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Full Length Research Paper

Evaluating the toxicity of permeability enhancers of polyethylene glycol brij ethers surfactants group on cellular membranes and some of their physicochemical properties

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The aim of this study is to evaluate the effect of polyethylene glycol brij ethers surfactants group on red blood cells as a model for biological membranes. Also in this study, physicochemical properties including emulsification index (E_{24}), foam producing activity (F_h) and critical micelle concentration (cmc) were studied. Surfactant solutions were prepared in McIvan's buffer in specific concentrations. 0.2 ml of red blood cells (RBC) was mixed with 0.2 ml of each surfactant solution. The four surfactant solutions had each been incubated differently at two different temperatures for three different times. Each test was done six times. The results were presented as mean absorbance ± the standard deviation. E_{24}, F_h and cmc were also determined for each surfactant solution. All of the surfactant solutions showed hemolytic activity. In comparison with the four studied surfactants, brij 56 had the highest hemolytic effect and brij 72 the lowest. The values of E_{24} and F_h had good correlation with hydrophilic-lipophilic balance values. According to the results of this study, brijs should be used at concentrations lower than cmc in formulations. Also, according to the results, the use of brijs with low hemolytic effect such as brij 72, is preferred in pharmaceutical preparations.

Key words: Brij, biological membrane, hemolysis, hydrophile-lipophile balance (HLB).

INTRODUCTION

Surfactants have many characteristics comprising groups with hydrophilic and hydrophobic characters. They have different uses in pharmaceutical formulations, such as co-solvent, humectant, emulsifying, solubilizing agent and enhancer. Regarding their hydrophilic part, they are divided into four groups: anionic, cationic, amphoteric and non-ionic (Dehghan Noudeh et al., 2008).

Absorption enhancing ability of surfactants in formulations with low absorption like peptides or proteins is used for drug delivery in non-injectable formulations. Broad spectrums of surfactants are used as enhancers including bile salts, anionic detergents, glycerides and lysophospholipids (lysolecithins); however, the efficacy of non-ionic surfactants with moderate polarity is better. On the other hand, it is reported that non-ionic polar surfactants do not have toxicity, while surfactants with moderate polarity showed toxic effects (Gould, 2000).

Morphological and biochemical studies on the membrane

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Abbreviations: E24, Emulsification index; **Fh,** foam producing activity; **Brij 52,** polyoxyeethylene2cetylether; **Brij 56,** polyoxy-eethylene10cetylether; **Brij 72,** polyoxyeethylene- 2stearylether; **Brij 92,** polyoxyeethylene2oleylether; **HLB,** hydrophile-lipophile balance; **cmc**, Critical Micelle Concentration.

absorption sites showed that surfactants enhance membrane transport followed by acute toxicity, but these effects were reversed after a long time. As a result, there is a pivotal relationship between permeability enhancement activity and acute toxicity. Moreover, permeability enhancing effect of surfactants is not only related to their nature, but also depends on other characteristics like electrical charge, polarity and the membrane (Galembeck et al., 1998; Gould, 1996).

Permeability enhancers are agents that decrease or remove extra cellular layer resistance reversibly and allow the drug to pass through and between epithelial cells towards blood and lymph. Recently, enhancing drug permeability through cellular membrane has become one of the main topics in pharmaceutical research (Muranishi, 1990).

Gould, (1996) showed that some non-ionic surfactants could increase mucosal absorption of drugs with low absorption. One of the suggested mechanisms is inducing a partial but reversible gap within cell membranes' and consequently increasing the permeability by surfactants or other enhancers. Various models exist for evaluation of membrane toxicity of surfactants including single cell models using erythrocytes, erythrocyte ghosts, or liposomes. The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of the erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood; moreover, its membrane has similarities with other cell membranes (Gould, 1996).

Evaluating the toxicity of permeability enhancers using biological membranes plays an important role in current pharmaceutical research. Consequently, in the present study we decided to determine the effects of polyoxyeethylene polyoxyeethylene2cetylether (Brij 52), polyoxyeethylene10cetylether (Brij 56), polyoxyeethylene2-stearylether (Brij 72) and polyoxyeethylene2oleylether (Brij 92) on cellular membrane using the erythrocyte model.

MATERIALS AND METHODS

Materials

All materials were of reagent grade unless otherwise mentioned. Brij 52, Brij 56, Brij 72 and Brij 92 were prepared from Fluka (Netherlands). Sodium chloride, di-sodium hydrogen phosphate, citric acid (monohydrate), di-sodium phosphate and liquid paraffin were purchased from Merck (Germany). Drabkin's agent was supplied from Chimi-Daru (Iran).

