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A thermodynamic micellization and hemolysis evaluation of polysorbate surfactants in combination with short-chain alcohols

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28 **Abstract:**

29 Surface active agents have been used in many pharmaceutical formulations for different purposes as
30 penetration enhancers. In this study, the penetration enhancement activity of four nonionic surfactants from
31 polyoxyethylene sorbitan fatty acid esters alone and in combination with some short-chain alcohols on red
32 blood cells were investigated. Hemolysis at different concentrations of each nonionic surfactant (0.1, 0.2, 0.5,
33 1, and 2%) and ethanol, isopropyl alcohol, glycerol, and propylene glycol (0.5, 1, 2, 5, and 10% w/v) were
34 investigated. Critical micelle concentration (CMC) and micellization thermodynamic parameters of tween 80
35 in presence of ethanol and glycerol at different concentrations were determined. The influence of the ethanol
36 and glycerol on nonionic surfactant micelle formation was evaluated by determining CMC and micellization
37 thermodynamic parameters of tween80 at different concentrations of alcohol-tween mixtures. The mixture of
38 Tween 20 and glycerol have the lowest hemolytic activity while tween 80-ethanol mixture has the highest
39 hemolysis activity. The self-aggregation of surfactant monomers was affected by short-chain alcohols. The
40 structure breaking ability of alcohol and their interactions with the hydrophilic-hydrophobic groups of
41 surfactants might be the main factors for changing the micelle formation. The CMCs of tween 80 was
42 incremented by increasing the concentration of alcohol. The results indicated that by adding and increasing
43 short chain alcohols the CMCs increased also the CMCs were increasing through raising the temperature
44 (while in the presents of surfactants alone the CMCs decreased by raising temperature).

45 **Keywords:** polysorbate surfactants, ethanol, isopropyl alcohol, glycerol, propylene glycol, hemolysis,
46 thermodynamic

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53 1. Introduction

54

55 Penetration enhancers (PEs) are chemical substances widely used in topical and transdermal preparations to
56 increase drug penetration to deeper layers of skin. Various kinds of PEs such as surfactants, alcohols, glycols,
57 fatty acids, and urea, among others have been utilized in such topical formulations [1]. Alcohols in various
58 concentrations have been reported as an efficient permeation enhancer in dermal and transdermal
59 preparations. Ethanol and isopropyl alcohol (IPA) are the two most common short-chain alcohols utilized in
60 topical products. Different mechanisms for the enhancement permeation effect have been suggested such as
61 acting as a co-solvent or alteration of stratum corneum structure. Other excipients that have been used widely
62 in topical formulations include glycols. Propylene glycol (PG) is the most commonly used as a PEs and co-
63 solvent for poorly soluble drugs. Surfactants are amphiphilic compounds utilized in many therapeutic and
64 cosmetic formulations in order to solubilize lipophilic ingredients. All kinds of surfactants such as anionic,
65 cationic, non-ionic, zwitterion, and biosurfactants have been investigated as PEs with different mechanisms
66 of action, among them the non-ionic surfactants possess the least toxicity and skin irritation [2]. They have
67 the potential to solubilize the stratum corneum lipids which enhances drug penetration to the inner layers of
68 skin. Surfactants and alcohols abilities to solubilize the lipidic components of biological membranes could
69 have some toxic effects on cells. Various models have been suggested for estimating membrane toxicity and
70 the erythrocyte (RBC) hemolysis model is one of them. The availability of blood, easy isolation of RBC, and
71 the membrane similarities of RBC to other cells membrane are advantages of this model. Surfactants are
72 known to produce supramolecular structures called micelles only when the concentration of surfactant is at or
73 greater than the CMC. CMC is one of the most important physical parameters of surfactants and can be
74 estimated through some physicochemical properties of surfactants such as interfacial tension, conductivity,
75 viscosity [3, 4], osmotic pressure, density [5], polarity, specific heat, UV–Vis spectrophotometric method [6]
76 refractive index [7], and solubilization power [8, 9]. During the formation of micelles, the effects of different
77 factors including surfactant structure, temperature, pressure, pH, and additives on micellization can be

