The Twofold Role of 12-Hydroxyoctadecanoic Acid (12-HOA) in a Ternary Water—Surfactant—12-HOA System: Gelator and Co-Surfactant

Katja Steck 1, Claudia Schmidt 2 and Cosima Stubenrauch 1,*

1 Institute of Physical Chemistry, University of Stuttgart, Pfaffenwaldring 55, 70569 Stuttgart, Germany; katja.steck@ipc.uni-stuttgart.de
2 Department of Chemistry, University of Paderborn, Warburger Straße 100, 33098 Paderborn, Germany; claudia.schmidt@uni-paderborn.de
* Correspondence: cosima.stubenrauch@ipc.uni-stuttgart.de; Tel.: +49-711-685-64470

Received: 19 July 2018; Accepted: 10 September 2018; Published: 12 September 2018

Abstract: Gelled lyotropic liquid crystals can be formed by adding a gelator to a mixture of surfactant and solvent. If the gel network and the liquid-crystalline phase coexist without influencing each other, the self-assembly is called orthogonal. In this study, the influence of the organogelator 12-hydroxyoctadecanoic acid (12-HOA) on the lamellar and hexagonal liquid crystalline phases of the binary system H2O–C12E7 (heptaethylene glycol monododecyl ether) is investigated. More precisely, we added 12-HOA at mass fractions from 0.015 to 0.05 and studied the resulting phase diagram of the system H2O–C12E7 by visual observation of birefringence and by 2H NMR spectroscopy. In addition, the dynamic shear moduli of the samples were measured in order to examine their gel character. The results show that 12-HOA is partly acting as co-surfactant, manifested by the destabilization of the hexagonal phase and the stabilization of the lamellar phase. The higher the total surfactant concentration, the more 12-HOA is incorporated in the surfactant layer. Accordingly, its gelation capacity is substantially reduced in the surfactant solution compared to the system 12-HOA–n-decane, and large amounts of gelator are required for gels to form, especially in the lamellar phase.

Keywords: gelled complex fluids; gelator; lyotropic liquid crystals; phase diagram

1. Introduction

Gelled complex fluids [1], in which the mechanical stability of a gel is combined with the microstructure of a complex fluid, are interesting candidates for (trans-)dermal drug delivery systems and tissue healing applications [1,2]. Additional applications of this class of materials can be found in a recent review by Stubenrauch and Gießelmann [1]. Gelled complex fluids can be obtained by either adding a gelator to a complex fluid or by replacing the solvent of a gel by a complex fluid. A special class of gelled complex fluids are gelled lyotropic liquid crystals (gelled LCs), such as gelled lamellar phases Lα (gelled Lα). Examples of gelled Lα phases, known in literature are the cell [3], lamellar biogels [4], and gelled lamellar phases of synthetic surfactants. Only two systems of the latter type have been studied to date: The gelled lamellar phase of the system H2O–n-decane/12-hydroxyoctadecanoic acid (12-HOA)—tetraethylene glycol monodecyl ether (C10E4) [5] and the gelled lamellar phase Lα of the system H2O–2C12DAB–12-HOA [6]. The cell surely is the most prominent example. Proteins form filaments, which provide mechanical stability of the cell and coexist independently with the lipid bilayer of phospholipids, i.e., the building block of the cell membrane [3]. Warriner et al. introduced lamellar biological hydrogels that consist of the lipid dimyristoyl phosphatidyl choline (DMPC), the co-surfactant pentanol and tiny amounts of poly (ethylene glycol)-derived polymer lipids.

Gels 2018, 4, 78; doi:10.3390/gels4030078 www.mdpi.com/journal/gels
(PEG-lipids) [4]. The domains of the lamellar membranes built by DMPC and pentanol are randomly orientated and provide the gel properties, while the PEG-lipids are attached to these membranes, but can diffuse freely within the lamellar bilayers, which are separated by water. These PEG-lipids stabilize defects occurring in high curvature regions and lead to highly flexible membranes with large distances from each other caused by long-range repulsive forces of the PEG-lipids. Xu et al. studied the gelled lamellar phase \( L_\alpha \) of the system \( \text{H}_2\text{O}-n\text{-decane}/12\text{-HOA}-\text{C}_{10}\text{E}_4 \) with a focus on the question whether the formation of the gel network and of the lamellar phase formation is simultaneous, but independent [5]. Koitani et al. tried to answer the same question by studying the lamellar phase \( L_\alpha \) of the system \( \text{H}_2\text{O}-2\text{C}_{12}\text{DAB}–12\text{-HOA} \) [6].

