HIGH-PRESSURE CLOUD POINT DATA FOR THE SYSTEM GLYCEROL + OLIVE OIL + N-BUTANE + AOT

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Abstract - This work reports high-pressure cloud point data for the quaternary system glycerol + olive oil + n-butane + AOT surfactant. The static synthetic method, using a variable-volume view cell, was employed for obtaining the experimental data at pressures up to 27 MPa. The effects of glycerol/olive oil concentration and surfactant addition on the pressure transition values were evaluated in the temperature range from 303 K to 343 K. For the system investigated, vapor-liquid (VLE), liquid-liquid (LLE) and vapor-liquid-liquid (VLLE) equilibrium were recorded. It was experimentally observed that, at a given temperature and surfactant content, an increase in the concentration of glycerol/oil ratio led to a pronounced increase in the slope of the liquid-liquid coexistence curve. A comparison with results reported for the same system but using propane as solvent showed that much lower pressure transition values are obtained when using n-butane.
Keywords: Phase equilibria; N-butane; AOT surfactant; Glycerolysis.

INTRODUCTION

The use of monoacylglycerols (MAGs) in food, pharmaceutical and cosmetic industries has been continuously increasing over the past decade. Currently, MAGs are being produced on a large scale by chemical glycerolysis of fats and oils at high temperatures, typically in the range of 170-250°C, using inorganic alkaline catalysts (Kaewthong et al., 2005). Chemical catalytic processes however have several drawbacks such as the formation of dark-colored, burned tasting by-products; their energy intensive nature, low yields (30-40%) and the need for product post-purification by molecular distillation (Yang et al., 2005).

Attempts to synthesize higher quality monoacylglycerols with higher yield processes and to minimize energy costs have been made using lipases as catalysts, especially in non-aqueous media, in an increasingly employed standard approach to the development of strategies for organic synthesis (Kaewthong and Kittikun, 2004; Kaewthong et al., 2005; Yang et al., 2005; Habulin and Knez, 2001; Oliveira et al., 2004; Oliveira et al., 2005). In fact, several studies have shown that many enzyme-catalyzed reactions can be conducted in liquid or supercritical carbon dioxide, and in some cases, rates and selectivities achieved are greater than those obtained in normal liquid or gas phase reactions (Oliveira and Oliveira, 2000 2001).

Nonetheless, a serious drawback of those applications may arise from the non-polarity of carbon dioxide, which means non-proper dissolution of both hydrophobic and hydrophilic compounds

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(Knez and Habulin, 2002). Furthermore, the hydrophilic characteristics of carbon dioxide over wide pressure ranges may negatively affect the activity of the enzyme, as the water partitioning between the enzyme and reaction mixture may be a key factor in conducting enzymatic reactions (Knez and Habulin, 2002; Nakaya et al., 2001). Also, it should be considered that CO_2 is mildly Lewis acidic, interacting with Lewis basic sites (often carbonyl groups) in a solute. This interaction enhances the effective steric bulk, resulting in enhanced conformal rigidity of the protein/support (Rayner, 2007).

Nevertheless, other compressed gases also seem to be adequate for biocatalysis (Kao et al., 1997). Actually, the comparable dielectric constants of n-butane and carbon dioxide (Habulin and Knez, 2001; Chen and Yang, 2000) and the higher pressure phase transition values generally found in systems formed of carbon dioxide with higher molecular weight compounds (e.g. triglycerides) than those found with the use of n-butane (Ndiaye et al., 2006) support a firm belief that n-butane may also be suitable as reaction medium for enzyme-catalyzed bioconversions. It should also be taken into account that to conduct enzyme-catalyzed reactions at high pressures the enzyme behavior in compressed fluids is of primary importance. For example, Oliveira and co-workers (Oliveira et al., 2006a,b) investigated the effect of high-pressure treatment on the activity of immobilized lipases in compressed carbon dioxide, propane and n-butane and found that the enzymes utilized were much more active in propane and n-butane than in carbon dioxide. Besides, the use of n-butane offers the advantages of low separation costs and also solvent-free products when compared to higher homologue hydrocarbons liquid solvents at ambient conditions.

