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Effects of the Biomolecules: Vitamins, Proteins, Amino Acids, and Surfactants: DTAB, MTOAC, TMSOI, Orcinol on Upper Critical Solution Temperatures

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Abstract
Upper critical solution temperatures ($UCST_S \pm 0.05$ K) and mutual solubilities of phenol + water systems are reported separately with 0.5 millimol kg^{-1} (mm kg^{-1}) proteins (casein, pepsin, egg-albumin), vitamins (B_1-thiamine, B_2-riboflavin, B_6-pyridoxine), amino acids (glycine, β-alanine, L-leucine) and surfactants (dodecyl trimethylammonium bromide-DTAB, trimethylsulphoxonium iodide-TMSOI, methyltrioctylammonium chloride-MTOAC, orcinol). The additives decrease the UCSTs by about 0.50-2 ^{0}C with slight enhancement in mutual solubilities but the leucine with two $–CH_3$ (methyl) and two $-CH_2-$ (methylene) groups produce negligible increase in the solubilities. The $–CH_3$ and $-CH_2-$ groups develop stronger hydrophobic interactions but the glycine develops stronger hydrophilic interactions due to $–N^+H_3$ (amino) and $–COO^-$ (carboxylic) groups and weaker hydrophobic due to single $–CH_2-$ group. The leucine increases the solubility by 0.009 mole fractions with a 0.7 ^{0}C decrease in USCT as compared to phenol-water. The mole fractions of additives restricted to 0.002 to 0.005 range, the conjugations of casein and vitamins in place of $–CH_3$ groups of amino acids enhance the solubility with lower UCST values.

Keywords: DTAB, TMSOI, critical solution, surfactant, hydrophilic interaction, hydrophobic interaction, upper critical solution temperature.

1. Introduction

Upper critical solution temperature (UCST) is a temperature below and above of which the two phase liquid system develops a single phase liquid. It is a system dependent parameter hence for a particular system like water and phenol its value is fixed and due to its nature. It is used as an effective yardstick for additives added to water and phenol and their UCST data are determined. The variation in UCSTs with an additive elucidates structural interactions in our systems. We have chosen vitamins, proteins and amino acids these molecules which are an important part of our body are referred to as biomolecules.

Similarly the DTAB, MTOAC, TMSOI and orcinol interact with water as well as phenol due to their hydrophilic and hydrophobic parts respectively, and hence are denoted as surfactants and reduce interfacial surface tension of immiscible phases and facilitate intermixing at lower UCST. Hydrophilic and hydrophobic interactions are always of interest due to several (Singh and Singhal, 2006) uses in nanotechnology, biotechnology and microscale processes. Ample studies on them at NTP are reported in the literature but no work in critical solutions is cited. Our work focuses on them in upper critical solutions using three different categories of biomolecules and a single category of cationic surfactants along with orcinol. However four different series were studied elsewhere where the 1st series consists mono-, di- and trivalalent salts; the 2nd carboxylic acids (mono, di and tribasic); the 3rd surfactants (CTAB), TEAB, CPC, CPB, LDS, SDS, CA, PEG 200, EGMDE and the 4th polynuclear aromatic compound: benzene, naphthalene, anthracene, chrysene, toluene, xylene (derivatives) (Singh, 2006a). Such study is highly recommended for green and supramolecular chemistries (Singh and Singhal,

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2006) as to how the latter molecules influence the mutual solubilities that would curtail an excess use of materials to develop microscale and miniaturized processes.

Further the proteins, amino acids and vitamins are frequently used in several solubility based processes (Singh, 2001; Borghesani et al 1986; Cascella, et al, 1990) like separation of proteins from their natural sources where the UCSTs are useful. The DTAB, TMSOI and MTOAC cationic surfactants and orcinol are highly used in biosciences for several purposes so their influence on solubility of solvents assists the separation and extraction processes. The UCSTs are industrially useful (Cascella et al 1990; Zhang et al 2007) to develop the proteins, amino acids and vitamins based bionanoparticle for biochips and DNA biotemplates technology (Lagi, et al 2007; Bini, et al 2007; Misra 1999; Fried et al 1977; Goodwin et al, 1979). The critical solutions with proteins and vitamins, amino acids and surfactants are useful for cloud point temperatures especially food scientists and biophysical scientists (Singh and Kumar 2006; Singh, 2006c; Singh et al 2007; Borghesani et al 1986). Temperature-dependent surfaces and interfacial kinetics remain relatively unexploited in thin-film sensing applications that rely on optical surface-sensitive techniques like surface plasmon resonance spectroscopy. Currently Singh has reported Survismeter and formulated the Man Singh equation for friccohesity that focuses on interfacial surface forces which influence the intermixing of the immiscible phases (Singh, 2006b; Singh, 2006c). These techniques are inherently sensitive to the optical properties of the bulk solution in contact with the thin films, hence UCST data for the latter at the conditions of interests are useful. The UCST and the refractive index may have interesting correlations.

The UCSTs with a wide range of additives would serve a wider purpose in solution engineering of immiscible solvents for interactions with industrially useful molecules. The UCSTs with ionic, hydrophilic and hydrophobic interactions define cloud points with additives of thermodynamic significance with potential uses in soaps, detergents, textiles, inks, paint and pigments, solvent extractions, and disinfectant solutions. Industrially phenol is widely used as a disinfectant where its solubility in different solutions is highly influenced by the ingredients of the solution. Its aqueous solutions with proteins, amino acids and vitamins could be of some use in biotechnology and biophysics as it is used to inhibit the early degradation of fish meat etc. Similarly the surfactants are chosen for study to investigate their influence on the intermixing of the phenol-water phases due to their hydrophilic and hydrophobic interactions. Here UCST is taken as a standard method to determine an influence of the additives on mutual and critical solubilities of the phenol-water systems.