Introduced by Laibinis et al. for alkanethiols and alkane carboxylic acids on gold and alumina, respectively, the simultaneous (but independent) formation of two coexisting structures is called orthogonal self-assembly [7]. However, the term orthogonal self-assembly is not restricted to surface chemistry. Orthogonal self-assembled structures have been studied in bulk systems as well [8–12]. The cell, once again, is the most prominent example of an orthogonal self-assembled system, since proteins self-assemble to fibers forming the cytoskeleton, while phospholipids self-assemble to membranes [3]. The expression orthogonal self-assembly was first used for gelled complex fluids by the group of van Esch [13]. They reported on surfactant micelles, worm-like micelles, liposomes and phospholipids entrapped in self-assembled fibrillary networks of low molecular weight gelators (LMWG) [13–17]. Laupheimer studied the system \( \text{H}_2\text{O}-n\text{-decane}/12\text{-HOA}-\text{C}_{10}\text{E}_4 \) and proved that the gelled bicontinuous microemulsion is an orthogonal self-assembled system in which the formation of the nanostructured microemulsion is nearly independent on the formation of the gel network [18–20]. In the special case of the gelled \( L_\alpha \) phase of the very same system, the question whether it is a truly orthogonal self-assembled system could not be answered satisfactorily. On the one hand, phase behaviour and rheological studies supported the idea of an orthogonal self-assembled gelled lamellar \( L_\alpha \) phase. On the other hand, freeze fracture electron microscopy revealed that the gel network and the lamellar \( L_\alpha \) phase influence each other: The presence of the gel network leads to a higher order of the \( L_\alpha \) phase while the presence of the \( L_\alpha \) phase results in gel fibers which are not twisted anymore [5]. Moreover, Laupheimer et al. showed in a previous SANS study of the system \( \text{H}_2\text{O}-n\text{-decane}/12\text{-HOA}-\text{C}_{10}\text{E}_4 \) that 12-HOA is only partly involved in the gel formation, since it also acts as co-surfactant, which results in a shift of the phase boundaries to lower temperatures [20]. Koitani et al. studied the gelled lamellar phase \( L_\alpha \) of the system \( \text{H}_2\text{O}-\text{didodecyldimethylammonium bromide} (2\text{C}_{12}\text{DAB})–12\text{-HOA} \) at surfactant mass fractions \( \gamma_d = 0.10 \) and \( \gamma_a = 0.20 \) in order to answer the question of orthogonal self-assembly [6]. However, they also found that 12-HOA seems to have two roles in surfactant-containing systems, namely as gelator and as co-surfactant. The slightly surface active 12-HOA molecules are partly incorporated in the surfactant bilayers of the system \( \text{H}_2\text{O}-2\text{C}_{12}\text{DAB}–12\text{-HOA} \) and are thus not available for the gel formation which leads to a weaker gel compared to the binary gel \( n\text{-decane}–12\text{-HOA} \). Additionally, the incorporation of 12-HOA in the bilayers caused a slightly larger interlayer spacing compared to the non-gelled \( L_\alpha \) phase. In order to elucidate the role of 12-HOA in surfactant-containing systems and its ability of gelling lyotropic liquid crystals of different phase structures, we chose the binary system \( \text{H}_2\text{O}–\text{heptaethylene glycol monododecyl ether} (\text{C}_{12}\text{E}_7) \) instead of the ternary system \( \text{H}_2\text{O}-n\text{-decane}-\text{C}_{10}\text{E}_4 \) or the system \( \text{H}_2\text{O}-2\text{C}_{12}\text{DAB} \), for the following reasons. Firstly, studying the system \( \text{H}_2\text{O}–\text{C}_{12}\text{E}_7 \) ensures easy handling and a broader access to gelled lyotropic liquid crystals as it forms three lyotropic liquid crystalline phases, namely the hexagonal phase \( \text{H}_1 \), the bicontinuous cubic phase \( \text{V}_1 \) and the lamellar phase \( L_\alpha \) in specific surfactant concentration ranges and with melting points at moderate temperatures (\( T \approx 50 \degree \text{C} \)) [21]. Secondly, it allows us to prove the concept of gelling lyotropic liquid crystals of binary water–surfactant systems with the organogelator 12-HOA at much higher surfactant concentrations as compared to the binary system \( \text{H}_2\text{O}-2\text{C}_{12}\text{DAB} \). We first investigated the influence of the 12-HOA concentration on the phase boundaries of the system \( \text{H}_2\text{O}–\text{C}_{12}\text{E}_7 \) by means of visual observation in water basins and determined qualitatively whether a gelled lyotropic liquid crystalline phase was
formed. $^2$H NMR spectroscopy complemented the visual phase studies, since it is a well-known and powerful tool to detect phase transitions of lyotropic liquid crystalline phases to an isotropic phase and the coexistence of two phases, respectively [22–30]. In addition, we investigated the rheological properties of the lyotropic liquid crystalline phases in the presence of 12-HOA in order to identify gelled lyotropic liquid crystalline phases.

2. Results and Discussion

2.1. Visual Phase Studies

The visual phase studies allowed us to determine the occurrence of lyotropic liquid crystals via optical birefringence. In addition, they helped identifying transition temperatures, namely the melting points of the lyotropic liquid crystals and the sol-gel transition of the gel network. Therefore, we were able to measure the influence of 12-HOA on the phase boundaries of the lyotropic liquid crystalline phases of the system H$_2$O–C$_{12}$E$_7$. As already mentioned, the system H$_2$O–C$_{12}$E$_7$ forms three liquid crystalline phases as a function of the surfactant concentration, namely the hexagonal phase H$_1$, the bicontinuous cubic phase V$_1$ and the lamellar phase L$_\alpha$. The visually determined $T$-$\gamma_a$ phase diagram of the binary system H$_2$O–C$_{12}$E$_7$, as well as those of the systems in the presence of 12-HOA, i.e., H$_2$O–C$_{12}$E$_7$–12-HOA with 12-HOA mass fractions $\eta = 0.015, 0.025, 0.05$, are shown in Figure 1.

The black circles refer to the binary system H$_2$O–C$_{12}$E$_7$ and we note that the phase boundaries are in good agreement with literature data [21]. The highly viscous and transparent hexagonal H$_1$ phase occurs at a surfactant concentration range of $\gamma_a \approx 0.40$–0.68 and melts at temperatures $T \approx 50$ °C. At higher surfactant concentrations, in a range of $\gamma_a \approx 0.72$–0.85, the lamellar L$_\alpha$ phase is formed. The L$_\alpha$ phase melts at about the same temperatures as the hexagonal H$_1$ phase, but is less viscous and appears slightly turbid. The highly viscous, transparent bicontinuous cubic phase V$_1$ occurs between the hexagonal phase H$_1$ and the lamellar phase L$_\alpha$ in a surfactant concentration range $\gamma_a \approx 0.68$–0.72 and melts at temperatures $T \approx 46$ °C. In contrast to the anisotropic H$_1$ and L$_\alpha$ phases, the bicontinuous cubic phase V$_1$ is characterized by optical isotropy. The V$_1$ phase will not be discussed further as it is difficult to identify, especially in the presence of 12-HOA. The upper miscibility gap ($2\phi$), which is typical for oligo (ethylene oxide) alkyl ethers, was detected at higher temperatures for surfactant concentrations up to $\gamma_a = 0.50$.

