

Characterization of Microemulsion Structures in the Pseudoternary Phase Diagram of Isopropyl Palmitate/Water/Brij 97:1-Butanol

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ABSTRACT

This research was aimed to characterize microemulsion systems of isopropyl palmitate (IPP), water, and 2:1 Brij 97 and 1-butanol by different experimental techniques. A pseudoternary phase diagram was constructed using water titration method. At 45% wt/wt surfactant system, microemulsions containing various ratios of water and IPP were prepared and identified by electrical conductivity, viscosity, differential scanning calorimetry (DSC), cryo-field emission scanning electron microscopy (cryo-FESEM) and nuclear magnetic resonance (NMR). The results from conductivity and viscosity suggested a percolation transition from water-in-oil (water/oil) to oil-in-water (oil/water) microemulsions at 30% wt/wt water. From DSC results, the exothermic peak of water and the endothermic peak of IPP indicated that the transition of water/oil to oil/water microemulsions occurred at 30% wt/wt water. Cryo-FESEM photomicrographs revealed globular structures of microemulsions at higher than 15% wt/wt water. In addition, self-diffusion coefficients determined by NMR reflected that the diffusability of water increased at higher than 35% wt/wt water, while that of IPP was in reverse. Therefore, the results from all techniques are in good agreement and indicate that the water/oil and oil/water transition point occurred in the range of 30% to 35% wt/wt water.

KEYWORDS: microemulsions, characterization, colloidal structure, DSC, cryo-FESEM, NMR.

INTRODUCTION

Microemulsions are optically transparent, low viscous, and thermodynamically stable dispersions of oil and water stabilized by an interfacial film of a surfactant, usually in combination with a cosurfactant. In pharmaceuticals, micro-

emulsions are used as vehicles to deliver many kinds of drugs because of their thermodynamic stability, ease of preparation, and good appearance. In general, microemulsions can be separated into 3 types: water-in-oil (water/oil), bicontinuous, and oil-in-water (oil/water).^{1,2} In contrast to the ease of preparation, the characterization of microemulsions is more complicated and requires a combination of several techniques. The experimental techniques reported to be used in characterizing microemulsion microstructure include electrical conductivity, viscosity, differential scanning calorimetry (DSC), small-angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR), freeze-fracture transmission electron microscopy (FF-TEM), and cryo-field emission scanning electron microscopy (Cryo-FESEM).³⁻¹⁰ Each technique provides different information that can be used to complement each other to investigate the type and structure of microemulsions. Understanding the relationship between microstructure and composition of microemulsion is important to prepare microemulsion formulations for a more efficient use in pharmaceutical applications such as in drug and antigen delivery of hydrophilic and hydrophobic substances.

The aim of this work was to determine the type and structure of colloidal samples formed in isopropyl palmitate (IPP)/water/ Brij 97:1-butanol (2:1) system. Brij 97 (polyoxyethylene-10-oleyl ether) is a nonionic surfactant frequently used in microemulsion preparation.^{11,12} At a constant concentration of the surfactant/cosurfactant mixture, the microemulsions of various ratios of water and oil were produced and characterized. Several techniques including conductivity, viscosity, self-diffusion NMR, DSC, and Cryo-FESEM were combined to investigate the types of microemulsions and transitions between them.

MATERIALS AND METHODS

Materials

Brij 97 and IPP were obtained from Uniquema (New Castle, DE). 1-Butanol was obtained from BDH Chemicals Ltd (Poole, UK). Distilled water was used throughout the experiments. All chemicals were of pharmaceutical grade and used as received without further purification.

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Construction of Pseudoternary Phase Diagram

In order to find the concentration range of all components (IPP/water/2:1 Brij 97:1-butanol) in which they form microemulsions, a pseudoternary phase diagram was constructed using the water titration method. Brij 97 and 1-butanol were mixed at a weight ratio of 2:1 to obtain the surfactant mixture. IPP and the surfactant mixture were then mixed at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. These mixtures were diluted dropwise with water, under moderate agitation. The samples were classified as microemulsions when they appeared as clear liquids.

Preparation of Samples

The concentration of surfactant mixture was kept constant at 45% wt/wt, while the concentration of water was varied from 0% to 55% wt/wt in 5% wt/wt intervals. All formulations of the prepared samples were represented by a line on pseudoternary phase diagram (Figure 1). Brij 97 and 1-butanol were mixed in a 2:1 weight ratio to obtain the surfactant mixture. Required amounts of surfactant mixture, IPP, and water were mixed using a vortex mixer for 5 minutes at room temperature. All samples were stored at room temperature at least 24 hours to achieve equilibrium before further investigation.