2. Materials and Methods

2.1 Solutions Preparations

Stock solutions of 0.5 mm kg^{-1} of vitamins (B_1, B_2, B_6), proteins (casein, pepsin, egg-albumin), amino acids (glycine, β-alanine, L-leucine) and surfactants (DTAB, TMSOI, MTOAC, orcinol) were prepared separately in ultra pure water, w/v. Initially water and phenol in a 2:8 ratios were taken in optical cells as reference phases, the cell was mounted on a stainless steel stand at normal temperature. The cell with contents was remained dipped in a paraffin oil of the thermostat with ± 0.05 ^{0}C control measured with a Beckmann thermometer. The contents were heated at a rate of 1 ^{0}C min^{-1} stirring with a glass stirrer 50 rpm till the phases disappeared where their miscibility increased and approaching to a point where both the components are completely dissolved with a homogenous single phase. This temperature is termed a mutual miscibility temperature (MST). The heating was stopped and the cell with contents was air cooled for reappearance of the phases. The reappearance and disappearance of the phases develop a cloud hence the temperature at which the cloud is developed is called a cloud point temperature it coincides with the MST. Hence the MSTs for disappearance of a cloud point or turbidity and a reappearance of the cloud point were recorded. About 30 subsequent additions of the $2x10^{-3}$ dm^3 water were made and the MSTs were recorded.

After the water-phenol system, the cell was cleaned and rinsed with the solution in question for cloud point temperature measurements. For MSTs with each additive, a corresponding stock solution was used in place of water. An optical cell was made up of Borosil glass of 15 cm in length and a 1.5 cm radius (r) or a 3 cm inner diameter, the cell capacity was determined with $\pi r^2 h$, the r = 1.5 cm and h = 15 cm, which gave 106 cm^3 cell volume that accommodates more than 30 subsequent additions. About 36 cm^3 vacant volume of the cell was left unoccupied for safety measures for preventing evaporation and an escape of solution. The top end of the cell was properly blocked after inserting contact and Beckman thermometers, and an immersion rod. A gentle heating from T = 298.15 K of 1 min^{-1} was made with a 25 watt-immersion rod connected to an automatic electric relay through a contact thermometer with smooth stirring (glass stirrer) with wiper motor. The solutions were prepared w/w, using $1x10^{-5}$ g accuracy 100

DS Dhona balance, Instruments Pvt. Ltd, Calcutta India, and a calibrated thermometer were used to note down the temperatures. The water with a conductivity of $1x10^{-6}\Omega^{-1}cm^{-1}$ was de-mineralized, de-ionized and degassed. The precautions enhance the accuracy in experimental work.

2.2 Turbidity Measurements

At a point slightly below the temperature of the disappearance of cloud point, the heating was reduced to 1 ^0C min^{-1}, due to a sharp disappearance (*Figure 1a* and *b*). The phase dissolution was viewed with an eye lens of a cathetometer kept 1 m away from the thermostat. Near a point of disappearance of the cloud, the heating was stopped and the temperature at which the turbidity vanished was noted and taken as the transition point temperature. After this, the mixture was air-cooled till a clear turbidity reappeared, the temperature of this point was also noted. For the air-cooling, a slightly colder air than the temperature of the paraffin bath was passed around the cell through a copper coil inserted in the paraffin wax bath. The air was propelled with a booster pump of flow rate 2000 cm^3 min^{-1} from an air cooler fitted separately.

2.3 Chemicals

Vitamins B_1 (Prod. No. 95160), B_2 (Prod. No. 95170), B_6 (Prod. No. 95180); casein (Prod No 22097), pepsin (Prod No. 77152), egg albumin (Prod No. 05438); Glycine (Merck, Prod No. 4201), β-Alanine (Spectrochem, Batch No. 3161846), L-Leucine (Merck, Prod N0 37121 3W), Dodecyl trimethylammonium bromide (Sigma, Prod No. D 5047), Orcinol (Sigma, Prod No. 1875), Trimethyl sulphoxonium iodide (Fluka, Prod No. 92763), Methyltrioctylammonium chloride (Fluka, Prod No. 69485) and phenol (Merck, Prod No. 1/17847) were used as received; demineralized water triply distilled with KOH and degassed. The chemicals were dried for 24 hours, before their mp and stored in a P_2O_5 filled desiccator till use.

2.4 Illustration of Systems

For each ratio the MSTs were plotted against corresponding phenol mole fractions (x_{ph}) in *Figure 2* and *Figure 3* to *6* are plotted similarly with additives where the mole fractions below the curve lead to two phase solutions and above the curve a to single phase solutions. The component designated as solvent dissolves another designated as solute either at normal or slightly higher temperatures but initially with compositions of each the cloud point temperatures are higher. Hence their wider compositions with lower differences are studied with several subsequent additions. The systems with additives are referred to as ternary systems and the mole fractions of each component are calculated at cloud point temperatures (*Figure. 1a* and *b*). The latter finds a difference with vitamin pepsin in an apex of a curve which denotes the UCSTs of the systems.

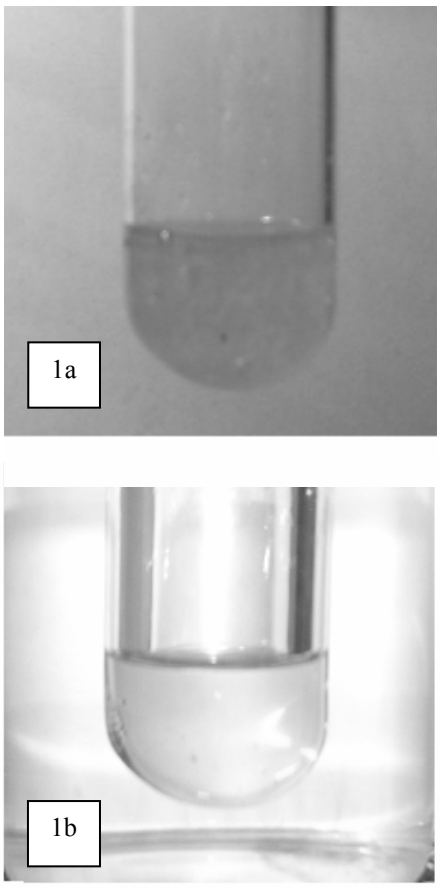


Figure 1a. and 1b. illustrate the states of the immiscible solvents phases, the 1a depicts the initial state of the phenol and water phase at 298.15 K and the 1b the single phase system at corresponding mutual miscibility temperatures.