The open circles in Figure 1 represent the phase boundaries of the system H$_2$O–C$_{12}$E$_7$ at three different 12-HOA concentrations. Comparing the binary system with the systems in the presence of 12-HOA, it is obvious that 12-HOA has an enormous influence on the phase boundaries of the liquid crystalline phases. For a 12-HOA mass fraction of $\eta = 0.015$, the melting point of the lamellar phase L$_\alpha$ is increased by $\Delta T \approx 5$ K, whereas at the same time the melting point of the hexagonal phase H$_1$ is decreased by the same value. The upper miscibility gap is also shifted towards lower temperatures in the presence of 12-HOA. The influence of 12-HOA on the upper miscibility gap at surfactant concentrations $\gamma_a < 0.3$ could not be determined via visual observations, because 12-HOA precipitated at these surfactant concentrations. After addition of 12-HOA ($\eta = 0.015$) the appearance and viscosity of the lamellar phase L$_\alpha$ did not change. It maintained its slight turbidity and also its flow ability, i.e., no gelled lamellar L$_\alpha$ phase was formed. On the contrary, the initially transparent hexagonal phase H$_1$ became turbid after addition of 12-HOA ($\eta = 0.015$), but whether a gel was formed, could not be detected by visual observation, because the viscosity of the hexagonal phase H$_1$ is high a priori. Moreover, the turbidity of the hexagonal phase complicated the visual observation, especially at low temperatures. For a 12-HOA concentration of $\eta = 0.025$ we observed the same trend, i.e., a stabilized lamellar L$_\alpha$ phase and a destabilized hexagonal H$_1$ phase, but a bit more pronounced as for $\eta = 0.015$. Again, even after increasing the 12-HOA concentration to $\eta = 0.025$, the appearance of the lamellar phase L$_\alpha$ did not change, i.e., still no gelled lamellar phase L$_\alpha$ was formed. In the case of the hexagonal phase H$_1$, the effect of 12-HOA is also slightly more pronounced for $\eta = 0.025$ than for
The hexagonal H\textsubscript{1} phase is marginally less temperature stable for $\eta = 0.025$, but again its appearance changes from transparent to turbid. For the highest 12-HOA concentration, i.e., $\eta = 0.05$, the lamellar phase L\textsubscript{\alpha} is extended to higher temperatures and lower surfactant concentrations $\gamma_a$, i.e., it additionally occurs in the range of $\gamma_a = 0.40$–0.60 at temperatures $T = 50$–70 °C. Unlike for $\eta = 0.015$ and 0.025, gelled L\textsubscript{\alpha} phases were obtained for $\eta = 0.05$ in a surfactant concentration range of $\gamma_a = 0.72$–0.85. In addition, gelled isotropic solutions were formed at lower concentrations of $\gamma_a = 0.40$–0.60, since the hexagonal H\textsubscript{1} phase did not form anymore in the presence of 12-HOA at $\eta = 0.05$. In Figure 1, bottom, the approximate sol-gel transition temperature line is added to the phase diagram of the system H\textsubscript{2}O–C\textsubscript{12}E\textsubscript{7}–12-HOA ($\eta = 0.05$) and it can be seen that the sol-gel transition temperatures decrease with increasing surfactant concentration $\gamma_a$.

![Figure 1. $T$–$\gamma_a$ phase diagrams of the binary system H\textsubscript{2}O–C\textsubscript{12}E\textsubscript{7} (black circles) and of the systems H\textsubscript{2}O–C\textsubscript{12}E\textsubscript{7}–12-HOA (open circles) with 12-hydroxyoctadecanoic acid (12-HOA) mass fractions $\eta = 0.015$, 0.025, and 0.05 (from top to bottom). The phase transition temperatures were determined by visual observations in water basins. The stars in the phase diagram of the system H\textsubscript{2}O–C\textsubscript{12}E\textsubscript{7}–12-HOA with $\eta = 0.05$ indicate the approximate sol-gel transition temperatures. Gelation could not be observed for H\textsubscript{2}O–C\textsubscript{12}E\textsubscript{7}–12-HOA with 12-HOA mass fractions of $\eta = 0.015$ and $\eta = 0.025$. The error is within the size of the symbols.](image-url)
Based on these results it is clear that 12-HOA has two roles in the system H$_2$O–C$_{12}$E$_7$–12-HOA. On the one hand, it acts as co-surfactant, which results in the stabilization of the lamellar phase L$_\alpha$ and at the same time in the destabilization of the hexagonal phase H$_1$. This effect can be explained by Israelachvili’s packing parameter, which is the ratio of hydrophobic area to the effective hydrophilic head group area [31]. By adding 12-HOA to the system H$_2$O–C$_{12}$E$_7$, a C$_{18}$ chain is introduced which increases the volume of the hydrophobic chains. As a consequence, the packing parameter is increased, and the surfactant layer has a lower curvature. This stabilizes the lamellar phase L$_\alpha$, and simultaneously destabilizes the hexagonal phase H$_1$ [31–33]. On the other hand, it seems that at sufficient high gelator concentrations, i.e., for $\eta > 0.025$–0.05, the bilayers of the lamellar phase are saturated by 12-HOA and 12-HOA starts to act as gelator. The sol-gel transition temperature of the system H$_2$O–C$_{12}$E$_7$–12-HOA ($\eta = 0.05$) decreases with increasing surfactant concentration $\gamma_a$ which can be explained by an increasing surfactant layer at higher surfactant concentrations $\gamma_a$. The higher the surfactant concentrations $\gamma_a$, the more likely 12-HOA is incorporated in the surfactant layer instead of forming the gelator network, which leads to weaker gels with lower sol-gel transition temperatures $T_{\text{sol-gel}}$. The system H$_2$O–C$_{12}$E$_7$ thus is a better solvent for 12-HOA at high surfactant concentrations $\gamma_a$ than at low ones. This concentration dependence is already evident at the lowest 12-HOA concentration $\eta = 0.015$. While the appearance of the lamellar L$_\alpha$ phase at high surfactant mass fractions does not change in the presence of 12-HOA at $\eta = 0.015$, the otherwise clear hexagonal H$_1$ phase becomes turbid, which may indicate the start of a gel formation. Therefore, we focused our study on the system H$_2$O–C$_{12}$E$_7$–12-HOA at $\eta = 0.015$, in order to answer the question whether 12-HOA acts as both gelator and co-surfactant already at the lowest 12-HOA mass fraction.