Appearance

Samples were observed for their visual appearance and their microscopic appearance using cross-polarized light microscopy (Nikon Optiphot PFX microscope, Tokyo, Japan).

Conductivity Measurements

Electrical conductivity of the samples was measured using a Riac CM/100 conductivity meter fitted with an YSI 3418 electrode (Yellow Springs Instruments Inc, OH), having a cell constant of 0.11 cm^{-1} . The measurements were performed in triplicate at 25°C .

Viscosity Measurements

Viscosity of the samples was measured using a Brookfield DV-III programmable cone and plate rheometer (Brookfield Engineering Laboratories Inc, Middleboro, MA) fitted with a CP-42 cone spindle. Brookfield Rheocalc operating software controlled the rheometer. The jacketed sample cup was connected to a circulating water bath operating at 25°C . A sample volume of 1 mL was used. The measurements were performed in triplicate.

Differential Scanning Calorimetry Measurements

DSC measurements were performed with a DSC TA Q100 equipped with a refrigerated cooling system (TA Instruments, New Castle, DE). Nitrogen with a flow rate of 50 mL/min was used as purge gas. Approximately 4 to 13 mg of sample was weighted precisely into hermetic aluminum pans. An empty hermetically sealed pan was used as a reference. Samples were cooled from 25°C to -50°C at a cooling rate of $5^\circ\text{C}/\text{min}$, held for 3 minutes at -50°C and then heated to 25°C at a heating rate of $10^\circ\text{C}/\text{min}$. All measurements were performed at least in triplicate.

Cryo-field Emission Scanning Electron Microscopy

Samples were loaded into copper rivets and plunge frozen in liquid propane with a Reichert KF 80 (Leica, Wetzlar, Germany). Samples were then transferred under liquid nitrogen to an Alto 2500 cryo-preparation chamber (Gatan UK, Abingdon, UK), fractured and transferred onto the cryo-stage of a JEOL 6700F field emission scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Samples were etched at -95°C for ~4 minutes and then transferred back into the cryo-chamber for sputter coating with platinum. Samples were then introduced back onto the cryo-stage of the microscope and viewed at an accelerating voltage of 2.5 kV.

Nuclear Magnetic Resonance Spectroscopy

Self-diffusion NMR measurements were performed on a Varian Inova (500 MHz) equipped with Performa II-Z gradient coils (Varian, Palo Alto, CA). The temperature was

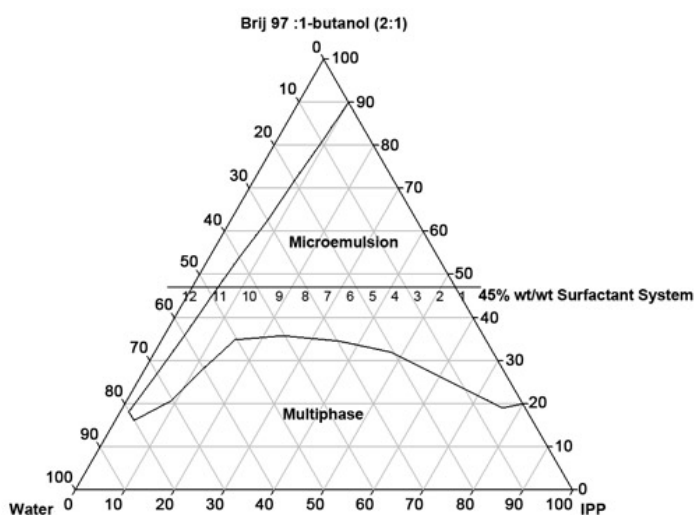


Figure 1. The pseudoternary phase diagram of IPP/water/Brij 97:1-butanol (2:1) and the dilution line for investigation at 45% wt/wt surfactant system.

kept constant at 25°C. Each sample was filled into a 3-mm diameter NMR tube (Wilmad-Labglass, Buena, NJ). 2,2-Dimethyl-2-silapentane-5-sulfonate sodium salt (DSS) dissolved in D₂O and sealed into a capillary was used as reference. ¹H-NMR spectra of the pure components were taken and compared with those of the microemulsions to obtain a characteristic peak frequency for each single component for the later analysis. Experiments were performed by keeping the z-gradient pulse length constant and gradually increasing the gradient strength in 17 steps. The gradient pulse length used was 100 ms to measure the diffusion coefficient of all components. The diffusion coefficients (*D*) were obtained from the slope of the equation:

$$\ln\left(\frac{I_g}{I_0}\right) = -[\gamma^2 d^2 G^2 (\Delta - d/3)]D \quad (1)$$

where *I_g* and *I₀* are intensities of the NMR signal in the presence and absence of field gradient pulses; γ is the gyromagnetic constant for ¹H; *d* is the duration of the z-gradient pulse; *G* is the gradient strength; and Δ is the time interval between the gradient pulses.