Buffer and reagents preparation

McIlvaine's buffer was prepared as follows: Solution 1, containing 21 g of citric acid (100 mmol) and 8.775 g of sodium chloride (150 mmol) made up to 1000 ml with deionized water, was mixed with solution 2, containing 28.4 g of di-sodium hydrogen phosphate (200 mmol) and 8.775 g of sodium chloride (150 mmol) made up to 1000 ml with deionized water, to produce the required pH of 7.0. Solution's pH was measured by electrical pH-meter (TWT Metrohm, Germany).

Preparation of red blood cells suspension

Human blood was collected from a healthy individual with 46.7% hematocrit and added to four heparinized tubes. After centrifuging at 3000 rpm for 10 min (Hermle 230 ZA,Germany), plasma and buffy coat were removed and the erythrocytes were washed three times in at least five times of their volume with McIlvaine's buffer, pH = 7.0. Afterward, by adding McIlvaine's buffer, an erythrocyte suspension with 12% hematocrit was prepared and kept in 4°C for experiments (Gould et al., 2000).

Hemolytic method

A suspension of erythrocyte (200 µl) within a micro-tube was incubated for the required times with an equal volume of the test sample of surfactants mixture, including 52, 56, 58, 72 or 92, prepared in McIlvaine's buffer, at 25 and 37°C. After incubation, the mixtures were spun in a microcentrifuge at 3000 rpm for 35 s (Spectrafuge 161M, England), and 200 µl of the resulting supernatants was added to 3 ml of Drabkin's reagent. To measure the amount of hemoglobin released, the absorbance of the samples was assessed at 540 nm wavelength using spectrophotometer (Shimadzu 3100, Japan). Positive controls consisted of 200 µl of uncentrifuged mixtures of erythrocyte suspensions and 200 µl of buffer, which were added to 3 ml Drabkin's reagent to obtain a value for 100% haemolysis. A negative control, included to assess the level of spontaneous haemolysis, comprised 200 µl buffer mixed with 200 µl erythrocytes and after centrifugation for 35 s, a 200 µl sample of supernatant was added to 3 ml of Drabkin's reagent. Haemolysis percentage for each sample were calculated by dividing sample's absorbance on positive control absorbance (compete haemolysis) multiplied by 100 (Gould et al., 2000).

Determination of emulsification Index

For estimation of the emulsification index, 5 ml of liquid paraffin was added to 5 ml of different concentrations of surfactants in a graduated tube and vortexed at high speed for 2 min. The emulsion stability was determined after 24 h. The E_{24} was calculated by measuring the emulsion layer formed (Carrillo et al., 1996).

Foam formation activity

Different concentrations of surfactants were dissolved with 5 ml disodium phosphate buffer and shaken with a vibrator for 5 s. The samples were put aside at 25°C for one minute. Foam activity was measured as foam height in graduated cylinder (Porter, 1994).

Critical micelle concentration (cmc)

For estimation of the cmc, surface tension was determined by tensiometer (KRUSS K100, Germany), (data not shown).

RESULTS

The results of haemolysis induced by the surfactants are

Table 1. Hemolysis induced by Brijs 52, 56, 72 and 92 after 15 min at 25°C (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	0.20	0.115	0.10	0.115	0.00	0.200	0.28	0.153
0.05	0.30	0.000	0.10	0.100	0.13	0.153	0.14	0.115
0.1	0.40	0.058	0.10	0.100	0.13	0.173	0.28	0.208
0.14	0.59	0.252	0.62	0.153	0.00	0.173	0.43	0.153
0.2	1.19	0.208	0.10	0.231	0.13	0.208	0.85	0.361
0.3	4.06	0.404	0.72	0.208	0.38	0.208	1.56	0.100
0.4	26.96	1.311	0.62	0.100	0.25	0.058	1.42	0.231
0.6	92.57	0.557	0.41	0.252	0.38	0.153	1.70	0.153
1	97.22	1.206	0.72	0.153	1.27	0.208	1.85	0.208
2	98.51	1.153	1.85	0.153	1.39	0.100	1.99	0.153
4	99.01	0.896	2.06	0.252	2.15	0.153	2.41	0.153
6	98.61	1.060	2.57	0.173	2.41	0.153	2.27	0.231
10	99.21	1.054	2.98	0.208	2.41	0.252	2.70	0.231