2.2. $^{2}$H NMR

We performed $^{2}$H NMR measurements in order to complement the visual phase studies of the system H$_2$O–C$_{12}$E$_7$–12-HOA at $\eta = 0.015$ as the determination of the phase boundary of the hexagonal phase H$_1$ at surfactant concentrations $\gamma_a \approx 0.40$ is difficult by means of visual observation, in particular, in the presence of 12-HOA which makes the sample turbid. For this purpose, H$_2$O needed to be replaced by D$_2$O, which, in turn, requires a phase study of D$_2$O–C$_{12}$E$_7$. The phase diagram of D$_2$O–C$_{12}$E$_7$ is available in the Appendix A (Figure A1). It was found that the lyotropic liquid crystalline phases were shifted to slightly smaller surfactant mass fractions $\gamma_a$. Moreover, the phase boundary of the hexagonal phase H$_1$ was shifted by $\Delta T = 1$–2 °C to lower values, whereas the phase boundary of the lamellar phase L$_\alpha$ was shifted to higher temperatures by $\Delta T = 2$–3 °C. The miscibility gap at low surfactant concentrations and at high temperatures was shifted to lower temperatures by $\Delta T = 2$–3 °C, i.e., it seems that the surfactant is more hydrophobic in the presence of D$_2$O [29]. The temperature shifts caused by replacing H$_2$O by D$_2$O are the same for the systems without and with 12-HOA. We performed temperature dependent $^{2}$H NMR measurements at $\gamma_a = 0.37$ and 0.38 for the system D$_2$O–C$_{12}$E$_7$ and at $\gamma_a = 0.36$ and 0.38 for the system D$_2$O–C$_{12}$E$_7$–12-HOA ($\eta = 0.015$). In the following, we will discuss the $^{2}$H NMR spectra of the systems D$_2$O–C$_{12}$E$_7$ and D$_2$O–C$_{12}$E$_7$–12-HOA ($\eta = 0.015$) at $\gamma_a = 0.38$. The spectra of the systems D$_2$O–C$_{12}$E$_7$ at $\gamma_a = 0.37$ and D$_2$O–C$_{12}$E$_7$–12-HOA ($\eta = 0.015$) at $\gamma_a = 0.36$ are shown in the Appendix A (Figure A2).

In the anisotropic hexagonal phase H$_1$, the quadrupolar $^{2}$H nucleus with a spin quantum number $I = 1$ shows a splitting which depends on the local orientation order of the D$_2$O molecules, as well as on the orientation of the director of the hexagonal phase with respect to the magnetic field. The D$_2$O molecules are on average slightly aligned, since they interact with the aligned surfactant molecules resulting in a non-zero residual quadrupole coupling. Hexagonal phases H$_1$ are not macroscopically aligned by the magnetic field, because of their high viscosity, i.e., domains with all possible orientations of the hexagonal axis are present in the sample. The superposition of the spectral contributions from all domains leads to a characteristic line shape, called the Pake powder pattern [34]. On the contrary, isotropic phases are indicated by a single peak, since the temporal average over the random orientations
of the molecules, which have no preferred orientation with respect to the magnetic field eliminates the quadrupole coupling.

In Figure 2a, the $^2$H NMR spectra of the system $D_2O–C_{12}E_7$ at $\gamma_a = 0.38$ as a function of temperature are shown. The bottom two spectra show the Pake powder pattern with two inner peaks and two outer shoulders, corresponding to domains with the hexagonal axis perpendicular and parallel to the magnetic field, respectively. The shape of the Pake pattern represents the isotropic distribution of domains in a macroscopically unaligned sample. As we studied the system $D_2O–C_{12}E_7$ by means of visual observation, and since the general phase behavior is known in literature, the splitting can be assigned to the hexagonal phase $H_1$. The spectrum at $T = 33 \, ^\circ C$ shows an additional peak that can be assigned to an isotropic phase that coexists with the hexagonal phase. The two phase region can be detected up to $T = 40 \, ^\circ C$, though the peaks that indicate the hexagonal phase are at minimum intensity. At $T = 41 \, ^\circ C$ the single peak corresponds to an isotropic phase, therefore this temperature is set as phase transition temperature from the hexagonal phase $H_1$ to the isotropic phase. The $^2$H NMR spectra of the system $D_2O–C_{12}E_7$ at $\gamma_a = 0.37$ shown in the Appendix indicate that the system consists of an isotropic and a hexagonal phase $H_1$ from room temperature up to $T = 42 \, ^\circ C$.

![Figure 2. (a) Temperature dependent $^2$H NMR spectra of the system $D_2O–C_{12}E_7$, recorded at $\gamma_a = 0.38$. (b) Temperature dependent $^2$H NMR spectra of the system $D_2O–C_{12}E_7–12$-HOA, recorded at $\gamma_a = 0.38$ and $\eta = 0.015$.](image)

The respective temperature dependent $^2$H NMR spectra of the system $D_2O–C_{12}E_7–12$-HOA ($\eta = 0.015$) at $\gamma_a = 0.38$ are shown in Figure 2b. As opposed to the spectra of the system $D_2O–C_{12}E_7$, the line shape is less sharp in the presence of 12-HOA, though the general phase behavior is maintained in presence of 12-HOA, but shifted to lower temperatures by $\Delta T = 8 \, ^\circ C$, which is in accordance with the visual phase studies. At $T = 25 \, ^\circ C$ up to $T = 31 \, ^\circ C$ again the Pake pattern can be observed and
can also be assigned to the hexagonal phase H1. At T > 32 °C an additional peak occurs, which can be assigned to the isotropic phase. At T = 34 °C, the Pake spectrum is still visible, indicating that the system is still in the two phase region. The phase transition temperature from the hexagonal phase H1 to the isotropic phase was set to T = 35 °C—since a single peak, indicative of a pure isotropic phase, was recorded. In the Appendix A the spectra of the 12-HOA containing sample at γa = 0.36 are shown.

In Figure 3, the phase transitions determined by ²H NMR are added to the phase diagram of the system H2O–C12E7–12-HOA (η = 0.015) determined by visual observation in water basins (see Figure 1, top). We added ΔT = +1 to the phase transitions determined by ²H NMR before we added them to the phase diagram, since D2O influences the phase boundaries in that way (see Figure A1). The results of visual observations and ²H NMR are in good agreement and complement each other. We were able to detect the phase boundaries of the hexagonal phase H1 at γa < 0.40 in the presence of 12-HOA, which was difficult by means of visual observations, due to turbidity caused by 12-HOA. Moreover, the ²H NMR measurements enabled the detection of the two-phase region of isotropic and hexagonal phase at γa = 0.38–0.40. To summarize, ²H NMR measurements confirm the phase behavior and the role of 12-HOA as co-surfactant determined by visual observations.

![Figure 3](image-url)

**Figure 3.** T–γa phase diagram of the binary system H2O–C12E7 (black symbols) and of the system H2O–C12E7–12-HOA (open symbols) at η = 0.015. The phase transition temperatures were determined by visual observation in water basins (circles) and by ²H NMR (crosses). For the sake of clarity, the two-phase region is not drawn for the system H2O–C12E7.