However, glycerolysis generally requires the use of surface-active agents due to the usually poor dissolution of both hydrophilic (glycerol) and lipophilic (triglyceride) substrates. Microemulsion systems offer many advantages as reaction media for biocatalysis because both hydrophilic and hydrophobic substances can be dissolved in high concentrations. Besides, reverse micelles (or water-in-oil microemulsions) have a relatively ordered structure; are characterized by a definite diameter; and provide an enormous interfacial area, which clearly favors lipase-catalyzed reactions, and it has been shown that lipases have high interfacial activity in micellar systems (Stamatis et al., 1999).

Microemulsions are thermodynamically stable, nanometer-size droplets dispersed in an organic phase stabilized by surfactant molecules. The majority of investigations with reverse micelles in biological systems has employed sodium(bis-2-ethyl-hexyl)sulfosuccinate (Aerosol-OT or AOT) as the surfactant (Stamatis et al., 1999; Bartscherer et al., 1995). This anionic surfactant has the ability to form reverse micelles in a great number of non-polar organic substances (e.g. oils) and several other polar solvents such as glycerol (Stamatis et al., 1999).

Recently, high-pressure phase equilibrium data for the quaternary system propane, AOT, glycerol and olive oil, was reported for a wide temperature range and pressures up to 30 MPa (Benazzi et al., 2006). The major objective was to investigate the phase behavior of such systems formed with potential alternative solvents in order to help elucidate the reaction mechanism as well as for a rational process design of lipase-catalyzed glycerolysis in compressed fluids.

The present report is part of a broader project aiming at developing new processes for the production of emulsifiers through enzyme-catalyzed glycerolysis reactions. Here, the aim is to report phase equilibrium (cloud point) experimental data on a glycerolysis reaction system composed of n-butane, AOT, glycerol and olive oil in the typical temperature range of enzymatic reactions, 303 K to 343 K. The effect of surfactant addition and glycerol/olive oil concentration in pressures up to 27 MPa was evaluated.

MATERIALS AND METHODS

Materials

N-butane (99.5 mol% purity) was supplied by White Martins S.A.; AOT surfactant (99 wt%) was purchased from Sigma-Aldrich and glycerol (99 mol%), from Nuclear. The olive oil (Otoyan) was purchased from a commercial establishment and was used without any additional treatment. The oil was stored in a dry place and protected against light before use. The fatty acid composition of the vegetable oil employed in this work is given elsewhere (Oliveira et al., 2005).

Apparatus and Experimental Procedure

Phase equilibrium experiments were conducted employing the static synthetic method in a high-pressure variable-volume view cell. The experimental apparatus and procedure have been used in a variety of studies (Corazza et al., 2003;

Franceschi et al., 2004; Ndiaye et al., 2006; Benazzi et al., 2006). Briefly, the experimental setup consists of a variable-volume view cell with two sapphire windows for visual observation, an absolute pressure transducer (Smar LD 301) with a precision of ±0.03 MPa, a portable programmer (Smar, HT 201) for pressure data acquisition and a syringe pump (ISCO 260D). The equilibrium cell contains a movable piston, which permits pressure control inside the cell. Phase transitions were obtained visually through pressure manipulation using the syringe pump and the solvent as pneumatic fluid.