The *Figure 1a* and *b* show a state of the phases with respect to temperatures, 1a shows the water and phenol phase separately at 298.15 K, but 1b shows a complete dissolution of the phases at particular MST. It is correlated to the molecular structure of the additives illustrated in *Figure 7*.

2.5 Calculations

The moles of each component were determined for mole fractions for each set of the solution along with critical compositions (CS_S) of corresponding MSTs. The MSTs were plotted on the y-axis against the corresponding x_{ph} on an x-axis and shown in *Figure 2* to *6*.

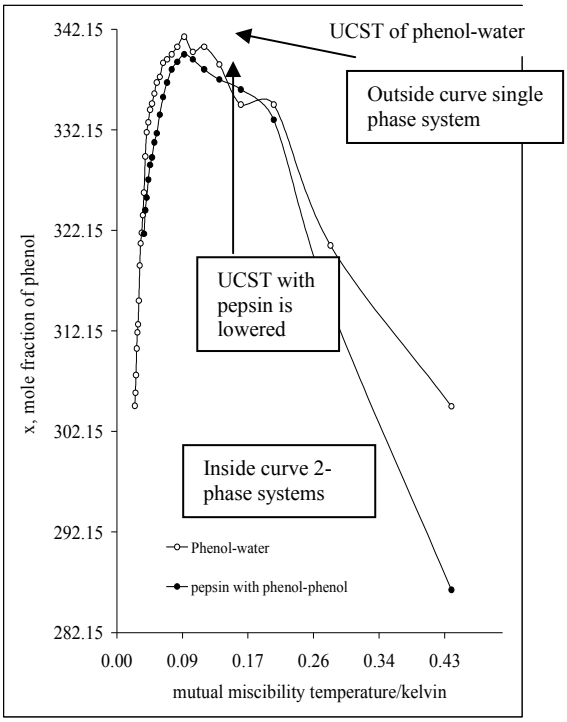


Figure 2. Plot of mutual miscibility temperatures against mole fraction of phenol (x_{phenol}) for each cloud point. The temperatures are plotted against corresponding phenol compositions and the apex of the curve depicts UCST.

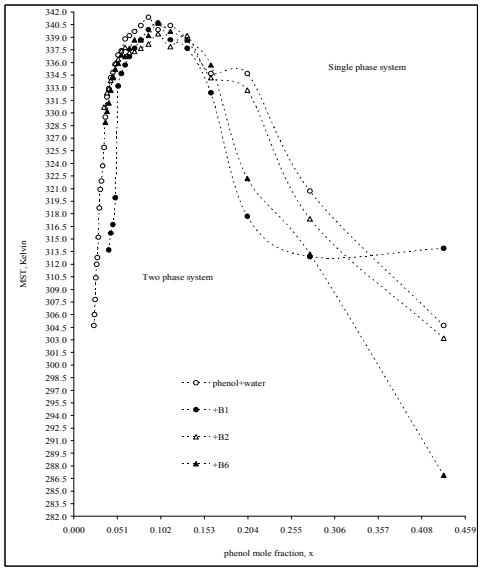


Figure 3. Mutual solubility temperatures, Kelvin and phenol mole fractions at corresponding single phase systems, the apex of graph depicts UCST value.

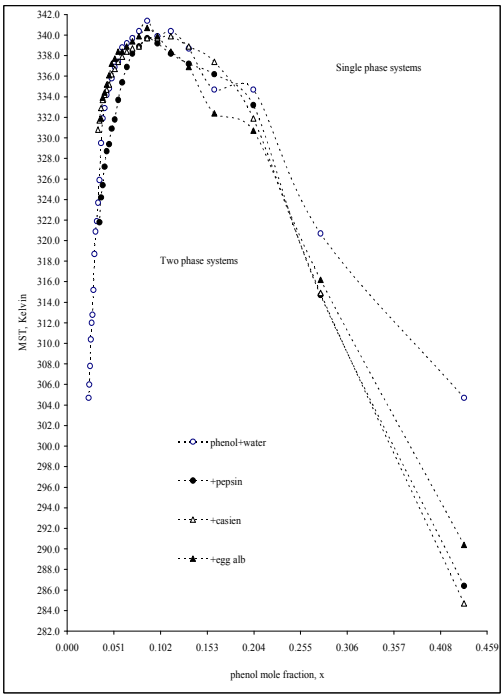


Figure 4. Mutual solubility temperatures, Kelvin and phenol mole fractions at corresponding single phase systems, the apex of graph depicts UCST value.

The apex of this curve gives values of the $UCST_S$ and CS_s, the data are given in TABLE I where the mole fractions of each additive were calculated including the amount of previous subsequent additions. The dTc/dx_{phenol} values were calculated from subsequent mole fractions and corresponding solubility temperatures (Tc) with the equation below.

$$\frac{dT_c}{dx_{ph}} = \frac{d\left((Tc)_2 - (Tc)_1\right)}{d\left((x_{ph})_2 - (x_{ph})_1\right)} \qquad (1)$$

The data are given in TABLE II.

3. Result and Discussion

The UCST and mutual solubility data for phenol + water show a close agreement with those of the literature (Singh, 2007) with T = (273.15 ± 0.05) K and ± 0.25 ^{0}C errors respectively. The phenol-water systems were repeated several times with standard experimental conditions for reproducibility in data. The UCSTs with additives remain near those of the water-phenol system (TABLE I), and the B_2 produces a lower UCST than that of B_1 and B_6 but B_1 and B_6 produce the same UCSTs (*Figure 3*).