### 2.3. Rheometry

Looking at the phase diagrams and the NMR spectra one can conclude that 12-HOA acts as co-surfactant up to η = 0.015. Somewhere between a 12-HOA concentration of η = 0.025 and η = 0.05 its second role of a gelator becomes obvious. However, at the lowest 12-HOA concentration of η = 0.015, the hexagonal H1 phase went turbid as discussed in Section 2.1, which could indicate the beginning of a gel formation. In other words, it can well be that 12-HOA acts already as both co-surfactant and gelator at η = 0.015. In order to determine whether or not the hexagonal phase H1 was gelled at a 12-HOA concentration of η = 0.015, we performed frequency sweeps by oscillation shear rheometry and compared the results with the pure hexagonal phase H1 at γa = 0.50 and with the binary gel n-decane–12-HOA at η = 0.015. For comparison, we also studied the lamellar Lα phase at γa = 0.76 under the same conditions. The results of the frequency sweeps are shown in Figure 4 for the lamellar phase Lα and in Figure 5 for the hexagonal phase H1.
which indicates a change from solid like, elastic behavior (\(G'\)). This shows that no gel was formed in case of the lamellar phase \(L\) since the slope of \(G'\) is higher than the loss modulus \(G''\) is observed at frequencies of \(\omega \approx 0.1\) s\(^{-1}\), which indicates a change from solid like, elastic behavior \((G' > G'')\) to liquid like, viscous behavior \((G' < G'')\) [35]. Moreover, \(G'\) of the pure hexagonal phase \(H_1\) changes with the frequency. Characteristic for gels and soft solids is the frequency independence of \(G'\) as can be seen in the frequency sweep of the binary gel \(n\)-decane–12-HOA [36–39]. The frequency sweeps of the pure lamellar phase \(L_{1\alpha}\), as well as of the lamellar phase \(L_{1\alpha}\) and the hexagonal phase \(H_1\) in presence of 12-HOA at \(\eta = 0.015\) show solid like behavior. In case of the pure hexagonal phase \(H_1\) a crossover point between \(G'\) and \(G''\) is observed at frequencies of \(\omega \approx 0.1\) s\(^{-1}\), which indicates a change from solid like, elastic behavior \((G' > G'')\) to liquid like, viscous behavior \((G' < G'')\) [35]. Moreover, \(G'\) of the pure lamellar phase \(L_{1\alpha}\) changes with the frequency. Characteristic for gels and soft solids is the frequency independence of \(G'\) as can be seen in the frequency sweep of the binary gel \(n\)-decane–12-HOA [36–39]. The frequency sweeps of the pure lamellar phase \(L_{1\alpha}\), as well as of the lamellar phase \(L_{1\alpha}\) and the hexagonal phase \(H_1\) in presence of 12-HOA show a slight frequency dependence of \(G'\). Comparing the absolute values of \(G'\) and \(G''\), one sees that the pure hexagonal phase \(H_1\) is much more viscous than the pure lamellar phase \(L_{1\alpha}\). This results from the densely packed cylinders compared to the parallel orientated lamellar bilayers, which can easily slip against each other [40]. Moreover, the absolute \(G'\) and \(G''\) values of the pure lamellar phase \(L_{1\alpha}\) are lower than those of the binary gel and do not change in the presence of 12-HOA. This shows that no gel was formed in case of the lamellar phase \(L_{1\alpha}\). In the frequency sweep of the hexagonal phase \(H_1\) in presence of 12-HOA no crossover between \(G'\) and \(G''\) can be seen anymore, since the slope of \(G'\) is lower, i.e., the solid like behavior predominates in the whole frequency range and indicates that a gel
was indeed formed. However, as the storage modulus $G'$ is still frequency dependent, a gel weaker (but stiffer) than the binary gel was formed. Note that the absolute values of $G'$ and $G''$ cannot be used as measure for gel formation as they are a priori high for the pure hexagonal phase $H_1$. In conclusion, one can say that for the lowest 12-HOA mass fraction of $\eta = 0.015$, 12-HOA is only co-surfactant in case of the lamellar $L_\alpha$ phase, but acts as both co-surfactant and gelator in case of the hexagonal phase. Again, it becomes obvious that 12-HOA is more likely incorporated in the surfactant layer of the system $H_2O\cdot C_{12}E_7$ at higher surfactant mass fractions than at low ones—since at higher surfactant mass fractions most of the 12-HOA molecules are dissolved in the surfactant layer, no gel can be formed.

3. Conclusions

Both visual observation and $^2$H NMR clearly show that 12-HOA stabilizes the lamellar phase and destabilizes the hexagonal phase of the binary system $H_2O\cdot C_{12}E_7$ (heptaethylene glycol monododecyl ether). The strong influence of 12-HOA on the phase behavior is clear evidence that the organogelator acts as a co-surfactant. With increasing mass fraction $\eta$ of 12-HOA the effect is more pronounced (at the highest gelator mass fraction investigated, namely $\eta = 0.05$, the hexagonal phase is completely absent), but the gelation capacity is increased, too. At $\eta = 0.05$, a sol-gel transition can be observed by visual inspection. The sol-gel transition temperature decreases with increasing surfactant concentration, which proves a decreasing gelation capacity, due to an increasing incorporation of 12-HOA in the surfactant layer. Note that the total gelator mass fraction is kept constant, while the amount of surfactant thus the surfactant layer increases.

The $^2$H NMR measurements show broadened features of the Pake powder pattern of the hexagonal phase, which indicates an increased viscosity, even at the lowest gelator mass fraction of $\eta = 0.015$. Dynamic rheometry proves that a weak gel is formed at this low gelator concentration in the case of the hexagonal phase (i.e., at low surfactant concentrations), whereas no gel is formed in the case of the lamellar phase (i.e., at high surfactant concentrations). This is in excellent agreement with the decreasing gelation capacity deduced from decrease of the sol-gel transition temperature as a function of surfactant concentration.

