sulfate) form lamellar phases with neutral lipids and water. However, low-polar MAGs undergo a phase transition from lamellar to reversed hexagonal mesophases in the presence of triacylglycerols. As reported by Mele et al.,10 very stable W/O emulsions can be prepared by gradual dispersing water in the lamellar and reversed hexagonal phases, formed by glycerol monooleate-based systems. These authors reported that a glycerol monooleate/trioleate mass ratio = 85/15 was optimal to form liquid crystalline phases first and then emulsions with high water content (up to about 80 wt %).

MAGs used in commercial W/O emulsions (such as margarine) form a crystallized film at the surface of water droplets. This way a strong barrier against coalescence is produced. According to Patino et al.,11 interfacial crystallization of glycerol monopalmitin, monoolein, and monolaurin, previously adsorbed at the oil–water interface, influences the reduction of the interfacial tension. Krog and Larsson12 have postulated that crystallization

* Corresponding author. E-mail: adamm@chem.pg.gda.pl. Telephone: +48 58 347 29 27. Fax: +48 58 347 26 94.
† Gdańsk University of Technology.
‡ University of Leeds.

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of MAG at the interface can be expected to be more rapid compared to the nucleation–crystal growth mechanism in the oil phase. The influence of MAG concentration on the interfacial tension was studied by Szelag and Zwierzynskij for paraffin oil–water systems. At a temperature of 45 °C, monolaurin reduced the interfacial tension to 15.9 mN/m, whereas the use of monostearate yielded 19.9 mN/m. These values were measured for MAG concentrations equal to the critical micelle concentration (CMC): $2.56 \times 10^{-3}$ and $3.8 \times 10^{-4}$ mol/dm$^3$, respectively. The most interesting result was the decrease of the interfacial tension to less than 2 mN/m when a mixture of monostearoyleglycerol and sodium stearate (molar ratio $9:1$) was used, showing the synergistic effect of the combination. Investigations on the effectiveness of acylglycerol emulsifiers, modified with sodium and potassium soaps, showed the possibility of using such emulsifiers for the stabilization of O/W emulsions.

As presented elsewhere, modification of the hydrophobic properties of acylglycerol emulsifiers can be realized by means of the controlled esterification of glycerol with fatty acids in the presence of zinc soaps. In contrast to the use of alkali soaps, the synthesized products are very effective in the stabilization of W/O-type emulsions. Preliminary studies on the phase behavior, dispersion distribution, and physical stability of W/O (paraffin and vegetable oil) emulsions, prepared with different acylglycerols/zinc carboxylate emulsifiers, have been reported in a previous publication. However, the detailed microscopic and thermal characteristics of the phase transitions were not discussed. It has been the aim of the present work to analyze the influence of temperature and water/oil ratio on the transition pathways in the dispersed systems, prepared with acylglycerols/zinc stearate emulsifier. Investigations were performed in order to characterize the structural changes of the systems that occurred during the dynamic emulsification processes. Time-dependent transitions in the structure of obtained O/W dispersion on storage were also studied.

II. Experimental Section

II A. Materials. Water used for the preparation of all dispersed systems was from a Milli-Q Elix system (Millipore). A mixture of paraffin oil and paraffin wax (9:1 wt/wt) was used as the oil phase. Both components of the oil phase were supplied by Sigma-Aldrich. Acylglycerols/zinc stearate (AGs/ZnSt) emulsifier was synthesized by means of direct esterification of glycerol with stearic acid (octadecanolic acid, C18:0; from Sigma-Aldrich), in the presence of zinc stearate. The composition of the emulsifier (checked by GC and HPLC) was as follows: 43.0 wt % of MAG (monostearate), 7.7 wt % of zinc stearate (ZnSt), 26.3 wt % of diacylglycerol (DAG, distearate), 6.1 wt % of triacylglycerol (TAG, tristearate), 9.9 wt % of stearic acid, and 7.0 wt % of glycerol. Details of the emulsifier synthesis have been presented in a recent report.

The hydrophilic–lipophilic balance (HLB) of the emulsifier was 4.7, determined experimentally via the Griffin method (the emulsion comparison procedure). The emulsion composition during estimation of the HLB number was as follows: oil phase (paraffin oil), 40 wt %; water, 55 wt %; blend of the AGs/ZnSt and standard emulsiﬁers, 5 wt %. TWEEN 60 (from Sigma-Aldrich; HLB = 14.9) was used as a standard emulsiﬁer. The contents of TWEEN 60 ($X_{T60}$ wt %) and AGs/ZnSt emulsifier ($X_{AGs/ZnSt}$ wt %), in their mixture, were calculated as follows:

$$X_{T60} = 100 \left( \text{RHLB} - \frac{H_{AGs/ZnSt}}{H_{T60}} - H_{AGs/ZnSt} \right)$$