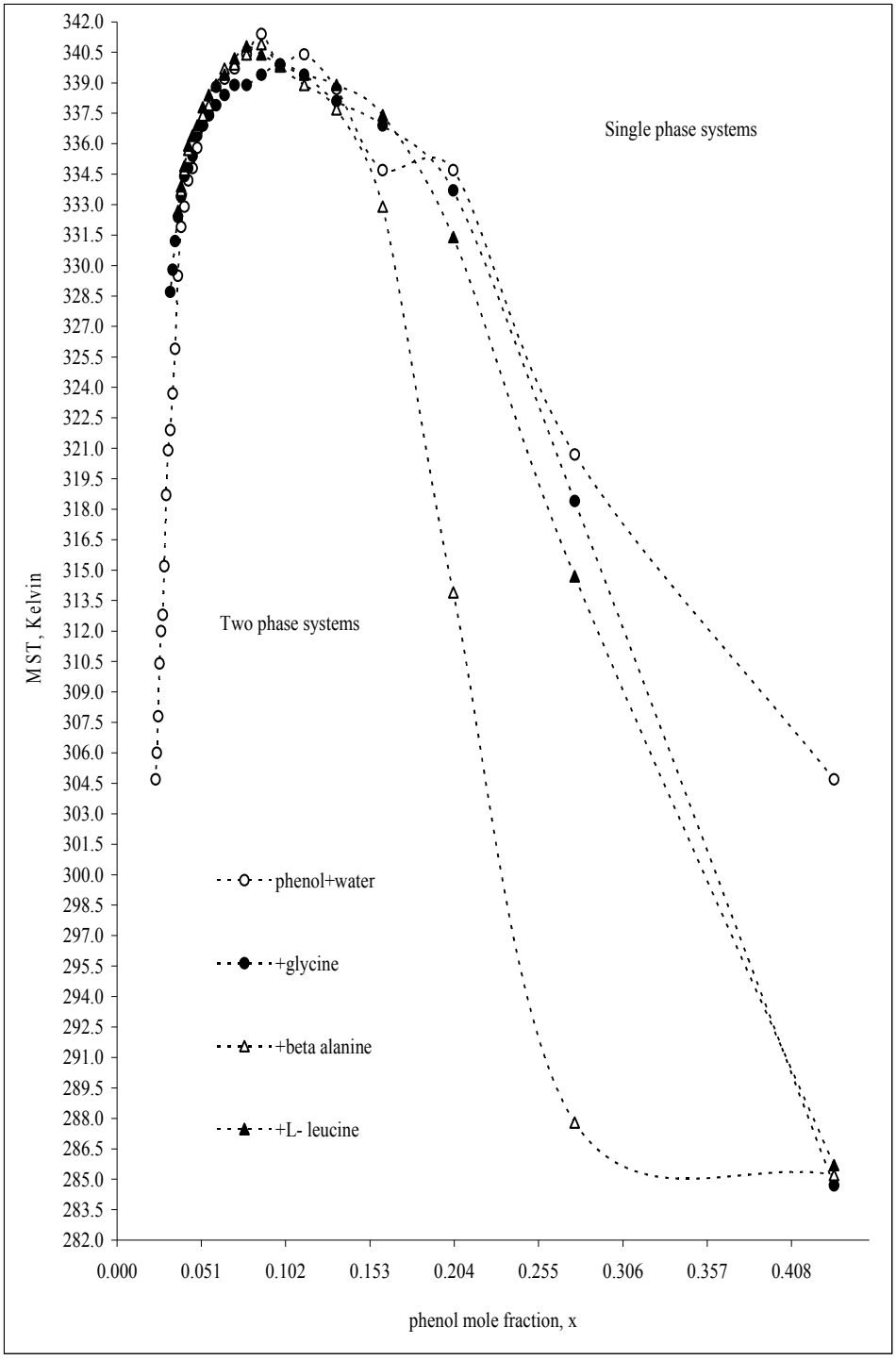


Figure 5. Mutual solubility temperatures, Kelvin and phenol mole fractions at corresponding single phase systems, the apex of graph depicts UCST value.

