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# Determination of phase transition temperatures of PEO-PPO-PEO block copolymer L62 in presence of fermentation media components

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This work investigates the effect of various additives/compounds present in fermentation media and products on phase transition temperatures [cloud point (CP), melting point (MP), double cloud point (DCP) and phase separation temperature (PST)] of non-ionic surfactant. Butanol fermentation media and L62 (tri-block copolymer) were considered as a model system. Results showed that the phase transition temperatures strongly depend on concentration of surfactant, butanol and glucose. Phase transition temperature (CP, MP, DCP, and PST) for control i.e. aqueous solution of L62 (3%–15% v/v) were 295–299 K, 297–307 K, 317–322 K, 342–343 K, respectively. Butanol (1%–5% v/v) significantly decreased the phase transition temperatures (7–8 K, 8–11 K, 8–12 K, and 27–29 K, respectively for CP, MP, DCP, and PST) followed by glucose (2–3 K, 2–3 K, 2–7 K and 5–6 K respectively for CP, MP, DCP, and PST) whereas other additives have negligible effect. Presence of both glucose and butanol together had synergistic effect on depression of phase transition temperatures of L62 (9–10 K, 10–14 K, 9–10 K, and 27–29 K, respectively for CP, MP, DCP, and PST). The results from this study provide the estimation of typical cloud point temperature for a range of components present in fermentation media/products. Further, these results will be useful for deciding the conditions for cloud point extraction.



## 1. Introduction

Surfactants find large number of applications in various industrial and daily applications. Surfactants are classified by their use, properties and chemical structure (the type of head group they possess). According to the polar head group, surfactants can be classified into ionic surfactants (anionic, cationic and zwitterionic) and nonionic surfactants. Cloud point is the characteristic of non-ionic surfactant only. Tri-block copolymers are commercially available as non-ionic surfactant. Polyethylene oxide (PEO) and polypropylene oxide (PPO) surfactants, often denoted as PEO-PPO-PEO or $(EO)_{n1}(PO)m(EO)_{n2}$ are water-soluble tri-block copolymers. Amount of EO and PO decide the hydrophile-lipophile balance (HLB) of the surfactants.

Non-ionic surfactants, due to its various advantages such as biocompatibility, significant reduction in process volume, high extraction efficiency, concentration of product and controlled release of substrate/product, are widely used in fermentation systems and bio-separations. They find large number of applications where product or substrate inhibition occurs [1–5]. Also, surfactant based aqueous two phase systems are preferred for separation of product from fermentation broth [6–9]. In case of extractive fermentation involving product inhibition, the product (e.g. butanol in butanol fermentation) is extracted into micelle during the fermentation. This relieves the butanol toxicity to the microbes. Non-ionic surfactants are also used for separation of biomolecules such as p-courmaric acid, ferulic acid [10] and butanol [11–13]. Other applications include, improving the yield in hydrolysis of lignocellulose [14,15], recycle of enzymes during enzymatic hydrolysis of lignocelluloses [16,17] and in simultaneous saccharification and fermentation process [16].

Extraction of product from fermentation systems is carried out by incubating the broth at a temperature called as cloud point temperature (CP); this process is referred as cloud point extraction. Cloud point of the system is affected by the presence of various compounds. Effect of various additives, such as inorganic electrolyte [18,19], non-electrolyte [20,21], mixed surfactant [22–25], alcohols [26–29], water soluble polymers [30], hydrotropes [31]and oil field performance chemicals [32] on CP of nonionic surfactants have been investigated.

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Fermentation medium is a complex system and consists of several compounds as substrate, nutrients, intermediate products and various by-products. It is anticipated that these compounds will affect the CP and also the phase separation phenomena. However, as per authors review of literature, the effect of complexity of fermentation media on CP and phase behavior are not investigated. Hence, this work was undertaken with an objective of evaluating the effect of nutrients and products on phase behavior (mainly cloud point) of non-ionic surfactant. Butanol fermentation and L62 was considered as a model system. Dhamole et al. [12], found that L62, a PEO-PPO-PEO tri-block copolymer, enhances butanol production and can also be used for separation of butanol from broth. This study also provides a temperature range over which cloud point extraction can be carried out in a fermentation system.

## 2. Materials and methods

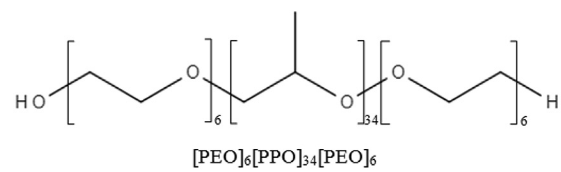
### 2.1. Chemicals

Glucose, yeast extract, $K_2HPO_4$(di-potassium hydrogen phosphate), $KH_2PO_4$(Potassium di-hydrogen orthophosphate), ammonium acetate, para-amino benzoic acid, thiamine, biotin, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$, $FeSO_4{\cdot}7H_2O$ and NaCl of analytical grade were used in this study and summarized in Table 1. Solvents (acetone, ethanol, and butanol) were purchased from Hi-media. Pluronic L62 (PEO-PPO-PEO) triblock copolymer was provided by BASF and used without further purification. L62 has a molecular weight of 2500 g mol$^{-1}$ and contains 20% PEO. Its molecular formula is represented as $PEO_6PPO_{34}PEO_6$ and hashydrophile-lipophile balance (HLB) value of 7.0. Chemical structure of non-ionic Pluronic surfactant L62 is as shown in Scheme 1.