$$X_{AGs/ZnSt} = 100 - X_{T60}$$

where RHLB is a required HLB of paraffin oil used as an oil phase (RHLB = 10.5), and HLB$_{T60}$ and HLB$_{AGs/ZnSt}$ are HLB values of Tween 60 and the examined AGs/ZnSt emulsifier, respectively. For each calculation of $X_{T60}$ and $X_{AGs/ZnSt}$, a different HLB$_{AGs/ZnSt}$ value was assumed. For each assumption of the HLB$_{AGs/ZnSt}$, the resulting $X_{T60}$ / HLB$_{AGs/ZnSt}$ ratio, calculated according to eqs 1 and 2, yielded the HLB of the emulsifier mixture equal to the RHLB value of the oil phase. Equality of both values is a rule of thumb in a preparation of stable emulsions. After preparation (emulsification at 500 rpm under continuous cooling from 80 to 25 °C), the emulsions were poured into 10-mL graduated test tubes and were observed for any sign of phase separation to occur. The HLB$_{AGs/ZnSt}$ value assumed in the calculation of the $X_{T60}$/HLB$_{AGs/ZnSt}$ ratio of the emulsiﬁer blend that yielded the most stable O/W emulsion was judged to be the HLB number of the AGs/ZnSt emulsifier. Stability of emulsions (stored 24 h at 23 ± 1 °C after preparation) was evaluated by centrifugation at 5000 rpm for 3 min (23 ± 1 °C) and subsequent determination of the volume of separated oil or water phase. At first, the emulsions were prepared with 0.5 HLB unit apart. After an approximate HLB value of the emulsifier was established, the experiments were repeated by making emulsions having 0.1–0.2 HLB unit apart until the exact HLB number was established. The estimated confidence interval of the determination of HLB was ±0.1.

A commercially available blend of glycercol tri- and diestearates (Fluka AG), consisting of 71.3 wt % triararitate, 20.2 wt % diararitate, 8.1 wt % stearic acid, and 0.4 wt % monostearate and glycerol, was used for the preparation of DAG-free product. For this purpose, the equivalent amounts of tri-/distaericate and stearic acid were mixed at 150 °C for 3 h (200 rpm) in order to esterify the hydroxy groups of the partial acylglycerols and glycerol. The use of the large excess of stearic acid allowed for almost complete esterification of di- and monostearate and glycerol to triararitate. The final product contained 53.9 wt % of triararitate, 46.0 wt % of stearic acid, and traces (0.1 wt %) of diararitate. The mixture obtained was utilized in the preparation of the emulsifying blends used in the investigations of the stability of W/O emulsions prepared in the absence of DAG and/or ZnSt. In these investigations, glycerol monostearate (99% purity), synthesized in our laboratory by the modified isopropylideneglycerol method, and purified by silica gel column chromatography, was also used.

Figure 1. DSC thermograms obtained for heating (a) and cooling (b) of AGs/ZnSt emulsifier.

below). This enabled the meticulous characterization of the results of the various dynamic transitions that occurred while mixing and simultaneous cooling of the emulsions. The corresponding transition temperature was determined from three preparations and measurements and showed a maximum standard error of ±0.3 °C. CLSM analyses were also performed for O/W dispersions stored at 23 ± 1 °C for couple days after preparation. Sudden and significant changes in the macroscopic appearance of the systems (e.g., homogeneity, phase separation, fluidity) were also investigated by visual observations.

Two different W/O emulsions (W:O = 40:60 wt/wt) were made: (i) with ZnSt (>95% purity), synthesized as reported elsewhere\textsuperscript{15} and (ii) with glycerol monostearate (99% purity). In both cases, the amount of the emulsifier was equal to 5 wt % of the total amount of oil and aqueous phases. The emulsions were made according to the procedure described earlier in this section. ZnSt and MAG were used separately in order to compare the microstructures of the emulsions stabilized by these compounds with the microstructure of the W/O system prepared with the AGs/ZnSt emulsifier.

II.C. Confocal Laser Scanning Microscopy (CLSM). Samples of dispersed systems were stained with Nile Red dye solution (0.4 μg of dye per 1 mL of the sample), placed into a well slide, and transferred to the microscope. A Leica TCS SP2 confocal laser scanning microscope was used. The confocal microscope was mounted on a Leica DM RXE microscope. A 20× dry objective with numerical aperture 0.7 or a 63× water-immersion objective with numerical aperture 1.3 were used in all experiments. This confocal microscope acquires images at a fast rate, because it scans the beam quickly along one dimension, by using an acousto-optic deflector. The acquisition time for each 1024 × 1024 pixel slice, (or the pixel size, 1 μm × 1 μm), was typically 3 s, although much faster images were acquired in order to compare the microstructures of the emulsions stabilized by these compounds with the microstructure of the W/O system prepared with the AGs/ZnSt emulsifier.

II.D. Differential Interference Contrast (DIC) Optical Microscopy. A DIC optical microscopy was used to characterize the shape and surface microstructure of the water droplets in the W/O emulsions. Images were obtained by using an Olympus BX-60 microscope with APO oil-immersion objectives with 60× and 100× magnifications and numerical aperture 1.3, equipped with an Olympus DP-50 digital camera. Micrographs were recorded at 23 ± 1 °C. The microscope was operated in transmission light mode. In the observations of O/W emulsions, the hydrophilic Evans Blue dye was used to expose the water phase. A staining aqueous solution of concentration of 0.1% (wt/v) was gently mixed with emulsion samples in amounts required to achieve the desire contrast between oil and water phases in microscopic visualizations.