represented by thermodynamics. Several investigations have been carried out on temperature and additives effects as these parameters significantly affect surfactants properties. Thermodynamic parameters of micellization include standard Gibbs free energy (ΔG_m°), enthalpy (ΔH_m°) and entropy (ΔS_m°) and have been used to quantify the relative importance of hydrophobic interactions and surfactant-water contacts [10]. These parameters can be derived from Van't Hoff equation that describes the dependency of CMC on temperature. CMC of nonionic surfactants decreases by increasing the temperature up to 50°C. Breaking hydrogen bonds between the water and hydrophilic chains of surfactants is the reason for the reduction of CMC a process that has been demonstrated in nonionic surfactants at increasing temperatures [11, 12]. Surfactants and alcohols are often used together as PEs and co-solvent in some formulations. Therefore, the effect of short-chain alcohols addition on the thermodynamic micellization parameters of nonionic surfactants has been considered by Ruiz and co-workers [13] and Glenn and co-workers [14] who evaluated the effect of ethylene glycol on micellization of tween surfactants 20, 40, 60, and 80. They reported that the structure breaking ability of ethylene glycol and its interaction with the hydrophilic groups of the surfactant were the main controlling factors in the micellization leading to increased micellization [13, 14]. In another study, it was shown by the addition of ethanol and ethylene glycol to anionic surfactant aqueous solution, the Gibbs free energy of micellization became less negative in comparison to treatments without any additives. The ΔS_m° was larger than the ΔH_m° in aqueous solution without any co-solvent while in the presence of co-solvent the enthalpic role predominates [15]. Since the polysorbates are used frequently in formulations and usually they are in combination with some alcohols, few studies appear in the literature on these mixtures. In this study, the simultaneous effects of some short-chain alcohols on erythrocyte lysis and the thermodynamics of micellization were investigated.

2. Materials and methods

2-1- Material

The surfactant Tween 20 (Polyoxyethylenesorbitan monolaurate), Tween 40 (Polyoxyethylenesorbitan monopalmitate), Tween 60 (Polyoxyethylenesorbitan monoestearate), 80 (Polyoxyethylenesorbitan

monooleate), citric acid (analytical grade), sodium chloride, potassium ferricyanide, potassium cyanide, PG (analytical grade), IPA (analytical grade) and potassium dihydrogen phosphate were obtained from Merck, Germany. Ethanol 96% and Glycerol were obtained from Sepidaj, Iran. Doubly distilled and deionized water was used to prepare all fresh solutions.

2-2- Hemolysis evaluation

2-2-1- Preparation of RBC suspension

Human blood was collected from a healthy volunteer in a heparinized tube. After centrifugation (Spectrafuge 161M, England) at 3000 rpm for 10 min RBC were separated, washed three times with McIlvaine's buffer (0.2 M Na₂HPO₄, 0.1 M citric acid adjusted to pH 7.0) and erythrocytes re suspended in the same buffer to achieve a suspension with 12% hematocrit.

2-2-2- Assessment of hemolysis

Mixtures of different ratios of each polysorbate (Tweens) and alcohols (200μl) were added to equal volume of RBC suspension and incubated at 25 and 37°C for 15 and 30min. After incubation intervals, the mixtures were centrifuged at 3000rpm for 35s and 200μl of supernatants were added to 3ml of Drabkin's reagent (200mg potassium ferricyanide, 50mg of potassium cyanide, 1 g of sodium bicarbonate, and 0.1% nonionic detergent in 1 liter of water). The absorbance of the samples at 540nm was read by a Synergy 2 microplate ELISA reader (BioTek Inc., Winooski, USA). Positive control included 200μl of non-centrifuged erythrocyte suspension and 200μl of McIlvaine's buffer which was mixed with 3ml Drabkin's reagent in which the whole RBCs were lysed. Negative control was prepared by a mixture of 200μl erythrocyte and 200μl buffer then centrifuged for 35s and 200μl of supernatant was mixed with 3 ml Drabkin's reagent. The hemolysis percentage was calculated using the following equation:

Equation 1

$$\% \text{Hemolysis} = [(Abs_s - Abs_{neg}) / (Abs_{pos} - Abs_{neg})] * 100$$

In this equation Abs_s is the UV absorption of the sample and Abs_{neg} and Abs_{pos} are UV absorption of negative and positive control respectively. All experiments were carried out triple times and every samples freshly prepared [4].

