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SUB-ZERO TEMPERATURE BEHAVIOUR OF NON-IONIC MICROEMULSIONS IN THE PRESENCE OF PROPYLENE GLYCOL BY DSC

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Abstract

The five-component system is quite unique since it allows formation of reverse micelles with hydrophilic ethoxylated alcohol in the presence of ethanol and it facilitates dilution by water/propylene glycol (1,2-propanediol, *PG*) aqueous phase, all the way from a water-in-oil (*W/O*) microemulsion *via* a bicontinuous phase to an oil-in-water (*O/W*) microemulsion.

The surfactant/alcohol/*PG* can strongly bound water in the inner phase so that it freezes below –10°C and acts in part as 'bound' water and in part as 'non-freezable' water. Upon dilution to >30 mass% aqueous phase (water/*PG* at constant mass ratio of 1/1) the system becomes bicontinuous and the aqueous layers are composed again from bound water. Even after complete inversion to *O/W* microemulsions the water in the continuous phase is strongly interacting with the *PG*/surfactant and remains bound or non-freezable. Water/*PG*/ethanol have a strong effect on the head groups (freezing below –10°C) and also on the hydrophobic tails (recrystallizing and melting) at lower temperature when dilution exceeds 45 mass% water/*PG* (1/1).

No free water was detected neither in the *W/O* microemulsion's inner droplet domains nor when the microemulsion was either bicontinuous or when it was inversed to *O/W*. Continuous phase of resulting *O/W* microemulsion apparently is based on water/*PG* at a mass ratio of 1/1.

Keywords: differential scanning calorimetry, interphasal water, non-freezable water, non-ionic microemulsions, propylene glycol

Introduction

Microemulsions have attracted in the last decade, considerable attention in many applications such as cosmetics, foods, pharmaceuticals, pesticides and coating materials [1–3]. There is growing interest in microemulsions as vehicles for food formulations, since they can solubilize large amounts of lipophilic and hydrophilic food additives (functional food additives i.e., nutraceuticals and antioxidants), enhance reaction efficacy and allow selective extraction [2, 4].

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Some surfactant molecules are known to self-assemble in polar organic solvents like propylene glycol (1,2-propanediol), glycerol, and formamide [5–10]. These solvents like water, form hydrogen bonds, have relatively high dielectric constants and are immiscible with hydrocarbon solvents [6, 7]. Critical micelle concentrations are higher in polar nonaqueous solvents than in water [11, 12].

It is known that in water/poly(oxyethylene)-type non-ionic surfactant ($C_i(EO)_j$)/hydrocarbon systems, microemulsions invert from oil-in-water (*O/W*) to water-in-oil (*W/O*) when the temperature increases [13, 14]. It was observed, in some investigations, that the structural transition of microemulsions from *O/W* to *W/O*, occurs when mixtures of water and glycerol, or propylene glycol are employed instead of pure water [7, 10].

Recently, we found [15] that the addition of short chain alcohols (such as ethanol) and polyols (glycerol or propylene glycol), induce the formation of both water-in-oil (*W/O*) and oil-in-water (*O/W*) microemulsions. The phase behaviour of a food-grade system of R(+)-limonene, ethanol, water/*PG* (1/1) and poly(oxyethylene) sorbitan monostearate (Tween 60) or cosmetic grade system of poly(oxyethylene) (10) oleyl alcohol (Brij 97, $C_{18:1}E_{10}$) is characterized by a single continuous microemulsion region starting from a pseudo binary solution (surfactant/oil phase) to the microemulsion water/*PG* (1/1) corner [15]. This means that all of the structural changes occurring in the isotropic phase develop continuously (no phase transition takes place).

Four-component non-ionic microemulsion systems based on poly(oxyethylene)-type alkyl non-ionic surfactants were investigated by a sub-zero temperature DSC in our previous works [16–18]. The present study is focused on the investigation of the thermal behaviour of a unique Winsor IV type five-component microemulsion [15] at sub-zero temperatures. It was also interesting to explore the effect of water-soluble polyol such as propylene glycol on the thermal behaviour at sub-zero temperatures.

The properties of water in microstructured fluids can be significantly different from those in the bulk phase due to the interface contributions to its free energy [19–21]. Understanding the interactions between water and ‘oil’ at interfaces may help to gain some insight into the behaviour of water in biomolecular processes, food constituents [22–24] and confined spaces [25].