II.E. Calorimetric Measurements. Differential scanning calorimetry (DSC) was applied to (i) determine melting and crystallization temperatures of AGs/ZnSt emulsifier and (ii) characterize the thermal behavior of O/W dispersions prepared with that emulsifier. A Mettler TA 3000 thermal analysis system, equipped with a TC 10 TA processor and a DSC 30 temperature cell, was used. Samples of the emulsifier or dispersions (30 mg) were weighed in standard 40 μL aluminum pans and sealed by a press. Measurements were performed in the endothermic and exothermic scanning modes. Samples were heated and subsequently cooled between 25 and 80 and 80–25 °C, respectively. The rates of heating and cooling were 2 °C/min. An empty pan was used as a reference.

When the thermal behavior of O/W dispersions was studied, subsequent reheating (25–80 °C; 2 °C/min) of the sample was also performed. That additional scan was performed in order to determine the difference in the heat content of the sample, between the first heating and the second heating. As found in the literature,\textsuperscript{7,25,26} for binary MAG–water systems, the enthalpy of melting of pure β-crystalline MAG coagel phase is about twice the value obtained for the melting of the liquid-crystalline α-gel phase. The coagel index (CI), defined as the ratio between the enthalpies of the heating (ΔH\textsubscript{H}) and reheating (ΔH\textsubscript{RH}) scan,\textsuperscript{2,6} indicates the amount of α-gel that converted to coagel. When the CI = 1, the examined material is in the α-gel state. For a pure coagel, this parameter is about 2. Both phases convert into the lamellar phase at melting temperature, but, when cooled, the lamellar phase converts only to the α-gel phase.\textsuperscript{23} Since the transition of α-gel to coagel usually requires days or weeks, the immediate reheating scan exhibits the melting of the α-gel.

In our experiments, we examined the kinetics of the similar phase transition in the O/W dispersion prepared with acylglycerol emulsifier (AGs/ZnSt). We defined the ratio ΔH\textsubscript{H}/ΔH\textsubscript{RH} as a parameter called the Dispersion Coagel Index (DCI).

II.F. Evaluation of the Stability of Dispersed Systems. Changes in the dispersity of emulsions were monitored by multiple light scattering (MLS) method using a Turbiscan LAb Expert analyzer (Formulation). A freshly prepared emulsion (ca. 25 mL) was placed into a flat-bottomed cylindrical glass cell and stored at 23 ± 1 °C over a period of about one year. The sample was scanned by using two synchronous optical sensors that detected light transmitted through the sample and backscattered by the sample (180° and 45° from the incident light, respectively). The reading head acquired backscattering and transmission data every 40 μm while moving along the entire height of the cell (i.e., 55 mm). The light source was an air–laser–luminescent diode (λ\textsubscript{exc} = 880 nm). The one-scan analyses were carried out at one-day intervals at a temperature of 23 °C (±0.5 °C). Additionally, the emulsion stability was assessed in the test, where a temperature was gradually increased from 25 to 60 °C. During this analysis, sample of emulsion was scanned every 1 min for about 100 min. The Lambert–Beer law gives an analytical expression of the transmitted flux, \( T \), measured by the Turbiscan as a function of the photon mean free path, \( \lambda \):

\[
T = T_0 e^{-\lambda/r}
\]

(3)

where \( r \) is the internal radius of the measurement cell, and \( T_0 \) is the transmittance of the continuous phase. The backscattered flux, BS, is directly linked to the photon transport length, \( \lambda^* \), through the dispersed system, by the following relation:

\[
BS \approx [(1 - B)/3]\left(\frac{h_d\lambda^*}{\pi}\right)^{1/2}
\]

(4)

where \( B = 0.2 \) is the boundary reflection factor in cylinderical geometry and \( h_d \) is the detector height. Thus, BS is inversely proportional to the square root of \( \lambda^* \). Both \( \lambda \) and \( \lambda^* \) depend on the physical features of the dispersed phase (mean particle diameter, \( d \), and particle volume fraction, \( \phi \)). The parameter \( \lambda \) represents the average distance traveled


by photons before undergoing a diffusion phenomenon. Whereas, \( \lambda^* \) corresponds to the distance of penetration of the photon in the dispersed system (i.e., the distance above which a photon is scattered in the backward direction)

\[
\lambda = \frac{2d}{3\phi Q_s}
\]

\[
\lambda^* = \frac{\lambda}{1 - g} = \frac{2d}{3\phi(1 - g)Q_s}
\]

The optical parameters \( Q_s \) and \( g \) are given by the Mie theory.\(^{27}\) Details of the measurement principles and the theory of the MLS method are given by Mengual et al.\(^{28,29}\)

The stability of dispersed systems was also evaluated in simple tests, where emulsion samples were centrifuged at 5000 or 10000 rpm for 5 min at 23 ± 1 °C. Afterward, the volume of separated water or oil phase was checked.