RESULTS AND DISCUSSION

Phase Diagram and Appearance

Figure 1 shows the pseudoternary phase diagram with the area inside the frame assigned on the phase diagram showing the microemulsion region. The area outside the frame indicates a turbid region with multiphase systems. It could be noted that the area of microemulsion region was considerably large since 1-butanol acted as a cosurfactant and interacted with the surfactant monolayer to increase the flexibility of the interfacial film.¹³

Sample 1 (Figure 1, 0% wt/wt water) was a hazy liquid and separated into 2 layers upon storage. Droplets were apparent under the light microscope at a magnification of $\times 40$, but no birefringence was detected; hence, sample 1 was determined to be an ordinary dispersion. Samples 2 to 12 (Figure 1, 5%-55% wt/wt water) were clear yellowish liquids that appeared dark under cross-polarized light microscopy (no birefringence) and were therefore classified as isotropic dispersion of spherical droplets, leading to assumption of spherical micelles or microemulsions. The visual appearance of microemulsions can be quite similar to that of liquid crystals, especially lamellar phases as they often have only slightly increased viscosities. Cubic phases on the other hand are highly viscous and can thus not be confused with a microemulsion. Cross-polarized light microscopy is the best way to distinguish lamellar liquid crystals from microemulsions and should always be a standard tool in the investigation of microemulsions.¹⁴

Conductivity

Figure 2 presents the relationship between water concentrations and conductivity values. In this work, the conductivity measurements were performed without deliberate incorporation of an electrolyte although the presence of electrolyte in a nonionic microemulsion is necessary to provide ions for a charge transport.⁴ However, the addition of salt may result in phase separation. The conductivity of the studied samples was very low as long as the water fraction was smaller than 10% wt/wt water. Above 10% wt/wt water, a drastic increase in conductivity was observed and furthermore a sharp increase of conductivity was found at 30% wt/wt water. The increase in conductivity at 30% wt/wt water is most likely caused by a transition from an oil-continuous microemulsion system to a water-continuous microemulsion system.¹⁵

Viscosity

The viscosity values of all samples were low and relatively constant at 5 to 11 mPas. All samples exhibited Newtonian flow behavior, as expected from microemulsions.⁴ For the systems of 0% to 30% wt/wt water, the viscosity was 5.29 to 9.02 mPas, while that of 35% to 50% wt/wt water systems was 8.28 to 11.17 mPas. It could be noted that the viscosity values tended to increase slightly when the water concentrations increased or when the system turned into oil/water type because oil/water microemulsions have higher viscosities than those of water/oil systems.⁹ The slight increment of viscosity might be the result of interaction of microemulsion droplets in oil/water systems. It is expected that the hydrophilic chains of Brij 97 are strongly hydrated and connected with hydrogen bonds allowing the interaction between the droplets.⁵

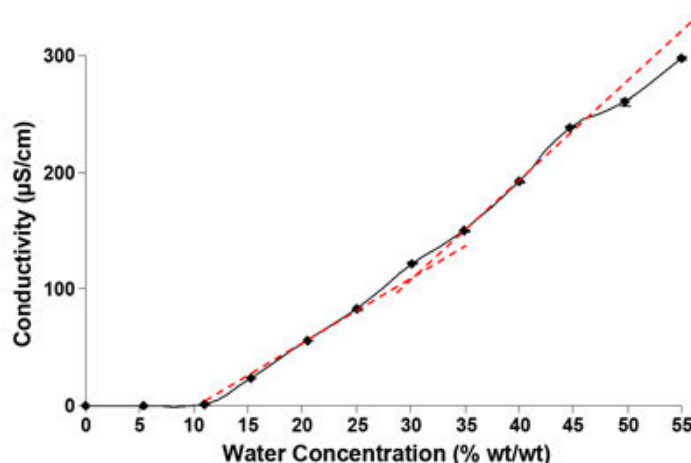


Figure 2. Conductivity as a function of the water concentration of the systems. The dotted line shows the tendency of changing in conductivity increment.