Differential scanning calorimetry (DSC) is widely used for studying low-temperature behaviour of surfactant-based multicomponent microemulsion systems [16–18, 26–28]. The operational (technique-dependent) classifications as suggested by Senatra *et al.* [29], differentiating between three types of water when describing the state of water in microemulsions, will be used in the present work. The differentiation is based on difference in melting (freezing) points of: (i) free water which melts at ~0°C; (ii) interphasal water, defined as water confined within the interface of the dispersed system (melts at about –10°C) [16, 17, 29]; (iii) bound water, which is associated to hydrophilic groups (melts at <–10°C). Note that interphasal and bound water are not necessarily distinguishable. The water that is not detected by DSC is termed ‘non-freezable’ water. Recently, the role of sub-zero temperature DSC (designated as SZT-DSC) in the investigation of the water behaviour in microemulsions was reviewed by Ezrahi *et al.* [17].

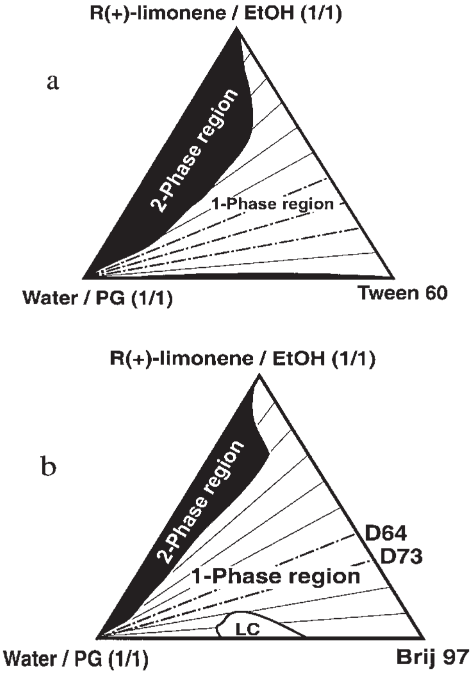

Fig. 1 Phase diagrams of the system: water/*PG*/R(+)-limonene/EtOH/ ethoxylated non-ionic surfactant at 25°C with constant mass ratio of water/*PG* (1/1) and a constant mass ratio of R(+)-limonene/EtOH (1/1). Ethoxylated surfactants used in these phase diagrams were: a – Tween 60; b – Brij 97

Water-soluble polyols such as propylene glycol (1,2-propanediol) and glycerol are cryoprotectants protecting biological systems from massive ice crystallization [30], since they lower the freezing point of water [30–34]. Menger and Rourk [35] developed low-temperature *O/W* non-ionic microemulsions based on $C_{12}E_{10}$, that resist freezing and phase separation at sub-zero temperatures by using mixtures of water and propylene glycol as the continuous phase. However, there was no attempt to fully describe the nature of water-polyol interaction on the microstructure of the system. In this study we are trying to explain the effect of water-propylene glycol hydrogen bonded complex on the thermal behaviour of both the head groups and the hydrophobic tails of the surfactant at the interface.

Experimental

Materials

Brij 97 (poly(oxyethylene) (10) oleyl alcohol, ($C_{18:1}(EO)_{10}$) was purchased from ICI, Specialty Chemicals (Essen, Germany). Tween 60 (poly(oxyethylene) (20) sorbitan monostearate), was commercial grade and obtained from Sigma Chemical Co. (St. Louis, Missouri, USA).

R(+)-limonene (98%) was purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Ethanol (EtOH) from Frutarom, Haifa, Israel, and propylene glycol, *PG* (1,2-propanediol, BDH, Poole, England) were used without any further purification. The water was double-distilled.

Methods

Phase diagrams

The five-component systems were described on a pseudo-ternary phase diagrams. They were constructed as reported recently at 25°C [15] and two systems are given in Fig. 1.

Calorimetric measurements

A Mettler TA 4000 thermal analysis system, equipped with a TC 11 TA processor and a DSC 30 low-temperature cell was used. The instrument was calibrated every two weeks with indium, lauric acid, water, and ethyl acetate to ensure accuracy of the caloric data. The heating rate of calibration was 10 K min^{-1}. The DSC measurements were carried out as follows: microemulsion samples (5–15 mg) were weighed, using a Mettler M3 microbalance, in standard 40 μL aluminum pans and immediately sealed by a press (Senatra and coworkers [29, 36]), and peaks representing various states of water were analyzed. The samples were rapidly cooled by liquid nitrogen at predetermined rate from ambient to –150°C (sometimes to –100°C) and then heated at 5°C min^{-1} back to ambient temperatures. An empty pan was used as a reference. The instrument determined the fusion temperatures of the solid components, and the total heat transferred in any of the observed thermal processes. The enthalpy changes associated with thermal transition were obtained by integrating the area of each pertinent DSC peak. DSC temperatures reported here were reproducible to ±0.5°C.