II.G. O/W Dispersion Consistency Measurements. A Feinmess OFD VEB penetrometer was used. A ball-shaped, steel punch, affixed to a steel stem and an aluminum rod (wt. 55.9 g) of the penetrometer, was initially held in a stationary position with the punch tip just in contact with the surface of the dispersion sample, contained in a glass cup (volume 100 mL). The cups used for measurements were filled with the dispersion just after its preparation (i.e., when the dispersion was in semi-fluid state), sealed with polyethylene film (to prevent water evaporation), and stored at 23 °C for 5 min at 23 ± 1 °C. Afterward, the volume of separated water or oil phase was checked.

III. Results and Discussion

The microscopic examination of the structure of the dispersed systems allowed identification of the pathways of the phase transitions during the emulsification processes. To determine the PIT, continuous measurements of temperature and electrical conductivity were also made. Based on the data obtained and the macroscopic appearance of the systems (homogenity, separation of water or oil phase, significant changes of fluidity), the phase diagram was formulated (Figure 2). It shows the dependence of type of the dispersed system on temperature and water phase content. As mentioned in the Experimental Section (II.B.), a temperature was reduced gradually from 80 to 25 °C during the emulsification processes.

At the beginning of the dispersion formation, a W/O emulsion was formed due to the hydrophobic character of the emulsifier (HLB = 4.7), even for the system that contained 80 wt % of water (Figure 2). On cooling the emulsion systems to 61−60 °C, a sudden, significant increase in fluidity was observed, attributed to the destabilization of emulsions induced by the crystallization of the emulsifier (i.e., formation of the considerable amounts of acylglycerols crystals). In the emulsions containing 60−80 wt % water, such changes in fluidity were preceded by flocculation of the water droplets (Figure 2). As shown in the DSC thermogram (Figure 1b), crystallization of the emulsifier began sharply at 60 °C. Thus, the thermal behavior of the emulsifier correlates with the first observed temperature transition. Emulsifier crystallization produced complete destabilization of W/O emulsion. Micrographs of the system obtained show regions with separated oil and water phases (Figure 3). Acylglycerols crystals are in the oil phase. The destabilization led (under continuous emulsification) to a phase inversion. The type of system subsequently formed was dependent on the water-oil ratio, as described below in the following sections.

III.A. Water:Oil Weight Ratios = 20:80±55:45. In the mixtures that contained up to 55 wt % of water, a decrease of fluidity was subsequently observed, as a result of the phase inversion at 58−57 °C. The inversion and formation of an O/W emulsion occurred simultaneously with some destabilization of the system, i.e., separation of the excess of oil phase. This was attributed to the relatively high content of oil (45−80 wt %) in the system plus the hydrophobic nature of AGs/ZnSt emulsifier. As shown in Figure 4, large flocs of irregularly shaped O/W emulsion aggregates were suspended in the separated oil.

Since the external phase of the whole system was still paraffin oil, the conductivity remained unchanged, at around 0−0.1 μS. Such a system has been designated as “O/W + O” (Figure 2). From the photographs in Figure 5a, it is seen that the system cannot be considered double emulsion due to the apparent lack of the macroscopic uniformity, characteristic of stable emulsions. In fact, samples of such mixtures (when placed in a test tube) showed a rapid sedimentation of O/W emulsion aggregates and

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location of the separated oil in the uppermost regions. This process was monitored by the MLS method using a Turbiscan apparatus (Figure 5). The “O/W + O” mixture (W:O = 40:60 wt/wt) used in this experiment was produced by emulsification carried out from 80 to 45 °C (see Figure 2), and subsequently, the system was immediately transferred to the MLS analyzer. The MLS measurements were performed at 45 ± 0.5 °C. A sample of the freshly prepared “O/W + O” system was opaque (light could not be transmitted through the sample, $T = 0\%$); however, the divergences in the backscattering (BS) signals indicated the aforementioned lack of uniformity of this system (Figure 5a). After a few minutes, the progressive sedimentation of the O/W emulsion aggregates could be easily detected from the increase in $T$ level and a resulting decrease in BS signals for the top part of the sample (formation of the oil layer), as well as the local increases of the BS values in the middle and lower regions (Figure 5b). After moderate centrifugation (1000 rpm, 1 min), the sedimented aggregates formed a layer of opaque O/W emulsion with the BS level of about 50% (Figure 5c). The separation of some clear oil phase was signaled by the detection of light transmitted through the uppermost regions of the sample ($T = 60\%$). Formation of the water phase, at the bottom of the test tube, was not observed. Monitoring of this sedimentation process gave an insight into the nature of the “O/W + O” system. From the changes in BS and $T$ profiles, one may conclude that observed sedimentation was not a simple separation of oil and water phases. For such a case, the $T$ reading for the lower section of the centrifuged sample would be also $>0$, together with low BS.