2-3- Critical micelle concentration and thermodynamic studies:

The surface tension of solutions at various concentrations and temperatures were determined by Krüss tensiometer K100, Germany with Wilhelmy platinum plate at constant conditions. The CMC was taken from the breakpoint in the plot of surface tension vs logarithms of concentration values. In order to ensure constancy of result the surface tensions of deionized water were periodically examined. The thermodynamic of micellization was evaluated using CMC results obtained from experiments.

3. Results

3-1- Hemolytic activity measurement

Hemolytic activity of tweens (20, 40, 60, and 80) and alcohols (ethanol, isopropyl alcohol, PG, and glycerol) at two temperatures and two incubation times are shown in table 1 and 2.

Table 1: Hemolysis percentage of tween 20, 40, 60, and 80 at two temperatures and incubation times (n=3)

Conc %	25°C, 15 min								37°C, 15 min							
	tween 20	SD	tween 40	SD	tween 60	SD	tween 80	SD	tween 20	SD	tween 40	SD	tween 60	SD	tween 80	SD
0.1	10	0.3	8	0.2	3	0.1	12	0.4	15	0.5	10	0.2	5	0.2	14	0.3
0.2	15	0.7	11	0.5	7	0.1	16	0.4	19	0.9	12	0.2	11	0.3	18	0.3
0.5	25	1.1	22	1.0	12	0.6	24	0.9	28	1.8	25	1.1	18	0.9	26	1.5
1	35	1.2	32	1.1	22	1.2	36	1.9	39	2.1	34	1.0	30	1.0	37	1.6
2	45	1.5	39	1.8	27	1.9	77	2.1	47	1.1	40	2.1	37	1.8	40	2.2
5	69	2.1	93	4.3	87	2.8	94	3.9	89	3.3	100	4.2	100	2.9	100	4.9
Conc %	25°C, 30 min								37°C, 30 min							
	tween 20	SD	tween 40	SD	tween 60	SD	tween 80	SD	tween 20	SD	tween 40	SD	tween 60	SD	tween 80	SD
0.1	12.5	0.7	9.3	0.2	5.3	0.1	13.2	0.3	16.3	1.1	9	0.2	6	0.1	15	0.5
0.2	17.3	0.6	12.4	0.3	9.2	0.1	17.4	0.2	20.01	1.0	10	0.3	10	0.6	20	1.0
0.5	27.9	1.0	22.4	1.2	12.4	0.6	25.2	1.0	28.4	1.8	21	1.1	22	1.2	27	1.0
1	37.8	1.7	39.9	1.6	24.2	1.5	38	1.4	40.2	2.3	31	2.1	33	1.5	38	2.1
2	55.2	1.9	49.4	2.2	29.4	1.4	81.3	3.5	45	2.1	38	1.8	39	1.3	41	2.3
5	79	2.7	95	3.8	90	4.3	97	3.3	100	3.8	100	3.1	100	3.3	100	4.1

Table 2: Hemolysis percentage of ethanol, IPA, PG, and glycerol in two temperatures and incubation times

Conc %	25°C, 15min								37°C, 15min							
	Ethanol	SD	IPA	SD	PG	SD	GLY	SD	Ethanol	SD	IPA	SD	PG	SD	GLY	SD
0.5	1.2	0.1	3.1	0.1	2.3	0.1	1.1	0.1	2.28	0.1	4.34	0.1	3	0.1	2.9	0.1
1	3.7	0.1	5.4	0.1	3.5	0.2	4.2	0.2	7.03	0.2	7.56	0.1	4	0.1	6	0.2
2	7.5	0.3	9.5	0.2	6.3	0.2	6.2	0.2	12	0.6	13.3	0.7	8.5	0.2	8	0.2
5	12.4	0.8	16.1	0.6	9.2	0.5	10.3	0.5	21	1.2	22.54	1.6	14	0.7	11.1	0.6
10	27.1	1.7	33.2	1.8	18.3	0.9	15.1	0.7	42	2.2	46.48	2.0	18	0.8	17	0.9
Conc %	25°C, 30min								37°C, 30min							
0.5	2.4	0.1	4.34	0.1	3.7	0.1	2.5	0.1	1.92	0.1	3.72	0.1	4.2	0.2	3.05	0.1
1	4.44	0.1	7.56	0.2	4.9	0.1	5.6	0.2	5.92	0.1	7	0.2	5.4	0.2	6.15	0.1
2	9	0.3	13.3	0.2	7.7	0.2	7.6	0.3	12	0.3	14	0.2	8.2	0.3	8.15	0.5
5	14.88	0.6	22.54	0.9	10.6	0.4	11.7	0.5	21	1.1	23	0.9	11.1	0.6	12.25	0.5
10	32.52	1.4	46.48	1.1	19.7	0.9	16.5	0.8	31	1.5	36	1.4	20.2	1.1	17.05	0.9

Hemolytic activity of tweens in combination with each alcohol are shown in figures 1–3.