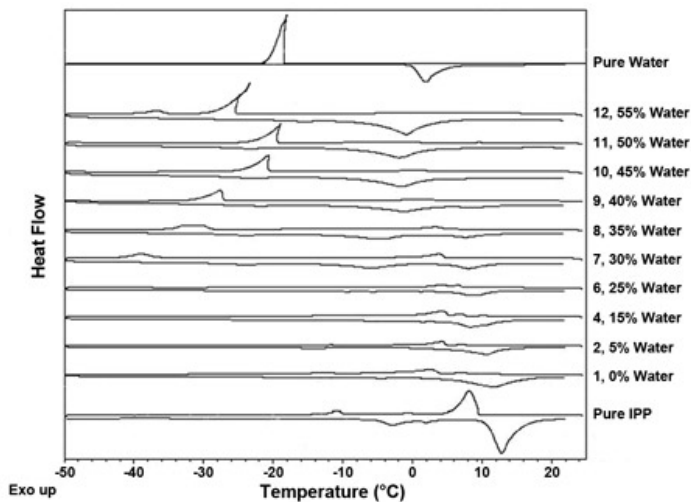


Figure 3. DSC curves of pure water, pure IPP, and IPP/water/Brij 97:1-butanol (2:1) microemulsions with various water concentrations.

Differential Scanning Calorimetry

When water is mixed into a microemulsion system, it can be either bound or free water depending on its state in the system, and differences in the melting and freezing behavior should be detectable with DSC measurements. The thermal behavior of water in the microemulsion systems

of this study was therefore investigated by DSC and compared with that of pure water (Figure 3).

Upon cooling, pure water showed a large exotherm at -18°C (freezing temperature) and the heating curve, showed an endotherm at 0°C (melting temperature). At water concentrations below 30% wt/wt, the freezing exotherm of water could not be observed. When the water fractions were increased, the exotherm became apparent in the cooling curve and gradually approached -18°C , the freezing point of pure water. Bulk (free) water is assumed to have similar physicochemical properties to those of pure water. For bound (interfacial) water, the presence of a nearby surface, however, will alter the thermodynamic properties such as freezing point, melting point, enthalpy, and heat capacity.^{5,16}

As the freezing exotherm of water in the IPP/water/Brij 97:1-butanol (2:1) systems could be observed in samples containing more than 30% wt/wt of water, it can be assumed that water changes from the internal pseudo phase into the external pseudo phase of the microemulsions at this point. It could also be noted that no samples showed a freezing exotherm at the same position of pure water (-18°C) and the partial miscibility of surfactants and 1-butanol with water is a likely reason for this.⁵