Figure 4. CLSM micrograph of the “O/W + O” (W:O weight ratio = 40:60) system from 45 °C (a). Arrows (b) indicate the numerous oil droplets dispersed within the water phase (black-colored regions represent water).

Figure 5. Schematic illustration and multiple light scattering (MLS) analysis of the “O/W + O” system (W:O weight ratio = 40:60): (a) freshly prepared system (the photographs show the macroscopic appearance of the system spread on the glass plate); (b) time-dependent sedimentation of the O/W emulsion aggregates; (c) separation of the O/W emulsion from the external oil, accelerated by the moderate centrifugation (the micrograph shows the separated O/W emulsion). BS and $T$ are the intensities of light backscattered by the sample and transmitted through the sample, respectively. $G$ is an electrical conductivity of the system. For more details see text.
modified with sodium and potassium soaps.14 The change from
with acylglycerol emulsifiers (acyl group: C16:0 and C18:0),
the cooling of the W/O emulsions (20°
- 735°C).

Formation of similar “O/W + O” systems was observed during
the cooling of the W/O emulsions (20–60 wt % of water) prepared
with acylglycerol emulsifiers (acyl group: C16:0 and C18:0),
modified with sodium and potassium soaps.14 The change from
W/O to “O/W + O” was induced by cooling the systems to
temperatures ranging between 55 and 45°C, depending on
acylglycerols fatty acid moiety and the type of soap cation.

Further cooling and emulsification of the 20–50 wt % water
content systems led to the formation of viscous, uniform W/O
emulsions (Figure 6a). The temperature at which formation of
the final emulsion was noted decreased with water content in
the mentioned range (Figure 2). This shear-induced transition of
unstable “O/W + O” systems into stable W/O emulsions took
place together with the formation of the small number of large
(10–40 μm diameter) water droplets that contained globules of
dispersed oily substances (Figure 6b). Thus, in the phase diagram,
the final emulsion system has been called “W/O + O/W/O”
(Figure 2). The CLSM views of the surface and cross-section of
selected water droplet (Figure 6c,d) verify the location of the oil
globules inside the droplets. Since the main surface-active
components of the emulsifier (i.e., MAG and ZnSt) are lipophilic
compounds, there was not any hydrophilic surfactant in the system
that could act as O/W-type emulsion stabilizer. Therefore, we
hypothesize that the observed formation of “O/W + O” systems,
which led finally to “W/O + O/W/O” systems (at 20–50 wt %
water content), was caused by crystallization of TAG (a component
of the AGs/ZnSt emulsifier) and paraffin wax in the
oil phase, and simultaneous dispersion of part of that solidified
material into the water phase. Water with these crystallized
substances was then dispersed in the oil phase (formation of
“W/O + O/W/O” systems due to continuous shearing of the
“O/W + O”). DAG and stearic acid (components of the AGs/
ZnSt emulsifier) are expected to adsorb at the water—oil interface,
together with MAG and ZnSt. Stearic acid and glycerol
monostearate have been found to exhibit strong synergism at the
water-paraffin oil interface.30 According to Awad et al.,31
glycerol distearate is able to form interfacial, molecular aggregates
with an emulsifier in emulsion systems.

Two different W/O emulsions, stabilized by (i) glycerol
monostearate and (ii) ZnSt crystals, were prepared in order to
compare the microstructures of the interfacial films of these
individual surfactants to the film formed by AGs/ZnSt emulsifier
in the emulsion systems (Figure 7). Figure 7a presents the
micrograph of the surface of selected water droplet from the
W/O emulsion prepared with AGs/ZnSt emulsifier. The formation
of crystallized, corrugated, and plate-like films of the emulsifier,
which coated the droplets, was observed. Solidification of the
emulsifier influenced the irregular (i.e., not spherical) shape of
water droplets. According to Rousseau,32 fats that crystallize
directly at the interface provide very good coverage of emulsion
droplets. Such a phenomenon, found in W/O dairy emulsions,
have been presented by Buchheim and Dejmek.33 They have
shown butter microstructures with the water droplets covered by
a layer of platelike fat crystals, as well as images of a thin
interfacial layer of crystalline fat and/or emulsifier around the
water droplets in low-fat spreads. Similar observations have been
also demonstrated for margarine.34,35

The images of crystallized AGs/ZnSt emulsifier films (Figure
7a) are similar to those observed for W/O emulsions prepared with
glycerol monostearate, Figure 7b, whereas the micrographs of
the emulsions obtained with ZnSt crystals (Figure 7c) show
typical solid-particle stabilized dispersed systems.36 The ZnSt
crystals are small compared to the emulsion droplets, which is
one of the key factors enabling the use of particulate solids as
emulsion stabilizers.37 They are able to adsorb and arrange at the
oil—water interface to form a rigid film. This also verifies that
ZnSt crystals are partially wetted by the both oil and aqueous
phases, ensuring their location at the interface. The presence of
MAG and ZnSt in the AGs/ZnSt emulsifier seems to produce the
coadsorption of both surfactants at the interface.