We followed the method used by Senatra *et al.* [29, 36] to identify various states of water in our systems. The contribution of the interphasal water is readily calculated by Eq. (1) see references [16–18]:

$$W_\text{I}=(\Delta H_\text{I}(\text{exp})/\Delta H_\text{I})100 \tag{1}$$

where W_I is the interphasal water concentration (in mass percent); $\Delta H_\text{I}(\text{exp})$ is the measured enthalpy contributions of interphasal water for the endothermic peak (~ –10°C); and ΔH_I is the heat fusion of interphasal water. We used as found by Senatra *et al.* [29, 36] the enthalpy: $\Delta H_\text{I}=312.28$ J g^{-1}. The same equation was used to calculate the mass percent of bound water (W_B). In a similar way, the concentration of free water is calculated, using Eq. (2) see references [16–18]:

$$W_\text{F}=(\Delta H_\text{F}(\text{exp})/\Delta H_\text{F}^\text{o})100 \tag{2}$$

where W_F is the free water concentration (in mass percent); ($\Delta H_\text{F}(\text{exp})$ is the measured enthalpy change for the 0°C peak, and $\Delta H_\text{F}^\text{o}$ is the heat fusion of pure water, measured at the same experimental conditions. We measured $\Delta H_\text{F}^\text{o}=327.17$ J g^{-1}. The amount of 'non-freezable' water (W_NF) was calculated using the material balance equation [17]:

$$W_{\mathrm{NF}}=W_{\mathrm{T}}-(W_{\mathrm{I}}+W_{\mathrm{B}}+W_{\mathrm{F}}) \qquad (3)$$

where W_{T} is the mass of total water.

Results and discussion

Phase diagrams

The pseudo-ternary phase diagrams for R(+)-limonene–ethanol–water–*PG*–Tween 60 or $C_{18:1}(EO)_{10}$ are shown in Fig. 1. It was found that short-chain alcohol (ethanol in food-grade systems) together with polyols (glycerol and propylene glycol) when added to a three component system (oil–surfactant–water) induce the formation of water-in-oil (*W/O*) that upon dilution inverts continuously to oil-in-water (*O/W*) microemulsions [15]. The phase behaviour of these systems is characterized by a monophasic region of the microemulsion starting from the pseudo binary surfactant-oil mixture and upon adding water/*PG* mixture, at a mass ratio of 1/1, can be diluted all the way to the water/*PG* corner. *PG* based microemulsions were found to be quite temperature insensitive systems [15]. The aqueous phase dilution line (see dilution line D64), investigated in this study, is indicated on the phase diagram in Fig. 1b.

DSC measurements

Endothermic events along the *PG*/H_2O dilution line

In order to understand the effect of the solubilized aqueous phase in the microemulsion, a binary mixture of water–*PG* was prepared with increasing *PG* content and its thermal behaviour was determined (Fig. 2). As expected, it was found that wa-

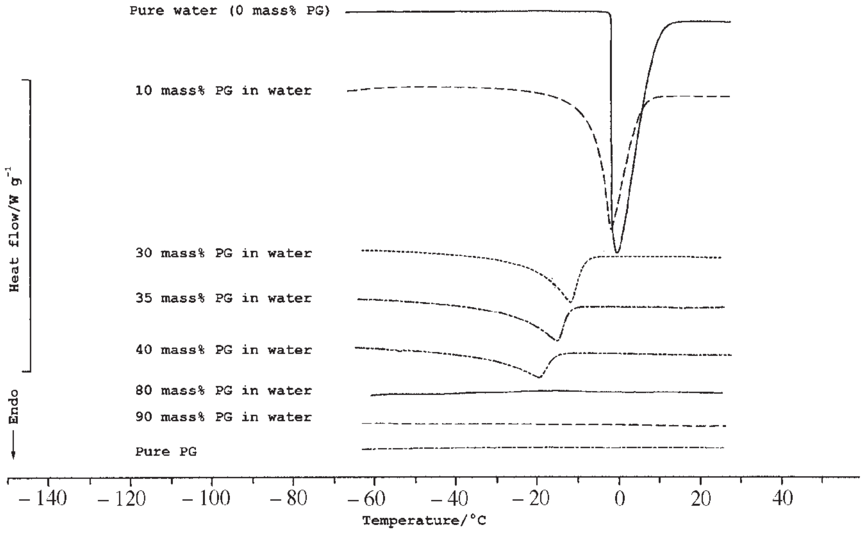

Fig. 2 Thermal behaviour of binary water–*PG* solutions of varying propylene glycol (*PG*) content

ter strongly binds to *PG* and the melting event (fusion temperature, T_{fusion}) of the binary system, containing 50 mass% *PG*, is lowered to –31.2±1°C.