All the solutions were prepared using distilled water. The detail composition of solution and procedure is mentioned in each subsection. Temperature at which change in phase occurs was noted down.

### 2.2. Phase diagram of L62 -water

Aqueous solutions (3, 6, 9, 12 and 15% v/v L62)were prepared by dissolving the required amount of L62 in distilled water. The mixture was then mixed and used for further studies. Aqueous solution (10 mL) was then taken into graduated glass test tubes (15 mL) sealed with glass stopper and incubated into circulating water bath(Polyscience Digital Temperature Controller, USA, MX07R-20-A-12E). The samples were heated with a temperature ramp of 1 K and held at each temperature for 2 min to attain thermo-equilibrium. CP, DCP, MP and PST were determined based on visual observation i.e. the change in the appearance of the surfactant solutions [27,30,33]. All the experiments were carried out in duplicate and the % error was less than 10%.




**Scheme 1.** Chemical structure of non-ionic surfactant Pluronic L62.

### 2.3. Effect of fermentation media components and products on phase transition temperatures

Effect of media components and products (at a concentration typically used in fermentation) was studied on cloud point of L62. The samples were prepared as per the composition mentioned below. The effect of (i) butanol (5% v/v) independently, (ii) glucose (60 g/L) independently (iii) combined acetone (20.25 g L$^{-1}$), butanol (40.5 g/L) and ethanol (6.75 g/L) (in the ratio of 3:6:1) (iv) fermentation media consisting of glucose (60 g/L), yeast extract (1 g/L), $CaCO_3$ (3 g/L), $K_2HPO_4$(0.05 g L$^{-1}$), $KH_2PO_4$ (0.05 g L$^{-1}$), ammonium acetate (0.22 g L$^{-1}$), $MgSO_4{\cdot}7H_2O$ (0.02 g L$^{-1}$), $MnSO_4{\cdot}H_2O$ (0.001 g L$^{-1}$), $FeSO_4{\cdot}7H_2O$ (0.001 g L$^{-1}$), NaCl (0.001 g L$^{-1}$), para-amino benzoic acid (0.00019 g L$^{-1}$), thiamine (0.00019 g L$^{-1}$) and biotin(0.00019 g L$^{-1}$). and (v) combined, fermentation medium (as mentioned above) and ABE products (i.e. acetone −20.25 g/L, butanol- 40.5 g/L and ethanol- 6.75 g/L) was studied. The conductivity-concentration data were obtained for aqueous solution of surfactant L62 and in the aqueous solution of L62 with additives. Conductivity measurements were carried out at constant temperature (298 K) by circulating water from water bath through a jacketed double walled glass vessel. The conductivity values were determined with conductivity meter (VSI-04) using a cell with a cell constant of 0.974.

### 2.4. Effect of varying butanol and glucose concentration on phase transition temperatures

A series of an aqueous solution containing a different concentration of L62(3, 6 and 9% v/v) and butanol (1%–5% v/v) were prepared. Solution (10 mL) was taken into graduated glass tubes

**Table 1**
Tested salts, corresponding manufacturer, and purity.

| Chemical Name | Chemical Formula | CAS No | Source | Purity (Mass %) | Purification Method |
|---|---|---|---|---|---|
| D-Glucose | $C_6H_{12}O_6$ | 50-99-7 | Hi-Media | 99.5 | None |
| Yeast extract | – | – | Hi-Media | – | None |
| di-Potassium hydrogen phosphate anhydrous | $K_2HPO_4$ | 7785-11-4 | Hi-Media | 99.0 | None |
| Potassium dihydrogen phosphate | $KH_2PO_4$ | 7778-77-0 | Hi-Media | 99.0 | None |
| Ammonium ethanoate | $CH_3COONH_4$ | 631-61-8 | Hi-Media | 99.0 | None |
| 4-Aminobenzoic acid | $C_7H_7NO_2$ | 150-13-0 | Hi-Media | 99.0 | None |
| Thiamine hydrochloride | $C_{12}H_{17}ClN_4OS{\cdot}HCl$ | 67-03-8 | Hi-Media | 99.0 | None |
| D-Biotin | $C_{10}H_{16}N_2O_3S$ | 58-85-5 | Hi-Media | 99.0 | None |
| Magnesium sulfate heptahydrate | $MgSO_4{\cdot}7H_2O$ | 10034-99-8 | Hi-Media | 99.0 | None |
| Manganous sulfate monohydrate | $MnSO_4{\cdot}H_2O$ | 10034-96-5 | Hi-Media | 99.0 | None |
| Ferrous sulfate heptahydrate | $FeSO_4{\cdot}7H_2O$ | 7782-63-0 | Hi-Media | 99.0 | None |
| Sodium chloride | NaCl | 7647-14-5 | Hi-Media | 99.9 | None |