Initially, a precise amount of the solute was weighed on a precision balance (Gilbertini E254 with 0.0001 g accuracy) and loaded into the equilibrium cell. The cell was then flushed with low-pressure n-butane to remove any residual air. A known amount of n-butane was added with the syringe pump (resulting accuracy of ± 0.005 g in n-butane loadings). Based on the uncertainty in n-butane loading and the weighing of other compounds, we estimate that the uncertainty in global concentrations of the mixtures is lower than 0.005 % on a weight basis. Then the cell content was kept under continuous agitation with the help of a magnetic stirrer and a Teflon-coated stirring bar. The temperature control was then turned on, and once the desired temperature was reached, controlled within 0.5 K; the pressure system was increased until a one-phase system was observed in the cell. At this point, the system was maintained at least 30 min to allow stabilization, and then the pressure was decreased slowly (typically at a rate of 0.1-0.3 MPa/min) until

incipient formation of a new phase. This procedure was repeated at least three times for each temperature and global composition.

RESULTS AND DISCUSSION

In order to study the quaternary system n-butane + AOT + glycerol + olive oil, the glycerol/olive ratio oil was kept fixed at the stoichometric value of the glycerolysis reaction, i.e. 2:1 on a molar basis. Results for the quaternary system at 5 wt% and 7.5 wt% of AOT in n-butane (glycerol/olive oil free basis) are presented in Tables 1 and 2, respectively. For both surfactant compositions, the occurrence of coexistence curves for biphasic, LLE and VLE, and also three-phase, VLLE can be observed.

Figures 1(a) and (b) contain pressure-temperature diagrams for the AOT concentrations investigated at glycerol/olive oil overall compositions of 20 wt% and 25 wt%, respectively. It can be seen from Figure 1(a) that an increase in AOT concentration results in a decrease in pressure transition values to such an extent that, in contrast to VLE, LLE and VLLE, only VLE is registered at higher AOT content. Also, one can observe in Figure 1(b) that the addition of AOT to n-butane shifts the LL coexistence curves to higher temperatures, resulting in a broader region of homogeneous one-bulk phase. Besides, comparison of these two figures shows that a rise in glycerol/oil content at a constant AOT concentration, either 5 wt% or 7.5 wt%, can cause a change in phase behavior with at least a dramatic modification of cloud point values.

**Table 1: Experimental phase equilibrium data for the system AOT/n-butane(1) + glycerol/olive oil(2)
at an AOT mass fraction of 0.050 in n-butane (glycerol/olive oil free basis).
Glycerol/olive oil molar ratio fixed at 2:1.**

T [K]	Pressure [MPa]	σ [Mpa]	Transition type	T [K]	Pressure [MPa]	σ [MPa]	Transition type
$w_2^* = 0.102$				$w_2 = 0.250$			
303	0.72	0.02	VLE	303	0.50	0.01	VLE
313	0.80	0.01	VLE	305	1.33	0.06	LLE
323	0.95	0.03	VLE	305	0.51	0.02	VLLE
333	1.03	0.02	VLE	308	10.09	0.02	LLE
343	1.24	0.04	VLE	308	0.55	0.01	VLLE
$w_2 = 0.152$				310	18.21	0.05	LLE
303	0.65	0.02	VLE	310	0.57	0.04	VLLE
308	0.72	0.03	VLE	313	0.59	0.01	VLLE
313	0.77	0.01	VLE	323	0.69	0.02	VLLE
323	0.88	0.01	VLE	333	0.82	0.02	VLLE
328	0.96	0.03	VLE	343	1.09	0.03	VLLE
333	1.00	0.04	VLE	$w_2 = 0.396$			
338	1.06	0.01	VLE	303	0.81	0.03	VLLE
339	1.24	0.04	LLE	313	0.88	0.06	VLLE
339	1.04	0.04	VLLE	323	1.06	0.05	VLLE
340	2.11	0.04	LLE	333	1.08	0.01	VLLE
340	1.09	0.02	VLLE	343	1.27	0.04	VLLE