Our investigations clearly show that 12-HOA is not a suitable gelator for binary systems consisting of $H_2O$ and an alkyl polyglycol ether ($C_iE_j$), since its gelation capacity is hampered by its competing role as co-surfactant. The search for a suitable gelator for $H_2O\cdot C_iE_j$ systems that solely acts as gelator is continued; tests of other organogelators, as well as hydrogelators, are in progress. Once a suitable gelator is found, we will investigate (a) if the lyotropic liquid crystalline phases serve as template for the gelator network leading to well aligned gelator fibers and (b) if the gel network influences the structure of the lyotropic liquid crystalline phases. For this purpose, the sol-gel transition line has to be adjusted via the gelator concentration, such that (a) the sol-gel transition temperature $T_{sol-gel}$ of the gelator is lower than the melting point of the lyotropic liquid crystalline phases, and (b) the sol-gel transition temperature $T_{sol-gel}$ of the gelator is higher than the melting point of the lyotropic liquid crystalline phases. First experimental evidence that the chronology—that is, whether gelation or liquid crystal phase formation occurs first—may indeed play a role is the work published by Kato et al. They studied gelled thermotropic liquid crystals (thermotropic LC), focusing on the influence of the chronology on the resulting structure [41].

4. Materials and Methods

4.1. Materials and Sample Preparation

We purchased heptaethylene glycol monododecyl ether ($C_{12}E_7$) from Sigma Aldrich ($\geq98\%$) (St. Louis, MO, USA), Nikkol (Tokyo, Japan) and TCI (Tokyo, Japan), 12-hydroxyoctadecanoic acid (12-HOA) from Alfa Aesar (95\%) (Karlsruhe, Germany), and $D_2O$ from euriso-top (99.9 atom \% D). Note that Alfa Aesar does not specify whether 12-HOA is a racemate or a pure enantiomer. We thus measured the optical rotation angle of 12-HOA in methanol and found out that the substance is the
(R)-enantiomer. In a previous study we proved that 12-HOA provided by Sigma Aldrich is also (R)-12-HOA. [42] All chemicals were used without further purification.

The composition of the systems is defined by the surfactant mass fraction,

\[ \gamma_a = \frac{m_{C_{12}E_7}}{m_{C_{12}E_7} + m_{H_2O}}, \]  

and the 12-HOA mass fraction,

\[ \eta = \frac{m_{12-HOA}}{m_{C_{12}E_7} + m_{H_2O} + m_{12-HOA}}. \]

We used \( \eta = 0.015, 0.025, 0.05 \) as 12-HOA mass fractions. The samples were weighed in glass tubes, equipped with a stirring bar and sealed with plugs. The samples had to be heated in a water bath up to \( T = 85 \, ^\circ C \) as 12-HOA has to be molten and they were then stirred at this temperature for at least 5 min in order to ensure homogenous mixing. Subsequently, the samples were put into an ice bath for gelation for 5 min. Gelation was indicated by a change of turbidity in the case of the otherwise clear hexagonal phase and a change in viscosity in the case of the lamellar phase \( L_\alpha \). It is important to note that changes in turbidity and viscosity are no proofs of gelation per se. However, in the present case they can be used to identify gelation, since there are no other (phase) transitions, which could explain these changes. The samples measured by \(^2\)H NMR were prepared with D\(_2\)O instead of H\(_2\)O, but the surfactant mass fraction, as well as the 12-HOA mass fraction were calculated in the same way.

### 4.2. Visual Phase Studies

The phase boundaries of the binary system H\(_2\)O–C\(_{12}\)E\(_7\) and of the systems H\(_2\)O–C\(_{12}\)E\(_7\)–12-HOA with \( \eta = 0.015, 0.025, 0.05 \) were determined by visual observation in water basins equipped with a thermostat (Thermo Scientific DC30, Waltham, MA, USA). The phase boundaries of the anisotropic lyotropic LC phases to the transparent, isotropic phase were detected with the help of a lamp behind the water basin and two crossed polarizers, one behind and one in front of the sample. The lyotropic LC phases were distinguished due to anisotropy, viscosity and turbidity. The hexagonal phase H\(_1\) is transparent and highly viscous, whereas the lamellar phase \( L_\alpha \) is slightly turbid and less viscous. Accordingly, the turbid upper miscibility gap at higher temperature was identified. The approximate sol-gel transition temperature was determined by fading turbidity in the case of the hexagonal phase H\(_1\) and by a change in viscosity in the case of the lamellar phase \( L_\alpha \).

### 4.3. \(^2\)H NMR

\(^2\)H NMR measurements were carried out with a Tecmag Apollo 300 MHz spectrometer (Erfurt, Germany) at a \(^2\)H resonance frequency of 46.02 MHz with a pulse width of 6.4 \( \mu s \) and a relaxation delay time of 2 s. The samples were filled in 4 cm glass tubes with a diameter of 5 mm. The tubes, equipped with a Teflon spacer and sealed with a Teflon plug and parafilm, were put in a goniometer probe such that its axis was perpendicular to the magnetic field. By applying a quadrupole echo pulse sequence, temperature-dependent \(^2\)H-NMR spectra of each sample were recorded. The sample temperature in the goniometer probe was set according to the temperature calibration of a reference probe, which was calibrated by a known phase transition of the system that was determined by visual observation before. The target temperatures were reached after a 30 min heating ramp followed by an additional 30 min equilibration time. 64 scans were averaged before Fourier transformation was applied.

### 4.4. Rheometry

The measurements were carried out with a Physica MCR 501 rheometer from Anton Paar (Ostfildern, Germany). A cone-plate geometry was used with an upper moving cone of 2.5 cm diameter and a cone angle of 1°. The samples were transferred to the plate with a spatula. After the
upper cone was lowered to the measuring position (gap width \( z = 1 \) mm), the samples were kept at \( T = 22 \, ^\circ\text{C} \) for 30 min to reach equilibrium. Then, frequency (\( \omega \)) sweeps were performed with a constant strain amplitude \( \gamma = 1\% \) and constant temperature \( T = 22 \, ^\circ\text{C} \) of the lower plate. The frequency \( \omega = 0.01 \, \text{s}^{-1} \) is the lower limit of our rheometer. The temperature was set with a precision of \( \pm 0.1 \, \text{K} \) by a Peltier element. The strain amplitude \( \gamma \) was set such that it was in the linear viscoelastic (LVE) region determined through prior oscillating stress sweeps. The storage modulus \( G' \) and the loss modulus \( G'' \) were determined in the frequency sweeps. The pure hexagonal \( H_1 \) phase at a surfactant mass fraction \( \gamma_a = 0.50 \) and the pure lamellar \( L_\alpha \) phase at \( \gamma_a = 0.76 \) as well as both lyotropic liquid crystalline phases in the presence of 12-HOA with a gelator mass fraction \( \eta = 0.015 \) were measured. For comparison, the rheological behaviour of the binary gel \( n \)-decane–12-HOA at \( \eta = 0.015 \) was investigated.