The existence of a solid shell around the water droplets prevents
coolescence. All of the final “W/O + O/W/O” emulsions were
physically stable for at least one year, when stored at 23 ± 1°C.

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From the MLS analysis (Figure 8a), it is seen that the intensity of light backscattered by the emulsion remained constant during the 1-year storage, demonstrating no changes in the emulsion structure (e.g., flocculation or coalescence of droplets). Lack of the local increases in the BS level for the lower part of the sample scanned (Figure 8a'), i.e., the signs characteristic for the sedimentation of dispersed water droplets, showed the crystallization of acylglycerols at the water/oil interface and in the continuous oil phase to be the factor yielding the spatial arrangement of droplets that prevented the gravitational migration of emulsified water during this long time period. The parameter $\Delta BS_{5-50 \text{ mm}}$ has been defined as a difference in mean BS value (recorded for the emulsion sample in the region from 5 to 50 mm of its height; $BS_{5-50\text{ mm}}$, Figure 8a'), between the first scan and $n$ scan.

The 1-year-old emulsions were stable also during the centrifugation at 5000 rpm for 5 min. However, in emulsions containing 40–50 wt % water, the increase to 10 000 rpm resulted in the formation of a thin oil film (less than 1% of the oil phase volume) at the top of test samples. It was a result of the increase of the concentration of droplets in lower regions of the emulsion samples, but, since a separation of water phase was not detected, the centrifugation seems to not affect significantly the coalescence rate.

Stability of the emulsions stored one year at 23 ± 1 °C was also evaluated in the test where the temperature was increased stepwise from 25 to 60 °C (Figure 8b). Changes in backscattering of the samples were monitored on-line (one scan every one minute of analysis). The progressive decrease in the $BS_{5-50 \text{ mm}}$ number observed was attributed to the flocculation of water droplets. The most considerable drop of $\Delta BS_{5-50 \text{ mm}}$ was noted when 55–60 °C was reached. At this temperature, melting of the AGs/ZnSt emulsifier began (Figure 1a); thus, with all probability, the change was the effect of the resulting increase of the degree of flocculation/coalescence. It is well-known that an aggregation of droplets (i.e., flocculation) can lead to their merger and the resulting creation of larger droplets (coalescence phenomenon). Such variations in particle size (both flocculation and coalescence) are detected by the MLS method as a decrease of BS over the whole height of emulsion sample as long as the change of particle size does not produce the migration of dispersed particles. When the temperature of the analysis reached 60 °C, gravitational sedimentation of the water droplets was not observed yet. About 20-min storage at this temperature was required to detect the onset of the sedimentation.

To assess the influence of DAG and ZnSt on the emulsion stability, three different emulsions were prepared and analyzed. They were obtained in the presence of (i) AGs/ZnSt emulsifier, (ii) emulsifying mixture A, or (iii) mixture B. The mixtures A and B were composed of the defined amounts of the same compounds that were present in the composition of AGs/ZnSt emulsifier (see section II.A.). The only difference was the absence of DAG (mixture A) or DAG + ZnSt (mixture B). All of the emulsions were 40 wt % water systems of the W/O type. After preparation, the emulsions were immediately transferred to the MLS analyzer and scanned at constant temperature of 50 °C. Under quiescent conditions, the increased temperature of analyses did not affect the emulsion type. It was applied in order to accelerate any signs of destabilization to occur. At around 23 °C, the emulsions were very stable against sedimentation of water droplets and other destabilizing mechanisms (e.g., flocculation).

As shown in Figure 9a, storage at 50 °C resulted in a relatively rapid decrease in the $BS_{5-50 \text{ mm}}$ number of all of the emulsions due to a flocculation of water droplets. Coalescence was rather not possible because the melting temperature of the emulsifier...
was above 50 °C (Figure 1a). Indeed, the three emulsions studied were stable against separation of free water phase for weeks at 50 °C. The monitoring of changes in BS_{5–50 mm} values was carried out until the moment when the sedimentation of water droplets and the separation of the continuous oil phase occurred. Graph b shows the kinetics of the subsequent formation of the separated oil phase layer in the uppermost regions of the emulsion samples (H is a thickness of the oil layer). Each point on the curves represents an average of three preparations and measurements.

Figure 9. MLS analysis of the time-dependent changes in the structure of emulsions (W:O weight ratio = 40:60) prepared with (○) AGs/ZnSt emulsifier, (+) mixture A, and (●) mixture B, and stored at 50 ± 0.1 °C. Graph a shows the backscattering profile of the emulsions (changes in BS_{5–50 mm} values observed prior to the sedimentation of water droplets and the separation of the continuous oil phase occurred). Graph b shows the kinetics of the subsequent formation of the separated oil phase layer in the uppermost regions of the emulsion samples (H is a thickness of the oil layer). Each point on the curves represents an average of three preparations and measurements.