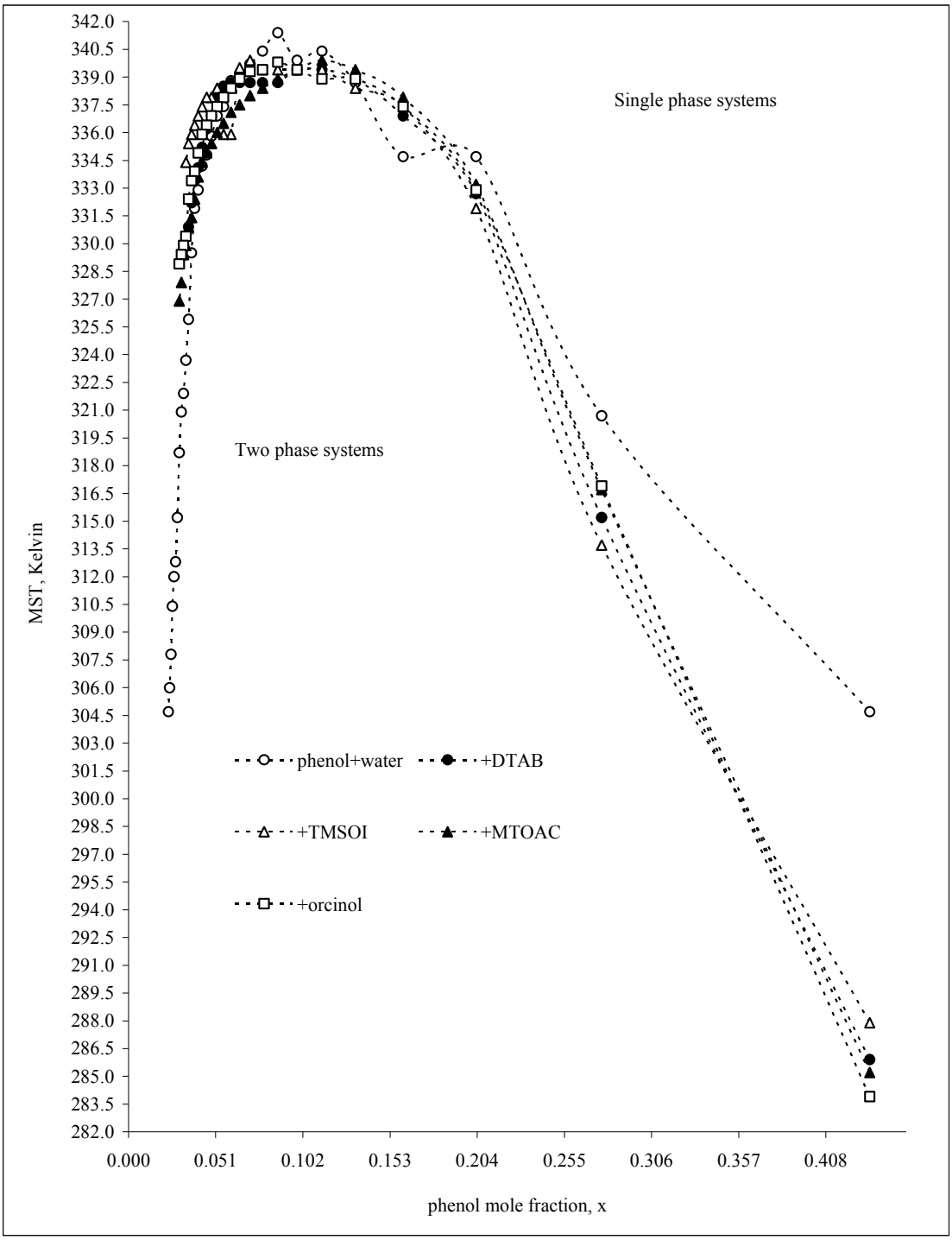


Figure 6. Mutual solubility temperatures, Kelvin and phenol mole fractions at corresponding single phase systems, the apex of graph depicts UCST values.

The 67.5 ^{0}C, 66.5 ^{0}C and 67.5 ^{0}C UCST values for B_1, B_2 and B_6 systems are noted at around 0.0986 x_{ph} which are slightly higher than those of the values without additive. This infers greater solubilities of the phenol with vitamins respectively at lower UCST. The vitamins enhance the x_{ph} by about 0.0112 compared to the blank system. But the B_2 decreases the UCSTs by 2.0 ^{0}C as compared to B_1 and B_6 because its 3 -OH, 2 -CO, -NH and 3N atoms develop slightly stronger hydrophilic interactions and 2–CH_3 and develop hydrophobic interactions (*Figure 7*).

However the 3-OH groups of B_6 show slightly higher UCSTs but as compared to B_2, the B_1 produces slightly higher UCSTs as it is not consisted of the hydrophobic –CH_3 groups. However it consists of 1–OH and 1-NH groups, 3 N atoms and 1 S atoms. Hence its hydrophilic and hydrophobic developing interaction strength remains weaker than that of the B_6, hence the B_2 acts as a mild surfactant for the phenol-water system. The UCSTs with B_1, B_2 and B_6 are noted for their 0.0047, 0.0054 and 0.0011 mole fractions respectively. These mole fractions are worked out for ternary systems. Their exact order is listed as (0.00545) B_2 > (0.00474) B_1> (0.00115) B_6 which decrease the UCSTs in order of (B_2, 2.0 ^{0}C) > (B_1, 0.7 ^{0}C) = (B_6, 0.7 ^{0}C) as compared to phenol-water.

The UCSTs with amino acids are listed as β-alanine (UCST) > L-leucine (UCST) > glycine (UCST) with $_{Xph}$ (glycine) > $_{Xph}$ (β-alanine) > $_{Xph}$ (L-leucine) order of the x_{ph} at corresponding UCSTs (*Figure 5*).

Hence glycine comparatively develops stronger hydrophilic as well as hydrophobic interactions because the x_{ph} are higher and UCSTs lower than those of the phenol-water by 1.5 ^{0}C and other acids. The COO^- and $–H_3N^+$ (*Figure 7*) groups of glycine develop hydrophilic and -CH_2- (methylene) group the hydrophobic interactions with higher phenol dissolution. Such action of the glycine facilitates their mutual mixing at lower temperatures but the β-alanine due to CH_3-CH_2 alkyl chain which develops slightly stronger hydrophobic and weaker hydrophilic interactions as compared to that of the glycine. Thus an elongation of the alkyl chain in the amino acids enhances the UCSTs which illustrate the weakening of hydrogen bonding strength of their $–COO^-$ and N^+H_3 groups. Specifically the 1–CH_3 of β-alanine enhances the UCST by 1 ^{0}C (*Figure 5*) due to its slightly stronger hydrophobic interaction with phenol that requires higher thermal energy for homogenous single phase solution. Hence solubility of phenol further decreases with L-leucine due to its longer alkyl chain (-CH_2-CH-CH-) with 2-CH_3 (*Figure 7*). Thus the alkyl chain of leucine enhances the UCSTs due to an induction and steric effects where strength of the hydrophobic interactions remains weaker. Because as compared to β-alanine the leucine decreases the UCST by 0.2 ^{0}C, because the x_{ph} with L-leucine are higher with stronger hydrophobic interactions due to 2-CH_2 and CH_2-CH, groups. The $x_{amino\ acids}$ for corresponding UCSTs are noted as glycine (0.00238) > β-alanine (0.00221) > L-leucine (0.00164), hence the UCSTs with the $x_{amino\ acids}$ decrease due to strengthening of hydrophobic interactions with concentration.

The proteins a 3rd series of additives produce the UCSTs as pepsin (1.7 ^{0}C) > casein (1.5 ^{0}C) > egg-albumin (0.7 ^{0}C) where the x_{ph} remains higher with the casein than those of the pepsin and casein (*Figure 4*), their order is listed as x_{ph} (casein) > x_{ph} (pepsin) = x_{ph} (egg-albumin). However their $x_{proteins}$ are listed as pepsin (0.00537) > egg-albumin (0.00493) > casein (0.00371) due to stronger interaction with egg albumin. Further the x_{ph} (B_1) = x_{ph} (B_2) = x_{ph} (B_6) trends with the vitamins illustrate almost same mutual solubility with UCSTs, perhaps the B_2 develops the stronger interaction with phenol as well as water and decreases the UCST. Therefore it acts as biosurfactants due to 2–CH_3, 3 benzene rings as hydrophobic and 3–OH, 4 N atoms as hydrophilic groups. Thus a less thermal energy is required to dissolve immiscible solvents which are a green chemistry step where vitamins behave as useful biosurfactants to enhance mutual miscibility of the solvents. The equal numbers of x_{ph} with vitamins illustrate the equal strength of both the hydrophilic and hydrophobic interactions which with equal response of phenol and water with them. Such features of vitamins with immiscible solvent are highly recommended for the systems where amount of water is to be controlled in vitamins. Their mole fraction are in order of B_2 (0.005456) > B_1 (0.004746) > B_6 (0.001056), hence the B_1 and B_6 cause a weaker solubiliging effects on the solvents but if benzene rings in the B_1 increase then this effect further weakened. This infers that the benzene rings are not so effective for intermixing or solubilization of the two immiscible phases.