As the *PG* content increases the amount of free water drops sharply (an increase of *PG* content in the binary system from 0.0 to 10.0 mass% leads to a 34.7% decrease in the free water concentration) and after 30 mass% of *PG* all the water was bound to the *PG*. Two types of *PG*–water interactions are detected in binary systems containing >30 mass% *PG*: 'non-freezable' and 'bound' water. As seen in Table 1, the non-freezable water increases while the bound or interphasal water drops. It should be noted that the binding of *PG* to water is so strong that free water is no more capable of freezing after adding 30 mass% *PG*, and after 80 mass% *PG* all the water in the system was non-freezable. The calculated molar ratio of non-freezable water to *PG* varies from 0.5 to 10.4.

Figure 3 summarizes the thermal behaviour of a system composed of R(+)-limonene, ethanol and poly(oxyethylene) (10) oleyl alcohol (Brij 97) at 1:1:3 mass ratio diluted with water/*PG* (as aqueous phase) from 0 to 50 mass% (dilution line D64 in the Fig. 1b).

Table 1 Thermal behaviour of the binary system water/*PG* at different *PG* content

PG/ mass%	T_{fusion}/ °C	Free water/	Bound water/	Interphasal water/	Non-freezable water/	Molar ratio non-freezable water/*PG*
		mass%				
0	0.00±1.00	100	–	–	0.0	0.0
10	–2.02	65.3	–	–	24.7	10.4
30	–11.27	–	–	31.2	38.8	5.5
35	–15.23	–	–	22.0	43.0	5.2
40	–19.60	–	16.0	–	44.0	4.7
50	–31.13	–	3.7	–	46.3	3.9
80	–	–	–	–	20.0	1.1
90	–	–	–	–	10.0	0.5

Figure 4 shows the thermal behaviour of microemulsion in which the aqueous phase was changed from 55 to 88 mass%, which is probably the region where bicontinuous or *O/W* microemulsions might be formed. The two endothermic events do not change practically. In all dilutions, the endothermic peaks are at –21.6±1.0°C and –33.0±1.0°C, suggesting that the microemulsion has only minor effects on the surfactant-water interaction. The intensity of the endothermic events becomes smaller due to the dilution effect (less surfactant is present at high water contents), but the both fusion temperatures (T_f's) do not change much. This indicates that the water/*PG* are at aqueous phase content of >55 mass% the continuous phase.

The surfactant (Brij 97) undergoes two different endothermic events (Fig. 5), the first peak occurring at ca –5.0°C (peak 1). The second occurring at 16.8°C (peak 2).

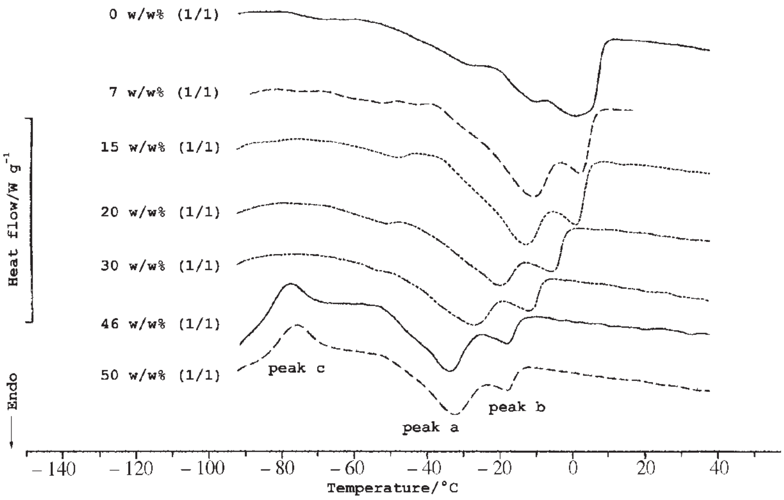

Fig. 3 Thermal behaviour of microemulsion prepared with Brij 97+R(+)-limonene+ethanol at a constant mass ratio of 3:1:1 (dilution line D64 in Fig. 1b) in the presence of various amounts (in the range 0–50 mass%) of the aqueous phase (water/*PG* at a mass ratio of 1/1)