Author Contributions: K.S. performed the experiments and wrote the first version of the article, C.Sch. and C.S. contributed to the discussion of the results and revised the first version of the article.

Funding: This research was funded by the Deutsche Forschungsgemeinschaft grant number STU287/6-1.

Acknowledgments: We thank Marco Grunwald for his help with the phase studies, Dmitry Kushnikovskiy for his help with the NMR measurements and Waldemar Keil for his help with the fine-tuning of the NMR spectra.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

\[ \text{Figure A1. } T-\gamma_a \text{ phase diagrams of the binary systems } H_2O-C_{12}E_7 (\text{black circles}) \text{ and } D_2O-C_{12}E_7 (\text{open circles}). \text{ The phase transition temperatures were determined by visual observation in water basins.} \]
Figure A2. (a) Temperature dependent $^2$H NMR spectra of the system D$_2$O–C$_{12}$E$_7$ recorded at $\gamma_a = 0.37$. (b) Temperature dependent $^2$H NMR spectra of the system D$_2$O–C$_{12}$E$_7$–12-HOA recorded at $\gamma_a = 0.36$ and at $\eta = 0.015$. The asymmetry seen in the spectra may have several reasons. One is the local anisotropy, which causes an anisotropy of the magnetic susceptibility and therefore an orientation dependent shift in addition to the orientation dependent quadrupolar splitting. Therefore, the isotropic peak is not exactly in the center of the two maxima (which correspond to domains aligned with the phase axis perpendicular to the magnetic field), and the maxima are not exactly in the middle of the two outer shoulders (which correspond to domains aligned with the phase axis parallel to the magnetic field). Additional asymmetries arise from shim and phase errors.

References

1. Stubenrauch, C.; Gießelmann, F. Gelled Complex Fluids: Combining Unique Structures with Mechanical Stability. *Angew. Chem. Int. Ed.* 2016, 55, 3268–3275. [CrossRef] [PubMed]
2. Kim, D.-H.; Jahn, A.; Cho, S.-J.; Kim, J.S.; Ki, M.-H.; Kim, D.-D. Lyotropic liquid crystal systems in drug delivery: A review. *J. Pharm. Investig.* 2015, 45, 1–45. [CrossRef]
3. Kelly, C.V.-D. Perturbations of Cellular Membranes with Synthetic Polymers and Ultrafast Lasers. Ph.D. Thesis, University of Michigan, Ann Arbor, MI, USA, 2009.
4. Warriner, H.E.; Idziak, S.H.J.; Slack, N.L.; Davidson, P.; Safinya, C.R. Lamellar Biogels: Fluid-Membrane-Based Hydrogels Containing Polymer Lipids. *Science* **1996**, *271*, 969–973. [CrossRef] [PubMed]

5. Xu, Y.; Laupheimer, M.; Preissig, N.; Sottmann, T.; Schmidt, C.; Stubenrauch, C. Gelled Lyotropic Liquid Crystals. *Langmuir* **2015**, *31*, 8589–8598. [CrossRef] [PubMed]

6. Koitani, S.; Dieterich, S.; Preissig, N.; Aramaki, K.; Stubenrauch, C. Gelling Lamellar Phases of the Binary System Water–Didodecyldimethylammonium Bromide with an Organogelator. *Langmuir* **2017**, *33*, 12171–12179. [CrossRef] [PubMed]

7. Laibinis, P.E.; Hickman, J.J.; Wrighton, M.S.; Whitesides, G.M. Orthogonal Self-Assembled Monolayers: Alkanethiols on Gold and Alkane Carboxylic Acids on Alumina. *Science* **1989**, *245*, 845–847. [CrossRef] [PubMed]

8. Hu, X.; Xiao, T.; Lin, C.; Huang, F.; Wang, L. Dynamic Supramolecular Complexes Constructed by Orthogonal Self-Assembly. *Acc. Chem. Res.* **2014**, *47*, 2041–2051. [CrossRef] [PubMed]

9. Kumar, D.K.; Steed, J.W. Supramolecular gel phase crystallization: Orthogonal self-assembly under non-equilibrium conditions. *Chem. Soc. Rev.* **2014**, *43*, 2080–2088. [CrossRef] [PubMed]

10. Li, S.; Xiao, T.; Lin, C.; Wang, L. Advanced supramolecular polymers constructed by orthogonal self-assembly. *Chem. Soc. Rev.* **2012**, *41*, 5950–5968. [CrossRef] [PubMed]

11. Saha, M.L.; De, S.; Pramanik, S.; Schmittel, M. Orthogonality in discrete self-assembly—Survey of current concepts. *Chem. Soc. Rev.* **2013**, *42*, 6860–6909. [CrossRef] [PubMed]

12. Wei, P.; Yan, X.; Huang, F. Supramolecular polymers constructed by orthogonal self-assembly based on host–guest and metal–ligand interactions. *Chem. Soc. Rev.* **2015**, *44*, 815–832. [CrossRef] [PubMed]

13. Heeres, A.; van der Pol, C.; Stuart, M.; Friggeri, A.; Feringa, B.L.; van Esch, J. Orthogonal self-assembly of Low Molecular Weight Hydrogelators and Surfactants. *J. Am. Chem. Soc.* **2003**, *125*, 14252–14253. [CrossRef] [PubMed]

14. Brizard, A.; Stuart, M.; van Bommel, K.; Friggeri, A.; de Jong, M.; van Esch, J. Preparation of Nanostructures by Orthogonal Self-Assembly of Hydrogelators and Surfactants. *Angew. Chem. Int. Ed.* **2008**, *47*, 2063–2066. [CrossRef] [PubMed]

15. Brizard, A.M.; van Esch, J.H. Self-assembly approaches for the construction of cell architecture mimics. *Soft Matter* **2009**, *5*, 1320–1327. [CrossRef]

16. Brizard, A.M.; Stuart, M.C.A.; van Esch, J.H. Self-assembled interpenetrating networks by orthogonal self-assembly of surfactants and hydrogelators. *Faraday Discuss.* **2009**, *143*, 345–357. [CrossRef] [PubMed]

17. Boekhoven, J.; Brizard, A.M.; Stuart, M.C.A.; Florusse, L.; Raffy, G.; Del Guerzo, A.; van Esch, J.H. Bio-inspired supramolecular materials by orthogonal self-assembly of hydrogelators and phospholipids. *Chem. Sci.* **2016**, *00*, 1–11. [CrossRef] [PubMed]

18. Laupheimer, M. Gelled Bicontinuous Micromelusions: A New Type of Orthogonal Self-Assembled Systems. Ph.D. Thesis, University of Stuttgart, Stuttgart, Germany, 2013.