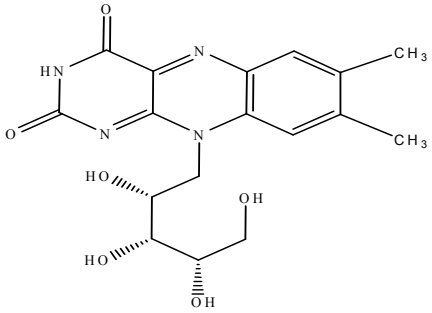


Figure 7a. vitamin B6.

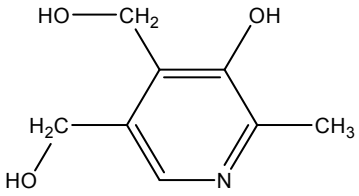


Figure 7b. vitamin B2.

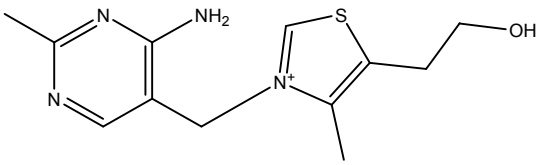


Figure 7c. vitamin B1.

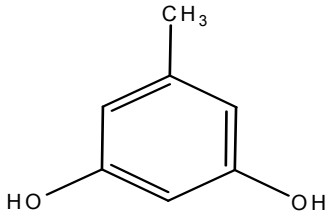
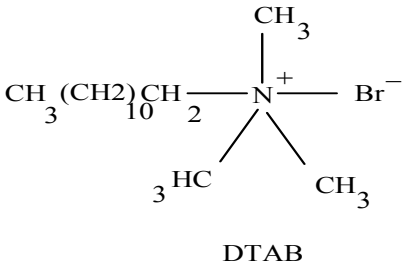
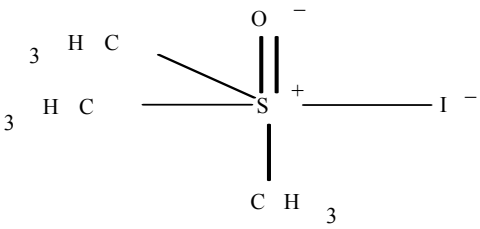


Figure 7d. orcinol.



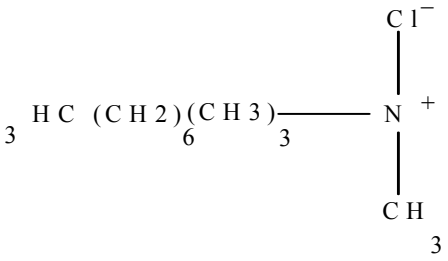
DTAB

Figure 7e.



T M S O I

Figure 7f.



M T O A C

Figure 7g.

Figure 7. Structure of the additives.

The x_{ph} with proteins is listed as x_{ph} (casein) > x_{ph} (pepsin) = x_{ph} (egg-albumin) where casein dissolves maximum phenol and the egg-albumin show a least effect on dissolution with a UCST as UCST (egg-albumin) > UCST (casein) > UCST (pepsin). It infers its stronger interaction with phenol which requires higher thermal energy with higher UCSTs for dissolution of the phenol-egg-albumin complex. The formation of phenol–egg albumin initiates salting out effect on water where phenol-egg albumin and water develop further well defined phases below cloud point. Thus the egg-albumin does not work as biosurfactant but does modify the hydrophobic interactions that occur between phenol and egg-albumin. However the casein behaves as biosurfactant and decreases the Tc and increase mutual miscibility of solvents with increase of the x_{ph} by about 0.0258 as compared to pepsin and egg-albumin. Hence the casein modifies the hydrophobic as well as hydrophilic interaction of phenol and water respectively. Since the casein due to 1-OH, develops hydrophilic and 4 benzene rings (*Figure 3*) support hydrophobic interactions with phenol. Further casein is small sized protein which requires higher thermal energy for molecular modeling due to torque and reorientation along with conformation. The torque and orientation in casein are quicker as compared to pepsin and egg albumin which is of large sized and requires higher thermal energy.

The x_{ph} and Tc are compared to the x_{ph} with vitamin and protein and their trend for amino acids and listed as x_{ph} and UCST trend for proteins- x_{ph} (casein) > x_{ph} (pepsin) = x_{ph} (egg-albumin) and UCST (egg-albumin) > UCST (casein) > UCST (pepsin), the x_{ph} (glycine) > x_{ph} (β-alanine) > x_{ph} (L-leucine) and UCST (β-alanine) > UCST (L-leucine) > UCST (glycine). The glycine behaves as a stronger hydrophilic and hydrophobic agent as it dissolves phenol in water and lowers the Tc due to two functional hydrophilic groups and one $–CH_2$ methylen hydrophobic group. It acts as a surfactant to enhance the mutual solubility of the solvents. However the β-alanine also dissolves immiscible solvents, but its dissolution capacity remains lower than that of the glycine. But the Tc of β-alanine is maximum among all amino acids, thus the $–CH_3$ of the β-alanine as compared to glycine develops stronger interaction with phenol rather than water. Similarly the L-leucine with $2–CH_3$, $2–CH_2$ (hydrophobic) and 1–CH hydrophilic groups also develops a strong hydrophobic interaction with phenol which requires higher thermal energy and remains lower than of the β-alanine. The L-leucine shows unique behavior for UCST and x_{ph}, perhaps induction and steric effects also contribute to the UCST values, but these effects leave the least input on the x_{ph}.