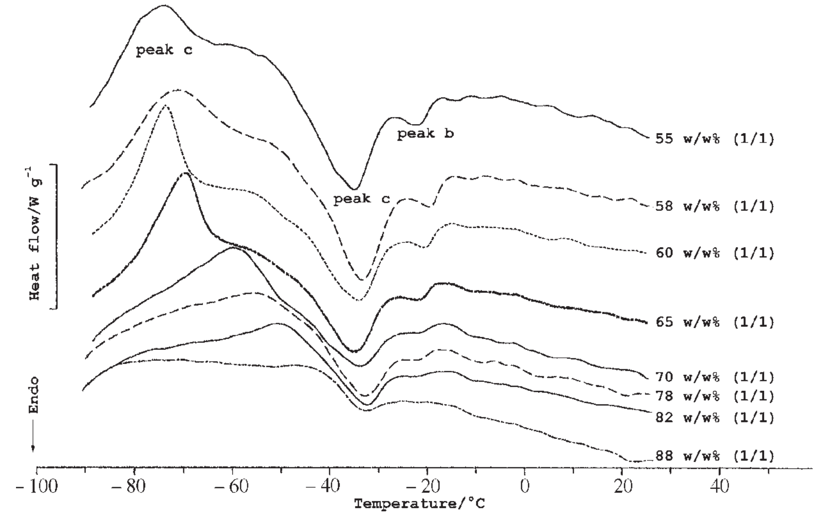

Fig. 4 Thermal behaviour of microemulsion prepared with Brij 97+R(+)–limonene+ethanol at a constant mass ratio of 3:1:1 (dilution line D64 in Fig. 1b) in the presence of various amounts (in the range 55–88 mass%) of the aqueous phase (water/*PG* at a mass ratio of 1/1)

It is important to stress that a blend of surfactant-water at 4:1 mass ratio melts at –16.64 and 4.93°C respectively. The surfactant, as expected, binds water to its hydroxyl and ether groups. The ethanol also binds to water and so does propylene

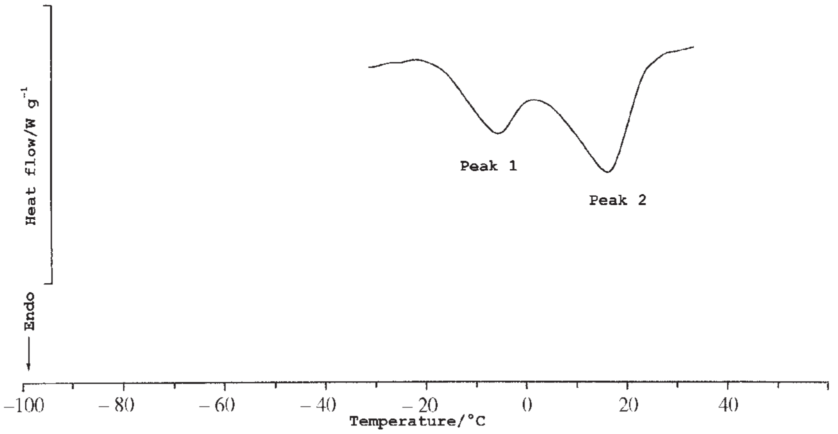

Fig. 5 Thermal behaviour of pure Brij 97

glycol. In both cases, the hydroxy groups reduce the melting point of the aqueous phase to lower temperatures (–5 to –15°C).

The microemulsion is a complex blend of five components – four of them strongly interacting between themselves *via* hydrogen bonds. The oil induces microstructure in the system and disturbs molecular-interactions and enhances interfacial associations of water to the *PG*, EtOH and the surfactant. Such interfacial association might also affect the 'organization' of the tails (fatty chains) of the surfactant.

Once a *W/O* microemulsion is formed, the endothermic peaks a and b (Fig. 3) are shifted to lower temperatures (lower melting point), with increasing the aqueous phase content (up to 50 mass%).

The fusion temperatures (T_f) of peaks a and b at their maximum onset in the microemulsion systems, as a fraction of the aqueous phase content, are presented in Fig. 6. The fusion temperatures of peaks a and b reach a plateau once the aqueous phase content is ca 46 mass%. At this *PG* content, in the binary water/*PG* system (Table 1), the endothermic peak appears at –31.2±1°C that is very close to the fusion temperature of the microemulsion at the plateau. It should also be noted that the increase in the aqueous phase content means also a relative decrease in the alcohol and the surfactant content and

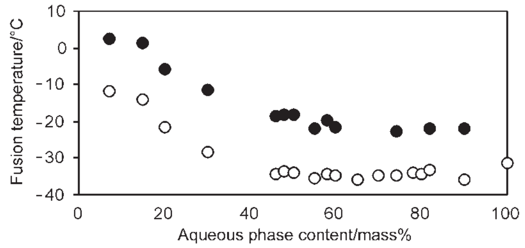

Fig. 6 Variation of the fusion temperatures of the endothermic peaks a – (o) and b – (•) (Figs 3 and 4) as a function of the aqueous phase content along the dilution line D64

a relatively higher *PG* fraction meaning lower fusion temperatures. It is well known that *PG* is associated to forming strong hydrogen bonds with water stronger than the surfactant and the ethanol water. Thus, the plateau in the fusion temperature beyond a certain amount of the aqueous phase (Fig. 6) in the system is related, most likely, to structural changes with the microemulsion. A transition from *W/O* microemulsion to *O/W* microemulsion occurs. This transition takes place once the aqueous phase becomes the continuous phase. We have shown, independently, by SD-NMR [37], conductivity [37], and viscosity [15] that an inversion to an *O/W* microemulsions occurs at 50–60 mass% aqueous phase along the dilution line D64.