Figure 1: hemolysis activity of tween 80 combination with different alcohols at various concentrations: a) ethanol, b) glycerol, c) IPA and, d) PG

Figure 2: hemolysis activity of tween 20 combination with different alcohols at various concentrations: a) ethanol, b) glycerol, c) IPA and, d) PG

Figure 3: hemolysis activity of tweens 40, 60 combination with different alcohols at various concentrations: a) ethanol, b) glycerol, c) IPA and, d) PG

3.2. Critical micelle concentration and thermodynamic study:

Thermodynamic study of nonionic surfactants was determined based on CMC to investigate the effect of alcohols on it. The surface tension (γ) of surfactants were measured at different concentrations and temperatures. The CMC values were calculated from the surface tension versus surfactant logarithmic concentration plot. The concentration which was the intersection point of the two linear portions of the plots was reported as CMC point. CMC data of Tween 80 in combination with different concentrations of glycerol and ethanol at different temperatures are listed in table 3.

Table 3: CMC of tween 80 in various ethanol and glycerol concentrations in aqueous solution at different temperatures

Additive Concentration %		CMC values (mmol/l)		
		293 K	308 K	323 K
Ethanol	0	0.013	0.011	0.008
	1	0.014	0.014	0.014
	10	0.016	0.017	0.019
glycerol	0	0.013	0.011	0.008
	1	0.013	0.013	0.014
	10	0.014	0.015	0.016

In order to estimate the thermodynamic parameters of micellization, the temperature dependence of the surfactants CMC could be used. The standard Gibbs free energy of micelle formation per mole of surfactant monomer (ΔG°_m) was determined according to the mass action model which related to the CMC by the following equation 2:

181 Equation 2

182
$$\Delta G_{mic}^0 = RT \ln X_{CMC}$$

183

184 where XCMC is the surfactant mole fraction in CMC point. By putting the Gibbs-Helmholtz equation (Eq. 3)
185 in equation 2 enthalpy of micellization could be calculated as follows.

186 Equation 3

187
$$\left(\frac{\partial \left(\frac{G}{T} \right)}{\partial T} \right)_P = - \frac{H}{T^2}$$

188 Equation 4

189
$$\Delta H_{mic}^0 = -RT^2 \left(\frac{\partial \ln X_{CMC}}{\partial T} \right)_P$$

190 Equation 5 could be used for calculation the entropy of micellization that enthalpy and Gibbs free energy
191 obtain from above equations.

192 Equation 5

193
$$\Delta S_{mic}^0 = \frac{\Delta H_{mic}^0 - \Delta G_{mic}^0}{T}$$

194 By using above equations, the calculated thermodynamic parameters for ethanol and glycerol at different
195 concentrations in water are presented in table 4 and 5.

196 The effect of additives on micellization could be estimated by free energy named free energy of transfer
197 (ΔG_{trans}^0) the free energy of transfer was obtained from equation 6 in which the additives were ethanol and
198 glycerol.

199 Equation 6

200
$$\Delta G_{trans}^0 = (\Delta G_{mic}^0)_{additive+H_2O} - (\Delta G_{mic}^0)_{H_2O}$$

201

Table 4: Micellization thermodynamics of tween 80 in various ethanol concentrations in water mixture

Ethanol (%)	T (K)	ΔG_{mic}^0 (kJmol ⁻¹)	ΔH_{mic}^0 (kJmol ⁻¹)	$T\Delta S_{mic}^0$ (kJmol ⁻¹)	ΔG_{trans}^0 (kJmol ⁻¹)
0	293	- 37.25	10.76	48.01	-
	308	- 39.52	11.89	51.42	-
	323	- 42.28	13.08	55.36	-
1	293	- 37.08	- 0.85	36.24	0.16
	308	- 38.93	- 0.94	37.99	0.59
	323	- 40.79	- 1.03	39.76	1.49
10	293	- 36.60	- 4.69	31.90	0.65
	308	- 38.20	- 5.18	33.02	1.32
	323	- 39.81	- 5.70	34.11	2.47