Continuation Table 1

T [K]	Pressure [MPa]	σ [Mpa]	Transition type	T [K]	Pressure [MPa]	σ [MPa]	Transition type
343	3.30	0.04	LLE				
343	1.12	0.01	VLLE	$w_2 = 0.594$			
$w_2 = 0.200$				303	0.79	0.01	VLLE
				313	0.91	0.01	VLLE
303	0.61	0.02	VLE	323	0.99	0.04	VLLE
308	0.58	0.02	VLE	333	1.08	0.02	VLLE
313	0.69	0.03	VLE	343	1.26	0.04	VLLE
318	0.99	0.04	LLE				
318	0.72	0.01	VLLE				
320	3.44	0.02	LLE				
320	0.74	0.04	VLLE				
323	7.81	0.04	LLE				
323	0.78	0.02	VLLE				
328	16.48	0.06	LLE				
328	0.88	0.03	VLLE				
330	20.33	0.03	LLE				
330	0.90	0.02	VLLE				
333	0.95	0.02	VLLE				
343	1.02	0.03	VLLE				

[*]w_2 stands for glycerol + olive oil overall mass fraction

Table 2: Experimental phase equilibrium data for the system AOT/n-butane(1) + glycerol/olive oil(2) at an AOT mass fraction of 0.075 in n-butane (glycerol/olive oil free basis). Glycerol/olive oil molar ratio fixed at 2:1.

T [K]	Pressure [MPa]	σ [Mpa]	Transition type	T [K]	Pressure [MPa]	σ [MPa]	Transition type
$w_2 = 0.201$				$w_2 = 0.305$			
303	0.81	0.02	VLE	303	0.81	0.01	VLE
308	0.85	0.01	VLE	308	0.85	0.01	VLE
313	0.91	0.02	VLE	310	3.58	0.02	LLE
318	0.97	0.01	VLE	310	0.79	0.02	VLLE
323	1.01	0.01	VLE	313	13.93	0.02	LLE
328	1.08	0.02	VLE	313	0.81	0.01	VLLE
333	1.15	0.02	VLE	318	0.83	0.01	VLLE
338	1.21	0.01	VLE	323	0.85	0.02	VLLE
343	1.30	0.04	VLE	328	0.89	0.01	VLLE
$w_2 = 0.249$				333	1.04	0.02	VLLE
303	0.55	0.01	VLE	338	1.12	0.02	VLLE
308	0.63	0.02	VLE	343	1.20	0.02	VLLE
313	0.70	0.02	VLE	$w_2 = 0.402$			
318	0.78	0.01	VLE	303	0.99	0.03	VLLE
323	0.83	0.01	VLE	308	1.02	0.02	VLLE
328	0.88	0.01	VLE	313	1.06	0.06	VLLE
331	1.07	0.02	LLE	318	1.09	0.08	VLLE
331	0.88	0.02	VLLE	323	1.13	0.05	VLLE
333	3.82	0.06	LLE	328	1.15	0.03	VLLE
333	0.90	0.01	VLLE	333	1.18	0.06	VLLE
338	9.90	0.05	LLE	338	1.21	0.04	VLLE
338	0.96	0.02	VLLE	343	1.25	0.04	VLLE
343	17.49	0.07	LLE				
343	1.01	0.01	VLLE				