Table 2 Variation of the molar ratio of non-freezable water to *PG* with the aqueous phase content along the dilution line D64

Aqueous phase content/mass%	Molar ratio non-freezable water/*PG*
50	3.2
55	3.3
58	3.5
60	3.6
65	3.6
70	3.8
74	4.0
82	4.0
88	4.1
90	4.1

In Table 2 we have summarized the results obtained for microemulsion systems, in which the aqueous phase composition contained 50 to 90 mass% of 1:1 mixture of PG and water. In all the samples the ratio of *PG* to water was kept constant. If all the *PG*/water would have been part of the continuous aqueous external phase the non-freezable water/*PG* molar ratio should have been 3.9, as found in the binary mixture (Table 1). However, one can see that only after reaching a 70 mass% of aqueous phase in the microemulsion this value was obtained, indicating that up to such dilution, part of the *PG* is present at the interface and thus does not bind water of all its capacity. Therefore, it seems that full inversion of the microemulsion from *W/O* to *O/W* takes place only once 70 mass% an aqueous phase has been used.

The exothermic events along the aqueous phase dilution line

The exothermic peaks (peaks type c) appearing at sub-zero temperatures in Brij 97 based system (Figs 3 and 4) is probably due to the so-called 'surfactant-related crystallization'. Ezrahi *et al.* [17] explained this phenomenon as a crystallization of the surfactant hydrocarbon (fatty) tail meaning an exothermic rearrangement of surfactant hydrophobic tail moieties into a more structured crystalline lattice. This crystallization is induced by heating the microemulsions in sub-zero temperature ranges. It can be seen from Fig. 7 that the temperature (T_{cry}) of exothermic peak (peak c) increases (i.e., it shifts to less negative values) as the aqueous phase content increases from 30.0 to 88.0 mass%. A possible interpretation is that the increase in the aqueous phase content leads to stronger *PG*/water interaction with the surfactant and the reorientation of surfactant molecules into crystalline structure will be more restricted so that higher temperatures will be needed. Similar behaviour was observed for the quaternary microemulsion system composed of Brij 97/water/butanol/dodecane with increasing the water content (system B in [17]), and in our ternary system water/*PG*/Brij 97 with increasing the aqueous phase. It was shown, for example, that diluting the system with solubilized water/*PG* (at a constant mass ratio of (1/1)) from 20 to 90 mass% increases the exothermic peak temperature (T_{cry}) in the ternary system from –60.09 to –40.08°C. It seems that once inversion occurs, the amphiphilic surfactant with its large head group has its hydrophobic fatty alcohol tails more tightly packed toward the core of the microemulsion which will lead to more restricted mobility and thus higher melting points for the tails (melting point of peak c in Figs 3 and 4). In addition, in the aqueous phase-rich region as water and *PG* interaction with the surfactant is enhanced, the mobility of surfactant molecules will be restricted and will result in retardation in reorganization of both Brij 97 and water, and inhibit the surfactant crystallization. It was reported that polyols such as *PG* and short-chain alcohols provide a ‘salting-in’ effect [10]. In our previous work [15], we found, from the phase behaviour of our five-component microemulsion systems, that a considerable fraction of *PG* molecules are incorporated into the surfactant layer and thus increase the interfacial fluidity and retard the surfactant crystallization, while other fraction of the *PG* molecules resides in the water and decreases the polarity of the water. It is known also that the resulting penetration of water-soluble polyols, such as *PG*, into the surfactant interface leads to flattening the interfaces and resulting in smaller or no liquid crystal phase regions [10, 11, 15].