Table 5: Micellization thermodynamics of tween 80 in various glycerol concentrations in water mixture

Glycerol (%)	T (K)	ΔG_{mic}^0 (kJmol ⁻¹)	ΔH_{mic}^0 (kJmol ⁻¹)	$T\Delta S_{mic}^0$ (kJmol ⁻¹)	ΔG_{trans}^0 (kJmol ⁻¹)
0	293	- 37.25	10.76	48.01	-
	308	- 39.52	11.89	51.42	-
	323	- 42.28	13.08	55.36	-
1	293	- 37.16	- 0.87	36.29	0.09
	308	- 39.04	- 0.97	38.07	0.49
	323	- 40.87	- 1.06	39.80	1.41
10	293	- 36.77	- 2.84	33.93	0.48
	308	- 36.65	- 3.13	33.52	2.87
	323	- 36.48	- 3.45	33.03	5.80

In order to evaluate the enthalpy–entropy compensation phenomenon, micellization enthalpy of tween 80 were plotted versus $T\Delta S_{mic}^0$, these data were obtained from micelle formation at different concentrations of ethanol and glycerol mixtures, a and b respected in Fig. 4. The enthalpy and the entropy change had a linear correlation that could be described by the equation 6.

Equation 7

$$\Delta H_{mic}^0 = \Delta H_{0,mic}^0 + \beta T\Delta S_{mic}^0$$

Figure 4: ΔH_{mic}^0 versus $T\Delta S_{mic}^0$ at 293 K for the micellization of tween 80 in different ethanol / glycerol-water mixtures (a: ethanol and b: glycerol)

4. Discussion:

Various components have been used in pharmaceutical formulations to improve the penetration of active ingredients to the inner layers of skin. Surfactants and alcohols are two main groups of PEs that are widely used in topical preparations as a solubilizer and penetration enhancer on stratum corneum by reversibly altering the bilayer cell membranes may potentially be toxic to the cells. In this study, we aimed to determine the toxicity of these materials on cells using the erythrocyte model technique. In this technique, we evaluated the extent of RBC hemolysis after exposure to different concentrations of surfactants and alcohol at two temperatures and durations. Surfactants and alcohols can lyse erythrocytes by enhancing penetration of some compounds through the cell membrane resulting in increased osmotic pressure inside cells which destroys the RBC [16, 17]. The degree of hemolysis depends on surfactants and alcohol (PEs) concentrations, temperature, and contact time with erythrocytes. In this study, we used different concentrations of surfactants (0 - 5 % w/v) and alcohols (0 - 10 % w/v) at two temperatures (25 and 37°C) and two exposure durations of 15 and 30 minutes. By increasing the concentration of surfactants and alcohol more hemolytic activity was observed. Similar results were reported by previous researches more hemolysis occurs when higher concentrations of PEs molecules are in contact with RBCs' cell membrane. Cell membranes consist of

bilayers that have liquid and jelly characteristic. These structures contain phospholipids which convert to the jelly mode when temperature decreases below 37°C. This conversion of phospholipids to jelly form results in a more stable cell membrane which increases resistance against cell lysis [18]. Therefore, the degree of hemolysis in samples at 37°C was more than that at 25°C except for glycerol and PG.

Increasing the exposure time and prolonging contact duration of PEs with the erythrocytes led to increased hemolytic effects which have also been reported by other researches [19]. When using short-chain alcoholic compounds while the number of hydroxyl groups increases, the hydrophilicity increases so their penetration into the cell membrane decreases. When alcohols have the same number of hydroxyl groups, other factors that may affect their hydrophilicity can cause differences in the levels of hemolytic activities. When alcoholic agents have higher molecular weights the compound consequently have higher melting point and accordingly lower hemolysis levels. When alcohol and tweens are used together, the combination of ethanol (a short-chain alcohol) and tween results in higher levels of hemolysis while when a mixture of glycerol (higher chain alcohol) and tween is used less hemolysis effects are observed. According to table 3, the CMC values of tween 80 decreased when the temperature increased which again is consistent with previous studies [11]. This occurs because of higher molecular movements at elevated temperatures as the chance of hydrogen bond formation decreased. The increase in temperature causes a reduction in hydration of the hydrophilic oxyethylene groups in tweens that is favorable for micellization. The hydrophilicity of the surfactant molecules was decreased by the reduction of hydrogen bonding so micelles formed at lower concentration. When the temperature increases further, the movement of water and surfactant molecules increases, which cause an increase CMC value [12]. The CMC has been affected by the addition of ethanol and glycerol. Hydrophobic bonds have important role in micelle formation in surfactant mixtures. In nonionic surfactant mixtures, the molecules that form micelles compact closely so penetration of ethanol molecules into the micelles would decrease. Therefore low concentration of ethanol and glycerol would have little effect on the CMC but by increasing ethanol and glycerol concentration, hydrophobic interactions between the hydrophobic tails of surfactants were gradually reduced. As a whole, the addition higher of ethanol and