To help visualize the experimental data presented in Tables 1 and 2, Figures 2(a) and (b) depict the pressure-temperature diagram for the system investigated at selected mass fractions of glycerol/ olive oil. As aforementioned, cloud point values are very sensitive to small variations in solute or solvent contents, considerably altering the phase equilibrium diagram. Accordingly, phase behavior together with other aspects, like enzyme activity and stability in compressed fluids (Oliveira et al., 2006a,b), will, of course, play a crucial role in determining the appropriate process parameters for conducting enzyme-catalyzed glycerolysis reactions.
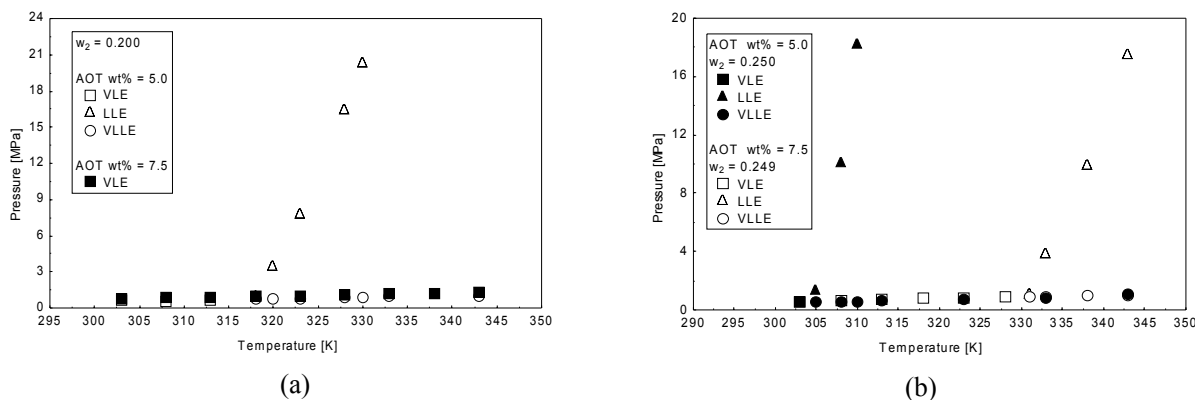
Figure 1: Pressure-temperature diagram for the system AOT/n-butane(1) + glycerol/olive oil (2) at the two AOT concentrations: a) glycerol/olive oil overall mass fraction of 0.200; b) glycerol/olive oil mass fraction of 0.250.
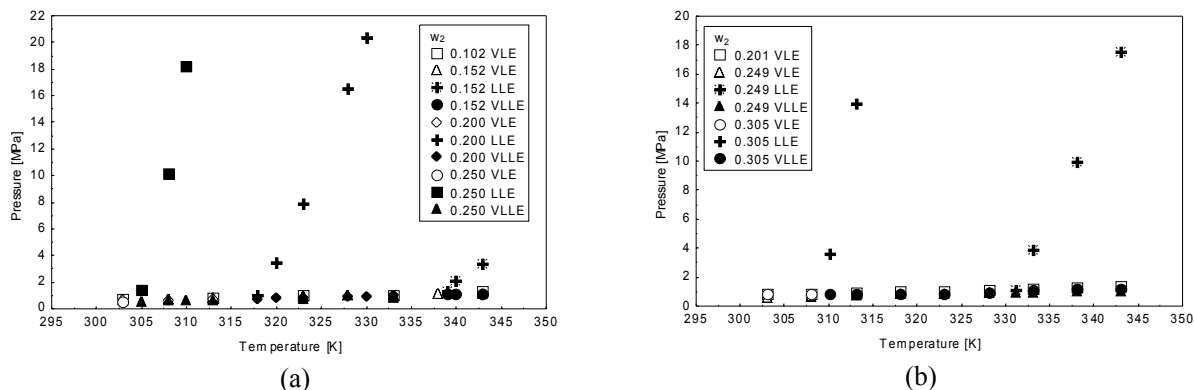

Figure 2: Pressure-temperature diagram for the system AOT/n-butane(1) + glycerol/olive oil(2) at selected mass fractions of glycerol/olive oil (molar ratio of 2:1): a) 5 wt% of AOT in n-butane (glycerol/olive oil free basis); b) 7.5 wt% of AOT in n-butane (glycerol/olive oil free basis).

Figures 3(a) to (c) provide a comparison of data obtained in this work with those reported by Benazzi et al. (2006) for the same system, except that instead of n-butane, propane was the solvent used. Inspection of Figure 3(a) (glycerol/olive oil overall mass fraction of approximately 0.100 and AOT mass fraction of 0.050) reveals that a completely different phase behavior was found for n-butane with only VLE phase transitions being observed in a wide temperature range. As immobilized enzymes are generally preferred in industrial applications (reutilization, easy recovery and recycling), this phase behavior may be of technological relevance if one intends to conduct the reaction in a one-phase system, allowing proper interaction between substrates and enzyme particles (in fact a solid-fluid medium) (Laudani et al., 2007). Additionally, it seems unnecessary in this case to emphasize that much lower pressure transition values are found for n-butane than for propane.