The temperature difference between the exothermic peak (peak c) and endothermic peaks (peaks a and b) in Figs 3 and 4 decreases with increasing the aqueous phase content. For example, there is a 43°C difference between peaks a and c in microemulsion containing 46 mass% aqueous phase, and this difference was de-

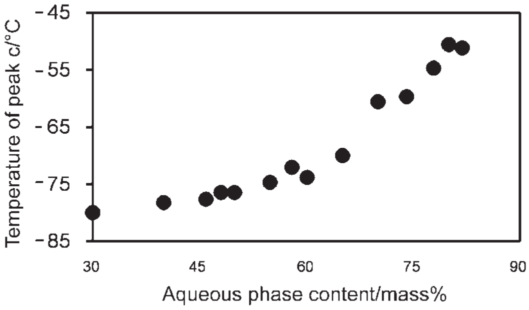

Fig. 7 Variation of the temperature of the exothermic peak (peak type c in Figs 3 and 4) as a function of the aqueous phase content along the dilution line D64

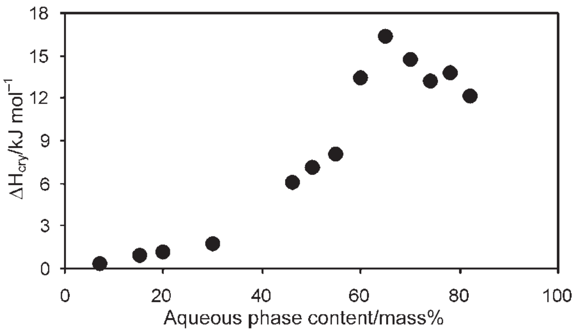

Fig. 8 Variation of the enthalpy of the exothermic peak (peak type c in Figs 3 and 4) as a function of the aqueous phase content along the dilution line D64

creased to 16°C in the microemulsion containing higher content of the aqueous phase (i.e., 80 mass%). The significant decrease in the temperature difference between exothermic and endothermic peaks could give us an indication to the strength of interaction of surfactant–water–*PG* in the system. The exothermic peak disappeared at high aqueous phase content (>88 mass%) as shown in Fig. 4. It is understood that at high aqueous phase content the freezing (crystallization) will be eligible due to the incorporation of *PG* into the surfactant and giving rise, to the appearance of 'non-freezable' water as will be seen. The change of the enthalpy of the exothermic peak with increasing the aqueous phase content along the dilution line D64 is given in Fig. 8. At low aqueous phase content *W/O* microemulsions are formed. The fatty chains are dangling in the oil phase (R(+)-limonene) and have relatively high degree of freedom. The fatty alcohol rearrangement from one polytype into another via a crystallization event needs at T_{cry} low ΔH_{cry} (0.7 kJ mol^{-1}) [38]. Upon increasing the aqueous phase content, the *W/O* microemulsion droplets have distorted and become more bicontinuous. This result in an increase in the ΔH_{cry} due to improved packing effect. However once an inversion occurs, the fatty alcohol tails are packed in a more restricted volume resulting in less mobility and lower possibility to rearrange in its melting and crystallization which shown a slight decrease in ΔH_{cry}.

Bear in mind that the dilute effect in the aqueous phase rich region, makes the estimations on the ΔH_{cry} more difficult and introduce large error effect.

State of water

A characteristic feature of DSC is its ability to distinguish clearly between water interactions with the surfactant and other ingredients at interfaces and in the bulk [17]. In the present study (Figs 3 and 4), no endothermic peak was found at 0°C at all aqueous phase contents, indicating that all the water is bound and there is no free water in the core of the *W/O* microemulsions and in the continuous aqueous phase of the *O/W* microemulsions at any aqueous phase content. Garti *et al.* [28] found that if the surfactant is very rich in water-binding groups (OH or ethylene oxides) all solubilized water in *W/O* microemulsion (based on ethoxylated poly(methylsiloxanes)) was confined in the vicinity of the interphasal region and no free water was detected in the aggregate core.

The present microemulsion systems are rich in water-binding functional molecules, such as EtOH, ethoxylated alcohol and *PG*, thus throughout all the dilution and solubilization stages (from inverse micelles, *W/O* microemulsion, bicontinuous phases, and direct micelles, *O/W* microemulsion) the water is strongly bound to any of these molecules or to all of them together. Bound water and interfacial water are detected at sub-zero temperature, but from calculations based on ΔH_f and the quantities of water added, it is clear that there is significant unaccounted quantity of water that was termed 'non-freezable' water. This indicates that the adduct 'water-polyol' does not freeze and thus does not thaw (melt). There is good correlation between the expected non-freezable water found in *PG*/water binary mixtures and the non-freezable water detected in diluted *O/W* microemulsion (above 70 mass%) indicating clearly that the aqueous phase (water plus *PG*) becomes the continuous phase.

From the intensities of the DSC peaks, the content of interphasal, and non-freezable water in Brij 97-based system microemulsions, along the dilution line D64 was plotted (Fig. 9).