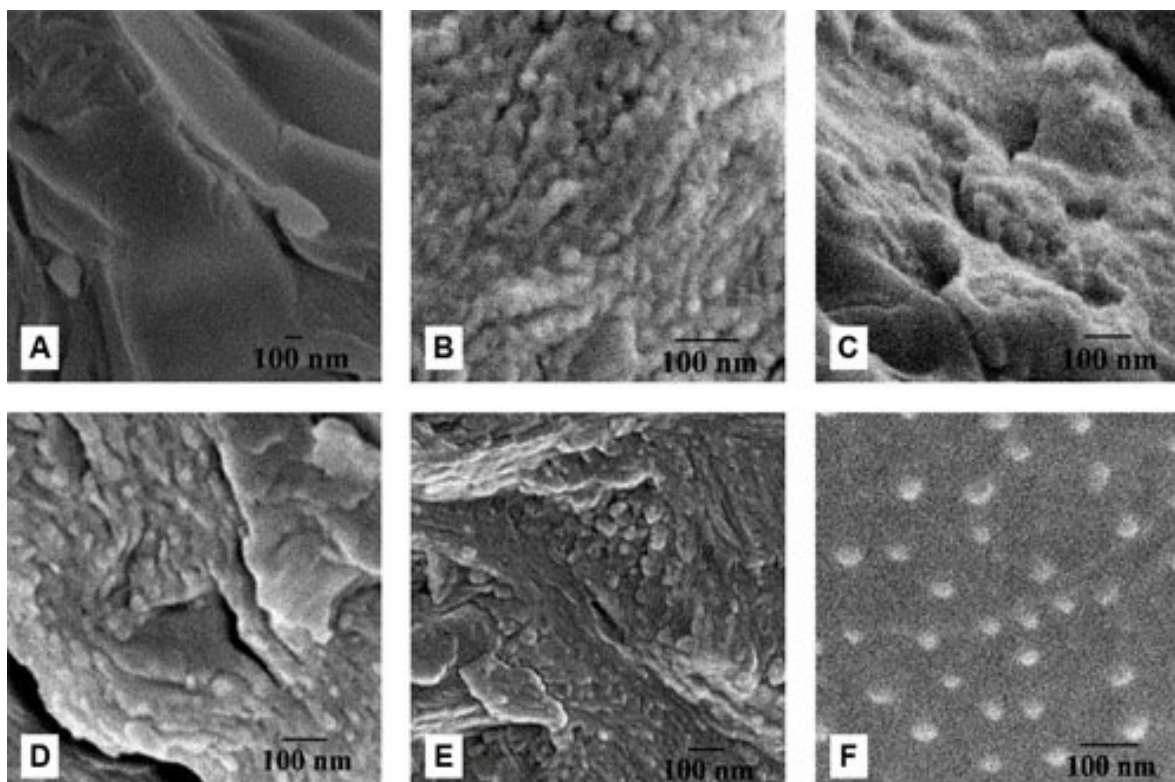


Figure 4. Cryo-FESEM micrographs of (A) sample 3 (10% wt/wt water), (B) sample 4 (15% wt/wt water), (C) sample 5 (20% wt/wt water), (D) sample 7 (30% wt/wt water), (E) sample 9 (40% wt/wt water), and (F) ice contamination artifact. Magnification is indicated on each photomicrograph.

Cryo-field Emission Scanning Electron Microscopy Photomicrographs

Cryo-FESEM photomicrographs of microemulsion samples prepared along the sampling path marked by the line on the pseudoternary diagram are shown in Figure 4. At water concentrations less than 15% wt/wt, no globular structures were detected (Figure 4A). Reversed micelles might have formed at low water concentration, but their size was too small to be detected. At water concentrations higher than 15% wt/wt, globular structures were observed as shown in Figures 4B-4E. These results implied that the droplet microemulsions were formed when the water concentration in the system was higher than 15% wt/wt; however, the microemulsion type (water/oil or oil/water) could not be differentiated in the cryo-FESEM photomicrographs.

Using this method requires careful interpretation since globular structures can also result from an ice contamination. Figure 4F is included for comparison and shows ice droplets align in a single direction on the surface of the sample rather than being embedded in the background structure, they are clearly different from those of microemulsions visualized in Figures 4B-4E.

Self-diffusion NMR

The self-diffusion coefficients measured for water, IPP, Brij 97, and 1-butanol in the microemulsion systems were compared with the self-diffusion coefficients measured for the pure components. The position of characteristic peaks

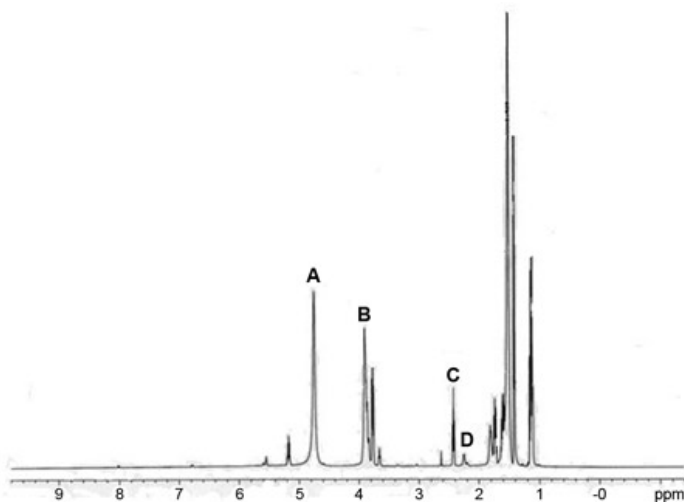


Figure 5. $^1\text{H-NMR}$ spectra of a sample composed of IPP, water, Brij 97, and 1-butanol. Symbols A, B, C, and D indicate characteristic peaks of water, 1-butanol, IPP, and Brij 97, respectively.

Furthermore, pure IPP showed freezing exotherm and melting endotherm at $\sim 8^\circ\text{C}$ and 12°C , respectively, indicating solidification and melting of IPP, respectively. In the systems of 0% to 25% wt/wt water, the IPP peaks could be observed and became smaller when the water concentration increased, owing to the dilution of IPP. The peaks disappeared at $\sim 30\%$ to 35% wt/wt water, suggesting that IPP changed from external to internal phase. Therefore, all DSC results indicate that transition from water/oil to oil/water would occur in the range of 30% to 35% wt/wt of water.