Figures 3(b) and (c) show that a rise in glycerol/oil mass fraction at a given AOT concentration results in the appearance of VLLE and LLE coexistence curves at lower temperatures. It may also be interesting to observe that the slope of

the LLE curve becomes more abrupt as glycerol/olive oil content is enhanced, which means that higher pressures must be applied to attain a one-phase system (homogeneous region).

In Figure 4 the phase behavior of the quaternary system AOT/solvent + glycerol/olive is shown for a glycerol/olive oil overall mass fraction of 0.200 and for 10 wt% and 5 wt% of AOT in propane and n-butane, respectively. In principle, it seems advantageous to conduct the enzymatic glycerolysis using n-butane as solvent, as lower pressures may be used along with a smaller amount of AOT in a wide temperature range, which may result in reduced capital equipment and energy (separation) costs. However, according to this figure, judgment of this question should also take into account the type of enzyme and its behavior in the pressurized fluid. For instance, Novozym 435 would be chosen over Lipozyme IM and n-butane instead of propane on the basis of enzyme behavior in the (pure) compressed fluid (Oliveira et al., 2006). On the other hand, if the temperature for achieving the optimal enzyme activity is considered, Lipozyme IM (40 °C), and not Novozym 435 (70 °C), could be the choice (Oliveira et al., 2005), as prohibitive pressures would be necessary.
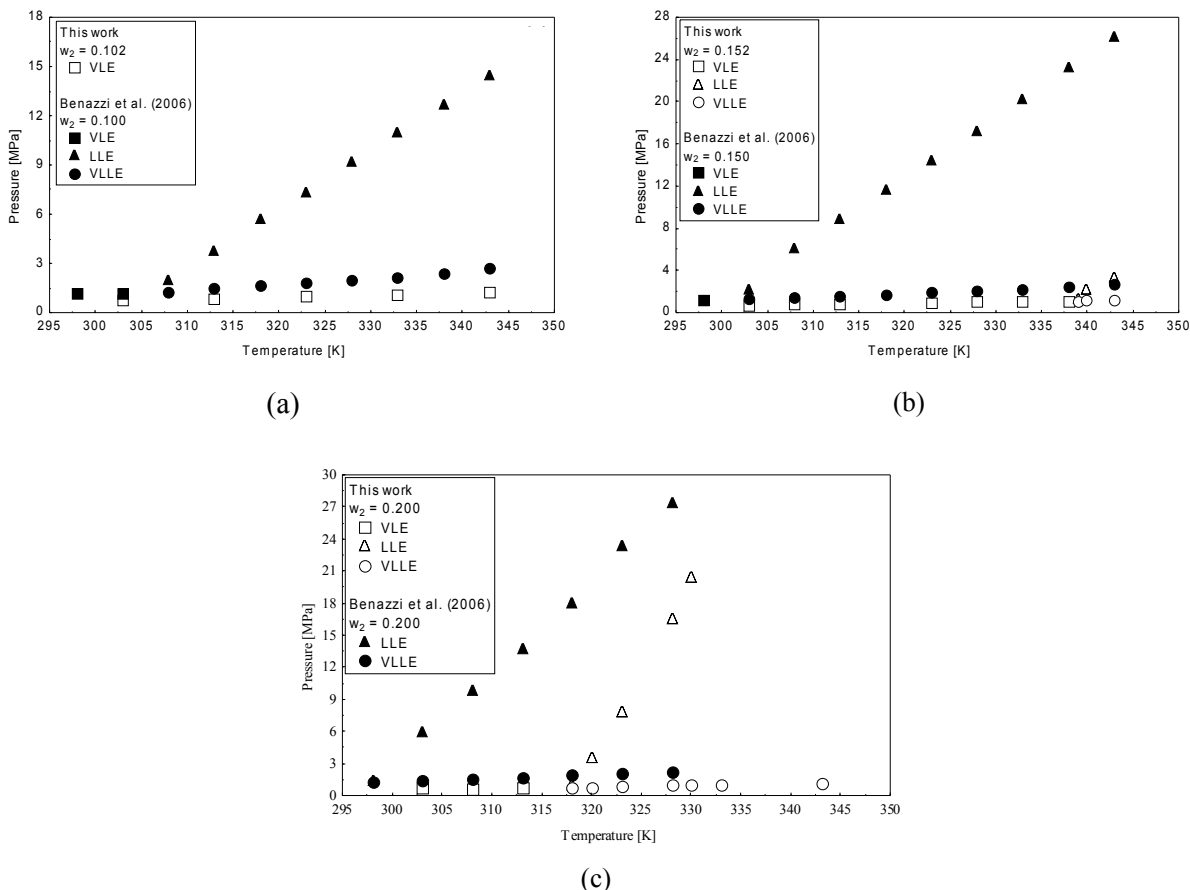
(a)   (b)