It can be seen from Fig. 9 that the interphasal water content increases until it reaches a constant value, which have been evaluated from the experimental data to be 9.1±1.0 mass%. We may see that when the total concentration of water equals 9.1 mass%, virtually all the water in the microemulsion system is of the interphasal type. At 25 mass% aqueous phase (based on the total sample mass) non-freezable water begins to appear and its fraction increases steadily between 25 and 90 mass% total aqueous phase content while the concentration of the interphasal water remains almost constant at 7.5±2.0 mass% until somewhere between 55 to 58 mass% total aqueous phase. At this point the concentration of interphasal plus bound water begins to decrease (Fig. 9b). The decrease in 'interphasal' water content could be attributed as was suggested in our previously published works [16, 17, 39] to a corresponding decrease in the available total surface area due to microstructural changes.

The molar ratio of total water/surfactant (referred as W_0) was evaluated at the beginning of non-freezable water formation in two ways as was suggested by Garti *et al.* [16]. The total aqueous phase content when the non-freezable water begins to appear is between 20 to 25(±1.0) mass% and thus W_0 is about 9.5±1.0. A similar molar ratio was obtained when the non-freezable water curve intersected the abscissa in Fig. 9a. This curve is a straight line (r^2=0.9975) and the intersection occurs at total aqueous phase content of 18.2±1.0 mass% and thus W_0 is 7.6±1.0. So on the average about 9 water molecules are bound per surfactant molecule before non-freezable water begins to form. This means that in this region 9/10≅1 water molecule is bound then per ethylene oxide (*EO*) group.

It was reported that the existence of non-freezable water in ethoxlyated alcohol based systems depends on the length of their hydrophilic head group [17, 40]. Schulz and Puig [40] detected non-freezable water in the non-ionic binary water–$C_{12}(EO)_{23}$ and water–$C_{16}(EO)_{20}$ systems, where some water is assumed to be trapped in the helicoidal structure of the hydrophilic chain and the space for this trapped water is very restricted. Thus, the water-surfactant interaction may be strong enough to prevent the water from freezing.

In our previous work, we did not find any clear evidence for non-freezable water in quaternary non-ionic microemulsion systems based on $C_{12}(EO)_8$ and $C_{18:1}(EO)_{10}$. The short head groups of both surfactants do not form such coils and thus no non-freezable water is expected [17, 41]. This is due to the fact that the water-surfactant interaction is weak for uncharged non-ionic surfactant [17]. A different trend of behaviour at low temperatures was found in our system from those found in system B [17] in spite of the fact that both systems were based on the same surfactant. The non-freezable water is related to the presence of *PG* and possible to alcohol which strongly bind the water and prevents its melting at 0°C (as free 'bulk' water).

This work has a significant contribution to the understanding of the behaviour of water in strongly interacting systems where its mobility is restricted. This immobilization of the water induces strongly the mobility of the head groups and has an effect on the surfactant tails ability to rearrange and recrystallize.

Immobilized water in the core of a *W/O* microemulsion might effect the activity of the water and thus shows down its functionality in chemical and enzymatic free water. The strong interaction of the water continuous phase in *O/W* microemulsions

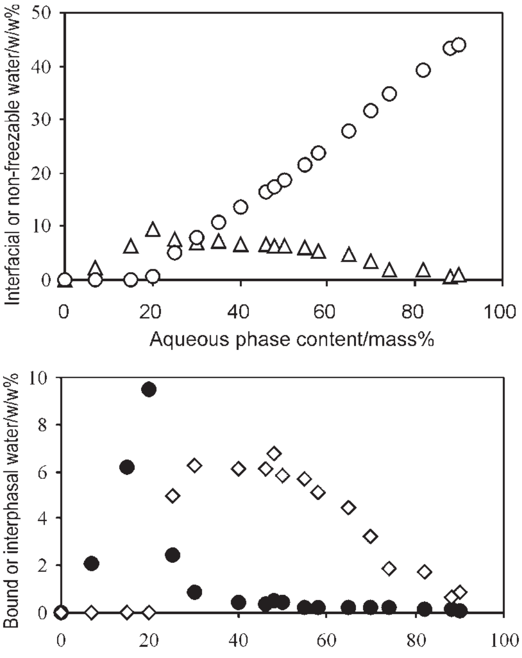

Fig. 9 Variation of 'non-freezable' (o) and 'interfacial' ('interphasal' plus 'bound') water (Δ) as a function of the aqueous phase content along the dilution line D64 (a). Variation of 'bound' and interphasal water along the dilution line D64. (◊) stands for bound water and (•) stands for interphasal water. Concentrations are calculated in mass percentage, relative to the mass of the microemulsion samples (b)

might have also an effect in reactions occurring in the continuous phase or at the interface of *O/W* microemulsions.

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