19. Laupheimer, M.; Jovic, K.; Antunes, F.E.; da Graça Martins Miguel, M.; Stubenrauch, C. Studying orthogonal self-assembled systems: Phase behaviour and rheology of gelled microemulsions. *Soft Matter* **2013**, *9*, 3661–3670. [CrossRef]

20. Laupheimer, M.; Sottman, T.; Schweins, R.; Stubenrauch, C. Studying orthogonal self-assembled systems: Microstructure of gelled bicontinuous microemulsions. *Soft Matter* **2014**, *10*, 8744–8757. [CrossRef] [PubMed]

21. Inoue, T.; Matsuda, M.; Nibu, Y.; Misono, Y.; Suzuki, M. Phase Behavior of Heptaethylene Glycol Dodecyl Ether and Its Aqueous Mixture Revealed by DSC and FT-IR Spectroscopy. *Langmuir* **2001**, *17*, 1833–1840. [CrossRef]

22. Blackburn, J.C.; Kilpatrick, P.K. Using Deuterium NMR Line Shapes to Analyze Lyotropic Liquid Crystalline Phase Transitions. *Langmuir* **1992**, *8*, 1679–1687. [CrossRef]

23. Davis, J.H. The description of membrane lipid conformation, order and dynamics by ²H-NMR. *Biophys. Acta* **1983**, *737*, 117–171. [CrossRef]

24. Dufoeur, E.J.; Parish, E.J.; Chitrakorn, S.I.C.P. Smith Structural and Dynamical Details of Cholesterol-Lipid Interaction as Revealed by Deuterium NMR. *Biochemistry* **1984**, *23*, 6062–6071. [CrossRef]

25. Jelinski, L.W. Deuterium NMR of Solid Polymers. In *High resolution NMR Spectroscopy of Synthetic Polymers in Bulk*; Komorowski, R.A., Ed.; VHC Publishers: New York, NY, USA, 1986; pp. 335–364.

26. Lukaschek, M.; Grabowski, D.A.; Schmidt, C. Shear-Induced Alignment of a Hexagonal Lyotropic Liquid Crystal as Studied by Rheo-NMR. *Langmuir* **1995**, *11*, 3590–3594. [CrossRef]
27. Seelig, J. Deuterium magnetic resonance: Theory and application to lipid membranes. Q. Rev. Biophys. 1977, 10, 353–418. [CrossRef] [PubMed]
28. Stubenrauch, C.; Frank, C.; Strey, R.; Burgmeister, D.; Schmidt, C. Lyotropic Mesophases Next to Highly Efficient Microemulsions: A 2H NMR Study. Langmuir 2002, 18, 5027–5030. [CrossRef]
29. Stubenrauch, C.; Burauer, S.; Strey, R. A new approach to lamellar phases (Lα) in water–non-ionic surfactant systems. Liq. Cryst. 2004, 31, 39–53. [CrossRef]
30. Stockton, G.W.; Polnaszek, C.F.; Tulloch, A.P.; Hasan, F.; Smith, I.C.P. Molecular Motion and Order in Single-Bilayer Vesicles and Multilamellar Dispersions of Egg Lecithin and Lecithin-Cholesterol Mixtures. A Deuterium Nuclear Magnetic Resonance Study of Specifically Labeled Lipids. Biochemistry 1976, 15, 954–966. [CrossRef] [PubMed]
31. Israelachvili, J.N.; Mitchell, D.J.; Ninham, B.W. Theory of Self-Assembly of Hydrocarbon Amphiphiles into Micelles and Bilayers. J. Chem. Soc. Faraday Trans. 2 1976, 72, 1525–1568. [CrossRef]
32. Mitchell, D.J.; Tiddy, G.J.T.; Waring, L.; Bostock, T.; McDonald, M.P. Phase behaviour of Polyoxyethylene Surfactants with Water. J. Chem. Soc. Faraday Trans. 1 1983, 79, 975–1000. [CrossRef]
33. Hoffmann, H.; Ulbricht, W. Faszinierende Phänomene in Tensidlösungen. Chemie Unserer Zeit 1995, 29, 76–86. [CrossRef]
34. Pake, G.E. Nuclear Resonance Absorption in Hydrated Crystals: Fine Structure of the Proton Line. J. Chem. Phys. 1948, 16, 327–336. [CrossRef]
35. Nishinari, K. Some Thoughts on the Definition of a Gel. Prog. Colloid Polym. Sci. 2009, 136, 87–94. [CrossRef]
36. Terech, P.; Rodriguez, V.; Barnes, J.D.; McKenna, G.B. Organogels and Aerogels of Racemic and Chiral 12-Hydroxyoctadecanoic Acid. Langmuir 1994, 10, 3406–3418. [CrossRef]
37. Terech, P.; Weiss, R.G. Low Molecular Mass Gelators of Organic Liquids and the Properties of Their Gels. Chem. Rev. 1997, 97, 3113–3160. [CrossRef]
38. Terech, P.; Pasquier, D.; Bordin, V.; Rossat, C. Rheological Properties and Structural Correlations in Molecular Organogels. Langmuir 2000, 16, 4485–4494. [CrossRef]
39. Weiss, R.G.; Terech, P. Molecular Gels: Materials with Self-Assembled Fibrillar Networks; Springer: Dordrecht, The Netherlands, 2005; ISBN 978-1-4020-3689-7.
40. Collings, P.J.; Hird, M. Introduction to Liquid Crystals: Chemistry and Physics; Taylor & Francis Ltd.: London, UK, 1997; ISBN 9780748404834.
41. Kato, T.; Hirai, Y.; Nakaso, S.; Moriyama, M. Liquid Crystalline physical gels. Chem. Soc. Rev. 2007, 36, 1857–1867. [CrossRef] [PubMed]
42. Laupheimer, M.; Preissig, N.; Stubenrauch, C. The Molecular Organogel n-Decane/12-Hydroxyoctadecanoic Acid: Sol–Gel Transition, Rheology and Microstructure. COLSUA 2015, 469, 315–325. [CrossRef]