III.B. Water:Oil Weight Ratios = 60:40–80:20. In the case of water-rich mixtures, destabilization of O/W emulsions, caused by cooling these systems to 61–60 °C, resulted in phase inversion and the formation of O/W dispersions (Figure 2). The transition was signaled by a sharp increase in conductivity, from 0–0.1 μS before inversion, to 20–25 μS as the O/W dispersion formed. CLSM examination of the dispersions revealed the coexistence of two types of structural components: hollow, spherical aggregates of flocculated lipid particles and separated droplets of the crystallized oil phase. In Figure 10, micrographs of the surface (a) and the cross-section (b, c) of large spherical aggregates are shown. These structures were built up from numerous small units (indicated by black arrows in Figure 10c), which are probably multimamellar spherulites of MAG. Formation of multilamellar vesicles in binary, water-distilled MAG systems, was first described by Larsson, for mixtures containing more than 50 wt % water. As reported by Krog, the phase behavior of MAGs in water depends on their purity, i.e., the presence of DAGs and TAGs. Distilled MAGs form liquid crystalline mesomorphic phases, whereas mono-diacylglycerols in most cases form emulsions due to a relatively high content of TAGs. According to Corkery and Hyde, metallic...
soaps, TAGs, and many DAGs do not exhibit lyotropic mesomorphism and are thus nonwetting in water. Taking into account these findings, we hypothesize that the formation of MAG spherulites in the O/W dispersion took place after separation of the glycerol monoester molecules from nonwetting components of AGs/ZnSt emulsifier (i.e., zinc stearate, DAG, and TAG). Saturated C16/C18 MAGs are dispersible in water between 55 and 70 °C in the form of lamellar aggregates or vesicles. As found by Borneé et al. for the ternary monoolein—sodium oleate—water (H2O) system, different vesicle phases can be formed spontaneously at high water contents upon diluting cubic and lamellar phases. According to van Duynhoven et al., fatty acid can be accommodated in the MAG bilayer of the swollen -crystalline gel phase, but upon transition to the coagel phase (crystals + water), it is separated into a relative "noncoagel" mobile phase, coexisting with the coagel crystal plates.

Besides MAG spherulites, crystallized droplets of the oil phase were also observed (see white arrows in Figure 10c). These droplets were formed probably as a result of crystallization of DAG, TAG, and paraffin wax in the oil phase. As mentioned earlier in the previous section, ZnSt crystals are able to effectively adsorb at the water—oil interface. Additionally, as reported by Awad and co-workers, glycerol distearate can form interfacial templates in O/W emulsions and accelerate the crystal nucleation rate in an emulsified oil phase. Thus, it is highly possible that the combination of these two compounds in the water-rich O/W dispersion could determine the formation of the interfacial layer that covered the droplets of crystallized oil phase.

From Figure 11, it can be seen that the structure of the spherical aggregates depended on the temperature. Cooling of the dispersion, from 50 to 25 °C, resulted in a decrease of a degree of dispersion of the O/W system. This was indicated by the drop in the BS50 mm value of the dispersion, observed during 3 days after preparation (Figure 14a). The subsequent increase of the BS50 mm value may be attributed to the gradual transformation of the coagel network in the crystalline structure of the spherical aggregates.

Figure 11. CLSM micrographs of surface (upper images) and cross-section (lower images) of selected spherical aggregates in O/W dispersion (O:W = 20:80 wt/wt). Comparison of the samples collected at 50 (a) and 25 °C (b).

Figure 12. CLSM images of O/W dispersion obtained after cooling the system (O:W = 20:80 wt/wt) from 80 to 25 °C. Arrows in image a indicate the fragments of the aggregates that were destroyed due to stirring the system. Some spherulites were found to adsorb at the surface of other aggregates (arrows in the micrographs b and c). Image c shows the magnification of surface of the aggregate with adsorbed spherulites.

seen to adsorb at the surface of those spherical aggregates that remained undestroyed in the system (Figure 12b,c). After 3 days storage at 23 ± 1 °C, dispersions revealed the presence of giant spherical and oval aggregates (Figure 13a). Crystallized droplets of the oil phase were separated from the aggregates. The close-packed structure of the thick walls of the aggregates can be clearly seen in Figure 13 (panels b and c).

The phenomena described are supposed to be caused by a gradual phase transition of the -crystalline gel phase of MAG, which formed spherulites, into -crystalline coagel. In the literature, a specific mode of crystallization has been suggested for the dispersion of monocaprin in water. It is proposed that, as a consequence of the contact of dispersion aggregates with the -crystalline surface, the bimolecular lipid leaflets unfold from the aggregates into the surface of the crystal. The crystallites then grow with discrete layers in a direction perpendicular to the surface plane. A similar mechanism of crystallization may be proposed as an explanation of our microscopic observations.

To obtain a quantitative measure of the changes in dispersion of the O/W system, MLS measurements were performed. The adsorption of small spherulites at the surface of undestroyed spherical aggregates and their fusion with the crystalline structure of the aggregates resulted in a decrease of a degree of dispersion of the O/W system. This was indicated by the drop in the BS50 mm level of the dispersion, observed during 3 days after preparation (Figure 14a). The subsequent increase of the BS50 mm value may be attributed to the gradual transformation of the coagel into a coagel network in the crystalline structure of the spherical aggregates. It is known that the MAG coagel phase is capable of enclosing large quantities of water. Hence, the effective volume fraction of the dispersed phase increased, yielding higher intensity of light backscattered by the dispersion.