The trends of x_{ph} and UCST, x_{ph} (glycine) > x_{ph} (β-alanine) > x_{ph} (L-leucine) and UCST (β-alanine) > UCST (L-leucine) > UCST (glycine). The L-leucine shows the least distribution of the phenol in water but Tc for leucine is higher among the additives except β-alanine.

TABLE I. UPPER CRITICAL SOLUTION TEMPERATURES (UCSTS) FOR BIOMOLECULES AND SURFACTANTS, MOLE FRACTION OF PHENOL AND ADDITIVES AT UCSTs. THE UCSTs WERE MEASURED WITH ± 0.05 K ERROR. SOLUTIONS WERE ACCURATE TO $1x10^{-5}$ w/w.

Additives	x_{phenol}, %	x_{water}	$x_{additive}$	UCST ± 0.05 ^{0}C
Phenol	0.0874, 33.33%	0.9126	0	68.3, 341.4 K
it B_1	0.0986, 36.33%	0.9014	0.00475	67.5, 340.7 K
Vit B_2	0.0986, 36.36%	0.9014	0.00546	66.5, 339.4 K
Vit B_6	0.0986, 36.36%	0.9014	0.0011	67.5, 340.7 K
Pepsin	0.0874, 33.33%	0.9126	0.00538	66.5, 339.7 K
Casein	0.1132, 40.00%	0.8868	0.00372	66.8, 339.9 K
Egg- al	0.0874, 33.33%	0.9126	0.00493	67.5, 340.7 K
Gly	0.0986, 36.36%	0.9014	0.00238	66.8, 339.9 K
β-Al	0.0874, 33.33%	0.9126	0.00222	67.8, 340.9 K
L- Leu	0.0784, 30.76%	0.9216	0.00165	67.6, 340.8 K
DTAB	0.0986, 36.36%	0.9014	0.00136	66.3, 339.4 K
TMSOI	0.0711, 28.57%	0.9289	0.00179	66.8, 339.9 K
MTOAC	0.1131, 40.00%	0.8868	0.00122	66.8, 339.9 K
Orcinol	0.0873, 33.33%	0.9126	0.00150	66.7, 339.8 K

Surfactants

The surfactant series including orcinol produces lower UCSTs than those of the vitamins, amino acids and proteins.

However vitamin B_2, pepsin, glycine show the UCSTs near the values of the surfactants (TABLE I).

This similarity of the UCSTs infers that B_2, pepsin and glycine as biosurfactants that equally develop hydrophilic and hydrophobic interactions like those of the surfactants

The UCSTs of surfactants are noted as TMSOI = MTOAC > orcinol > DTAB, here DTAB effectively develops the hydrophilic and hydrophobic interaction, which enhance mutual solubility.

However the orcinol with 2-OH groups and 1-CH_3 group (*Figure 7*) develops slightly weaker hydrophilic and hydrophobic interaction. The MTOAC and TMSOI show slightly higher UCST values by 0.5 than those of the phenol-water, hence these develop weaker hydrophilic and hydrophobic interactions. A close comparison of the molecular structure (*Figure 7*) of DTAB with an alkyl chain of 12 carbon atoms develops a stronger hydrophilic interaction but MTOAC and TMSOI with an alkyl chain of 12 carbon atoms and an alkyl chain with 3 carbon atoms respectively develop weaker hydrophobic and stronger hydrophilic interactions due to N^+ and Cl^- with MTOAC and the O atom of TMSOI.

It infers that surfactants with a longer alkyl chain develop stronger hydrophobic interaction due to higher solubility in phenol rather than that of the water. Hence the surfactants with a longer alkyl chain develop weaker hydrophilic interactions. Further the comparison of halide atoms of the functional groups of the surfactants visa-vis UCSTs show their placement as – I^- = Br^- > Cl^-. Hence the critical review of UCSTs vis-à-vis structures of TMSOI and MTOAC infer that hydrophobic 3-CH_3 groups of TMSOI outweigh the hydrophilic interactions due to $O=S^+$-I^-.

Hence UCST is higher due to higher thermal energy needed for dissolving $O=S^+$-I^--water boundaries. Perhaps the $O=S^+$-I^- likes the geometry of the $O=S^+$-I^- - H_2O towards the water phase that develops stronger dipolar interactions with H_2O but the I^- also develops stronger induced potential or polarizability but MTOACs Cl^- atom has a slightly stronger hydrophobic interaction than that of $O=S^+$-I^- due to a 10 carbon atom chain that dominates over the weaker hydrophilic interactions of N^+-Cl, where the Cl^- is a small sized anion and does not induce any potential. Thus $O=S^+$-I^- compensates more share to the higher UCSTs and N^+-Cl does not, but the 12 carbon chain do compensates for the same.

Atomic size d_0 influences the UCST

The UCST of surfactants with halide atoms are listed is as I^- = Br^- > Cl^-, and is correlated to the nonbonding electron transitions due to the greater electro negativity of the Cl^- atom. The nonbonding electrons on Cl^- hardly undergo energy change but of I^- on TMSOI is loosely bound and can undergo energy change. They strongly reorient the solvent structure due to the cage model of water that lead to develop weaker interaction with the protein. Perhaps it is difficult to get fited the hydrophobic parts of chosen additives in water, but align towards phenol. Similarly the hydrophilic parts align to water the H_2O. Thus the loose film between phenol and water interface is developed that initiates a soap action on the liquid-liquid interaction (LLI) model. Hence our study seems to be restricted to surfaces only where the surfactant molecules get dispersed in phenol-water phases but not at all to penetrate bulk to either. The additives were in water which on contact of phenol aligns to phenol and water surfaces.