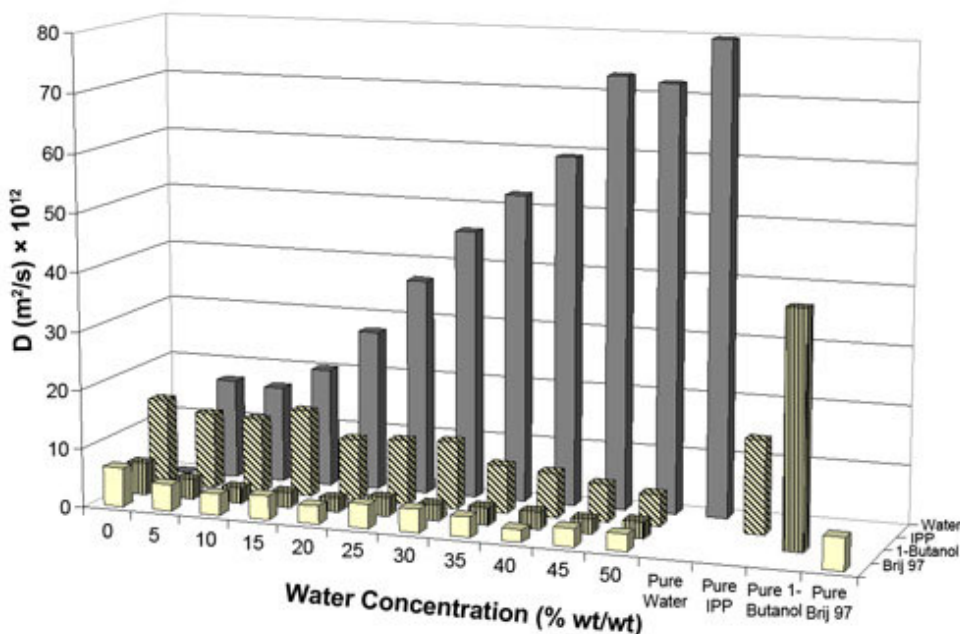


Figure 6. The self-diffusion coefficients of each component in microemulsion samples and in pure form.

for each component is shown in Figure 5. By comparing the self-diffusion coefficient of component in a sample with that of the single component, the microemulsion type can be identified.^{8,10,17,18}

If the microemulsion is of droplet-type, the self-diffusion coefficient of the internal pseudo phase is determined by the diffusion of the droplet and will therefore be slower than that of the pure components. In addition, the diffusion of the surfactant or surfactant mixture is also slow because of the formation of a monolayer around the droplet. In a bicontinuous microemulsion, where both oil and water are forming larger domains, the diffusion of these 2 components is high and often of the same magnitude as that observed for the pure components, while the surfactant is the slowest diffusing component.¹⁹

The self-diffusion coefficients of water and IPP in all samples were very low (10^{-11} - 10^{-12} m²/s range) as shown in Figure 6; therefore bicontinuous microemulsions were not likely to have formed. From the results of cryo-FESEM, droplet microemulsions did not originate at water concentration lower than 15% wt/wt. In sample 4 to sample 7 (Figure 1, 15%-30% wt/wt water), self-diffusion coefficients of IPP were in the range of 10^{-11} m²/s, and when the water concentration was increased in sample 8 to sample 11 (35%-50% wt/wt of water), self-diffusion coefficients of IPP decreased to the range of 10^{-12} m²/s. The self-diffusion coefficients of water did not change obviously; however, it could be noticed that the self-diffusion coefficients of water increased up to the range of 10^{-10} m²/s when the water concentration was higher than 35% wt/wt. Therefore, self-diffusion NMR measurements suggest the transition from water/oil to oil/water microemulsion type occurred at this point.

CONCLUSION

The results from different methods (ie, conductivity, viscosity, DSC, cryo-FESEM, and NMR) were useful to determine the type and colloidal structures of IPP/water/Brij 97:1-butanol (2:1) systems. In conclusion, the results from all techniques agree well and indicate that the systems containing less than 15% wt/wt water are likely to consist of reversed micelles. The systems containing between 15% and 30% wt/wt water were expected to be of the water/oil microemulsion type, while those containing more than 35% wt/wt water were expected to be of oil/water microemulsion type. The water/oil and oil/water transition point was in the range of 30% to 35% wt/wt water.

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