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As reported by Chupin et al., the $^2$H NMR study on lipid organization and dynamics of the binary, deuterated monostearoylglycerol-water system, showed the liquid crystalline-to-gel phase transformation at temperature lowered from 67 to 55 °C. According to the authors of these investigations, upon further cooling of the gel below 40 °C, the transition into the coagel phase took place. This transition occurred gradually in time, indicating the metastability of the gel phase. The transformation of the $\alpha$-gel into a coagel, in water-distilled MAGs mixtures, is relatively quick if the pH is as low as 5–6. In O/W dispersions examined by us, the pH varied in the narrow range 4.8–5.0, during the dispersion (80–25 °C) and storage at 23 ± 1°C. The acidic pH value was due to the presence of some free stearic acid. The formation of coagel phase can be also promoted by mechanical agitation.

The increased BS$_{50}$ level of the dispersion remained constant for prolonged time (~1 year at 23 ± 1 °C, Figure 14a), showing no detectable changes in the size and migration of the dispersed particles. Similarly, the “W/O + O/W/O” emulsion presented in the previous section (Figure 8b), the 1-year-old O/W dispersion required heating to 55–60 °C for the most considerable flocculation/coalescence-induced decrease in the BS$_{50}$ level to be observed (Figure 14b). The onset of creaming and resulting progressive separation of water phase at the bottom of test tubes was observed after the maximum temperature of analysis, i.e., 60°C, was reached and kept constant for about 15 min (data not shown).

After storage at 23 ± 1 °C for several days after preparation, the O/W dispersions developed a firmer consistency. It was observed that the penetration depth of the measuring punch into the samples dropped to less than 1 mm. The change of consistency, from an initial semifluid to a final semisolid state, took 4–135 days, depending on the water content (Figure 15). It appears that such an increase in hardness was caused by the transition in the MAG-crystalline phase, as proposed above. All of the dispersions stored one year at 23 ± 1 °C were stable during the centrifugation at 10 000 rpm (5 min). Phase separation was not observed in this test. As postulated by Heertje and co-workers, the increase in hardness, observed also for liquid crystalline phases of distilled MAGs and water, may be attributed to the phase change from $\alpha$-gel to coagel. They have argued that the change of consistency arises from a diffusion-controlled growth of the MAG crystals and their subsequent interconnection into a fine-mashed network. The similar change of the rheological properties of MAG gels, attributed to the $\alpha$-gel–coagel conversion, was also demonstrated by Sein et al.

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The proof for the α-gel → coagel transition in O/W dispersion prepared with AGs/ZnSt emulsifier seems to come from the results of the DSC measurements (Figure 16). From thermograms obtained for heating and reheating of dispersion samples (O:W phase ratio = 20:80, wt/wt), DCI values were calculated (see the Experimental Section, II.E.). As shown in Figure 16A, almost complete conversion to coagel (DCI ≈ 2), at 23 ± 1 °C, took about 90 days. In this case (Figure 16B), the endothermic peak in the first heating curve, corresponding to the melting of the coagel phase (curve a), presents about twice the heat content of the sample, compared to the melting of α-gel phase, represented by the peak in the second heating curve (c). Reheating was performed just after controlled cooling of the dispersion sample (curve b). DSC experiments were not focused on the examination of mechanical, chemical, or thermal treatments of the dispersion on the kinetics of coagel formation but rather on the investigation of possibility of the α-gel → coagel transformation in the MAG-based, multicomponent (complex emulsifier/water/oil), dispersed system. Formation of the MAG coagel phase was previously studied mainly for the binary MAG–water gels.4,7,25,26,42

IV. Conclusions

The water/oil ratio as well as the change of temperature strongly determined the character of the phase transitions that took place during the emulsification in the presence of acylglycerols/zinc stearate emulsifier.

The emulsifier crystallization-induced destabilization of W/O emulsions produced a phase inversion and subsequent formation of (i) mixtures of W/O emulsions and double (O/W/O) emulsions (“W/O + O/W/O”), for 20–50 wt % water systems, and (ii) complex O/W dispersions (60–80 wt % of water) that contained large spherical aggregates of flocculated lipid particles, and crystallized droplets of oil phase.

CLSM, DSC, MLS, and consistency experiments showed the possible transition of monostearoylglycerol-based α-crystalline gel phase to a coagel state, in O/W dispersions over time. The change could be monitored via the evaluation of a dispersion coagel index (DCI), and microscopic inspection of the dispersion microstructure. CLSM was effective in the visualization of the MAG aggregates.

Both, “W/O + O/W/O” emulsions and O/W dispersions seem to be stable over considerable time (at least one year at 23 ± 1 °C). The maximum water uptake in stable “W/O + O/W/O” system was 50 wt %.

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