The *Figures. 3* to *6* depict greater scattering in MST with amino acids, vitamins and proteins, and *Figure 6* depicts smaller scattering in MST values for the surfactant. Comparatively, proteins also show smaller scattering. Such trends of MSTs infer a different stage of hydrophobic and hydrophilic interactions with phenol and water where the surfactants behave with same the strength. Further the negligible scattering of the MST values up to 0.153 x_{ph}, this infers mutual enhancement of phenol-water soluble in each other of enhancement MST for lower x_{ph} + additives remains abreast similarly. However for higher phenol mole fractions the rate of change of USCT slightly decreases. Hence the additives are more effective with lower x_{ph} but with lighter x_{ph}. The phenol interaction might weaken the sharper effects of additives. The comparison of MST and UCST of biomolecules with surfactants infer their lower values similar to surfactants. Therefore our studies are useful in the bioscience process occurring at slightly higher temperatures.

3.1 UCST and $X_{vitamins}$

The UCSTs of vitamins are B_1= B_6> B_2 and the $x_{vitamins}$ B_2 > B_1 > B_6, which infer a sequence of strength of (biomolecules + water) interactions. Thus these influence the mutual solubilities of water and phenol to achieve a critical solvent-like behavior with specific UCST values. The biomolecules undergo structural changes due to different electron densities on

their atoms which develop polar centers with positive and negative charges. Therefore the charged molecules behave as molecular ions where the vitamins develop multiple centers with negative and positive charges. The vitamins decrease the UCSTs by about 0.7 ^{0}C as compared to phenol-water with higher water and phenol solubilities in each other.

3.2 UCST and $X_{proteins}$

Their UCSTs are pepsin > egg-albumin > casein and the $x_{proteins}$ in egg-albumin > casein > pepsin which infers a sequence of strength of (biomolecules + water) interactions. These slightly enhance the mutual miscibilities of solvents to achieve a critical solvent-like behavior with specific UCSTs. The biomolecules undergo structural changes due to different electron density on different atoms which develop polar centers with positive and negative charges, therefore the charged molecule behave as molecular ions. The protein egg-albumin, casein and pepsin decrease the UCSTs by 0.7, 1.5 and 1.7 ^{0}C with slightly higher enhancement of water and phenol in each other due to egg-albumin.

3.3 UCSTs and $X_{amino\ acids}$

The UCSTs are listed as glycine > β-alanine > L- leucine and the $x_{amino\ acids}$ in order of β-alanine > L-leucine > glycine which infers a sequence of strength of (biomolecules + water) interactions. The amino acids due to zwitterions develop hydrophilic and hydrophobic interactions where the β-alanine, L-leucine and glycine decrease the UCSTs by 0.5, 0.6 and 1.5 ^{0}C, respectively with higher mutual miscibilities due to β-alanine.

3.4 dTc/dx_{phenol} Values

The dTc/dx_{phl} values (TABLE II) for vitamins are $B_1 = B_6 > B_2$, and are negative, which infers a decrease in Tc with x_{ph} with each vitamin.

The $(Tc)_2(x_{ph}+x_{additive})_2$ and $(Tc)_1$, (x_{ph}) are values with additives and phenol, respectively).

The dTc/dx_{ph} values for both the B_1 and B_6 are -71.4 but the value B_2 is -160.7. Thus the vitamin B_2 develops higher hydrophobic and hydrophilic interactions. Similarly the proteins produce the dTc/dx_{ph} values egg-albumin > casein > pepsin and the pepsin, casein and egg-albumin show -1.8, -58.1 and -0.8, respectively. Hence the casein mole fraction produces a slightly higher value than that of pepsin and egg-albumin with comparatively stronger interactions with water, which allows water to mix homogenously at a slightly higher temperature. The dTc/dx_{ph} values for the amino acids are listed as –leucine > beta alanine > glycine, where the glycine shows a -133.9 value due to stronger hydrophilic and hydrophobic interactions. The β-alanine owns the -0.5 dTc/dx_{phl} value with weaker interaction but the value for l-leucine is 77.8 with weaker hydrophilic interactions.

TABLE II. THE SOLUBILITY TEMPERATURE (Tc), CORRESPONDING PHENOL MOLE FRACTION ($dTc/d_{x(phenol)}$) WITH AND WITHOUT ADDITIVES RESPECTIVELY.

Additives	$d((Tc)_2-(Tc)_1)$
Vit B_1	-0.7
Vit B_2	-2.0
Vit B_6	-0.7
Pepsin	-1.8
Casein	-1.5
Egg-Al	-0.8
Glycine	-1.5
β-Alanine	-0.5
L-Leucine	-0.7
Additives	$d((x_{ph})_2-(x_{ph})_1)$
Vitamin B_1	0.0112
Vitamin B_2	0.0112
Vitamin B_6	0.0112
Pepsin	0
Casein	0.0258
Egg-Al	0
Glycine	0.0112
β-Alanine	0
L-Leucine	-0.009
Additives	dTc/dx_{ph}
Vit B_1	-62.5
Vit B_2	-178.6
Vit B_6	-62.5
Pepsin	-1.7
Casein	-41.8
Egg-Al	-0.7
Glycine	-133.9
β-Alanine	-0.5
L-Leucine	66.7

4. Conclusion

The vitamins B_1 and B_2 decrease the UCSTs by about 0.7 ^{0}C, with a favorable effect of mutual miscibility and the egg-albumin, casein and pepsin decrease the UCSTs by 0.7, 1.5 and 1.7 ^{0}C. The amino acid β-alanine, L-leucine and glycine decrease the UCSTs by 0.5, 0.6 and 1.5 ^{0}C. The UCSTs do illustrate a cloud point and infer a pre-solubility state of immiscible solvents and the lower UCSTs with biomolecules reported in our studies investigate a favorable or unfavorable influence of vitamins, proteins and amino acids.

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Nomenclature

CS	critical solution
CPTs	cloud point temperatures
MST	mutual miscibility temperature
UCST	Upper critical solution temperature
Ss	surfactants
dTc/dx_{phenol}	mole fractions
(Tc)	corresponding solubility temperatures
(CS_S)	critical compositions
(x_{ph})	corresponding phenol mole fractions
mf	mole fraction
(LLI)	liquid-liquid interaction
w/w	weight by weight

Greek letter

β-alanine	biomolecule

Subscripts

r	radius of borosil glass

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