(c)

Figure 3: Effect of solvent on phase equilibrium for the system AOT/solvent(1) + glycerol/olive oil(2):
a) glycerol/olive oil overall mass fraction of approximately 0.100; b) glycerol/olive oil overall mass
fraction of approximately 0.150; c) glycerol/olive oil overall mass fraction of 0.200. Glycerol/olive
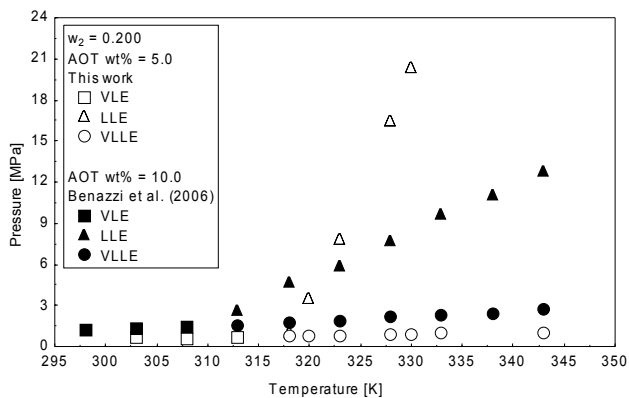oil molar ratio of 2:1 and 5 wt% of AOT in solvent (glycerol/olive oil free basis).

Figure 4: Comparison of AOT/solvent + glycerol/olive oil phase behavior at glycerol/olive oil
overall mass fraction of 0.200 and 10 wt% and 5 wt% of AOT in propane and n-butane,
respectively. Glycerol/olive oil molar ratio fixed at 2:1.

CONCLUSIONS

Phase equilibrium measurements for the system containing n-butane, AOT, glycerol and olive oil were reported in this work. Commonly found LLE, VLE and VLLE were experimentally observed within the variable ranges investigated. Pressure transition values were found to be very sensitive to changes in surfactant and substrate contents. Comparison with the quaternary system using propane showed that the use of n-butane might be advantageous depending on the experimental condition. The experimental data reported here might be relevant for conducting enzyme-catalyzed glycerolysis in an alternative medium composed of a compressed fluid in the presence of AOT surfactant.

NOMENCLATURE

Latin Letters

AOT	sodium(bis-2-ethyl-hexyl)sulfosuccinate	Aerosol-OT
P	pressure	MPa
T	temperature	K
w_i	mass fraction of component (or mixture of components) *i*	(-)
LLE	liquid-liquid equilibrium	(-)
VLE	vapor-liquid equilibrium	(-)
VLLE	vapor-liquid-liquid equilibrium	(-)

Greek Letters

σ	experimental error (standard deviation calculated from replicated measurements)	(-)

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