glycerol concentration increase gradually the CMC of the mixture [20, 21]. Increasing the temperature of the surfactant mixture in the presence of glycerol or ethanol makes the CMC go up which is opposite to that surfactant mixture with water. This CMC increasing is attributed to the disruption of the solvent structure at higher temperature [13, 14]. The thermodynamic parameter has an important role in realizing the micellization process. The interpretation of these parameters is necessary to consider the effects of co-solvents and temperature on the CMC value [22]. According to the results, the free energy of micellization in surfactant mixtures alone and with additives (ethanol and glycerol) is negative and becomes less negative as the content of the additives in the solution increases. The enthalpy value of micellization indicates that it is an exothermic process and decreases with increasing ethanol and glycerol concentrations. In Micellization endothermic and exothermic processes are effective. As ethanol and glycerol added to the surfactant mixture breaking up the hydrophobically structured water surrounding the hydrocarbon chains is an exothermic effect while the endothermic effect due to the transfer of the hydrophobic groups from water to the aqueous co-solvent mixture and the exothermic process is dominant over the endothermic process. Entropy change if micelle formation is related to aggregation of surfactant molecules so its change is predictably negative. On the other hand, the hydrophobically structured water around the hydrophobic tail becomes free or destructured when micelles are formed. The entropy of micellization is a combination of the above two effects. Thus, with increasing content of co-solvent in the surfactant mixture, the entropy values decrease meanwhile the values of enthalpy become more negative. Therefore, at the higher co-solvent concentration enthalpy is the dominating factor in the forming micelles process as shown in tables 4 and 5. In order to evaluate the effect of co-solvent on the micellization, the free energy of transfer (ΔG_{trans}) could be used. The values of ΔG_{trans} is positive for all systems [23]. The ΔG_{trans} value increases with increasing concentration of co-solvent because the hydrophobic tails of the tween interact with the hydrophobic part of co-solvent and hydrophilic heads of tween interact with water that decreases the aggregation of surfactant monomers [15, 23]. In enthalpy–entropy compensation phenomenon, micellization is divided into “solvation” and “chemical” part [24]. From the slope of the enthalpy-entropy compensation plot, the solvation part of

micellization can be obtained and the intercept of this plot indicates the chemical part of the micelle formation [25]. As shown in Fig 4, compensation between the $T\Delta S_{mic}^0$ and ΔH_{mic}^0 values is found in different concentration of ethanol and glycerol which ΔH_{0mic}^0 is the intercept of ΔH_{mic}^0 against $T\Delta S_{mic}^0$ plot and its slope is β . The ΔH_{0mic}^0 and β for ethanol - water mixture were $-35.61 \text{ kJ mol}^{-1}$ and 0.96 and for glycerol-water mixture were $-36.05 \text{ kJ mol}^{-1}$ and 0.97 respectively. In equation 7, β represent a quantitative correlation of the enthalpy–entropy compensation. The negative value of ΔH_{0mic}^0 (the intrinsic enthalpic gain) confirms that the micellization is desirable although the entropic gain is not exciting.

5. Conclusion:

Presence of four short chain alcohols with non ionic surfactants (Tweens) affected on the RBC lysis and micellization properties. Different actions of lysis on RBCs were seen in alcohols and poly sorbates combination such as higher lysis in mixture of short chain alcohol (ethanol) in comparison with higher chain alcohol (glycerol). The thermodynamic study of these mixtures was shown alcohols reduced micellization tendency in Tween 80 due to structure breaking ability of alcohols.

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Conflict of interest statement:

The authors report no conflict of interest.

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