Table 2. Hemolysis induced by Brijs 52, 56, 72 and 92 after 30 min at 25°C (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	0.40	0.153	0.00	0.058	0.00	0.173	0.14	0.153
0.05	0.40	0.115	0.10	0.208	0.00	0.153	0.00	0.173
0.1	0.60	0.200	0.10	0.173	0.00	0.115	0.28	0.153
0.14	0.71	0.208	0.10	0.153	0.00	0.153	0.14	0.000
0.2	0.91	0.321	0.21	0.115	0.00	0.321	0.57	0.153
0.3	6.05	0.400	0.10	0.153	0.13	0.265	2.97	0.379
0.4	72.28	0.252	0.21	0.173	0.13	0.058	3.39	0.153
0.6	97.28	0.693	0.21	0.100	0.25	0.115	3.68	0.252
1	98.69	0.306	1.14	0.153	0.51	0.000	3.96	0.208
2	99.29	0.907	2.59	0.100	0.88	0.200	3.96	0.321
4	99.60	0.907	4.56	0.252	2.90	0.200	4.10	0.300
6	99.29	1.305	6.85	0.153	2.90	0.173	4.24	0.173
10	99.50	0.513	7.37	0.265	3.16	0.252	4.24	0.100

shown in Tables 1-6. Tables 1-3 are related to haemolysis after 15, 30 and 45 min (incubation temperature 25°C). Tables 4 - 6 are related to haemolysis after 15, 30 and 45 min (incubation temperature 37°C). Results of emulsification index and foaming formation are presented in Tables 7 and 8, respectively.

DISCUSSION

Despite the fact that not all is known about surfactant's hemolytic activity, it is proposed that it may consist of the following processes: Absorption of surfactant molecules on cellular surface, penetration of surfactant molecules

Table 3. Hemolysis induced by Brijs 52, 56, 72 and 92 after 45 min at 25°C (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	0.40	0.100	0.00	0.000	0.14	0.231	0.00	0.100
0.05	0.61	0.208	0.00	0.058	0.00	0.231	0.15	0.058
0.1	0.61	0.153	0.10	0.100	0.14	0.153	0.00	0.000
0.14	1.31	0.115	0.10	0.058	0.29	0.153	0.15	0.058
0.2	1.41	0.306	0.10	0.173	0.29	0.058	1.05	0.153
0.3	8.28	0.252	0.10	0.100	0.72	0.115	5.98	0.265
0.4	77.78	0.666	0.21	0.289	1.30	0.100	5.83	0.100
0.6	97.07	0.100	0.10	0.115	1.73	0.252	5.68	0.115
1	97.27	1.767	2.15	0.115	3.03	0.265	6.28	0.153
2	98.68	0.781	3.90	0.306	4.05	0.200	5.98	0.208
4	98.38	0.458	5.44	0.321	6.50	0.153	5.98	0.000
6	98.89	0.557	5.74	0.404	6.79	0.153	6.13	0.265
10	99.09	0.404	8.31	0.115	7.08	0.153	6.58	0.289

Table 4. Hemolysis induced by Brijs 52, 56, 72 and 92 after 15 min at 37°C (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	0.51	0.115	0.00	0.058	0.24	0.153	0.29	0.100
0.05	0.61	0.115	0.00	0.058	0.12	0.306	0.43	0.115
0.1	0.51	0.121	0.10	0.058	0.24	0.058	0.29	0.200
0.14	0.61	0.153	0.20	0.058	0.24	0.173	0.43	0.208
0.2	1.73	0.458	0.31	0.265	0.37	0.058	0.86	0.208
0.3	6.72	0.624	0.31	0.200	0.37	0.100	1.87	0.153
0.4	49.39	0.493	0.31	0.153	0.37	0.100	2.44	0.115
0.6	97.45	0.231	0.61	0.153	0.61	0.115	2.30	0.173
1	99.39	1.015	1.33	0.153	0.98	0.200	2.15	0.300
2	99.19	0.451	1.84	0.153	1.22	0.200	2.44	0.265
4	98.98	0.954	2.66	0.153	2.08	0.153	2.87	0.200
6	99.59	0.500	3.59	0.153	2.08	0.300	3.16	0.058
10	99.90	0.100	4.20	0.252	2.20	0.208	3.44	0.252

into cellular membrane, initiation of alterations within cellular membrane, increasing permeability of cellular membrane, gradual increase of osmotic phenomenon followed by destruction of cellular membrane and haemolysis.

According to the above explanation, two different effects from surfactants in hemolytic studies can be observed; the first one is increasing cellular membrane permeability and the latter is cellular lysis. Surfactants which induce haemolysis can alter the membrane permeability for hemoglobin. This alteration occurs in a specific spectrum of the surfactant concentration while in lower concentrations hemolytic effects cannot be seen; in these concentrations, cellular membrane is permeable for low molecular weight molecules. Destruction due to surfactants is the result of cellular membrane breakage by alteration of structural molecules of the membrane. Subsequently, the membrane permeability for macro molecules similar to smaller molecules increases. In this chain reaction mechanism, surface active agents adhere to the erythrocyte surface and enter inside, changing the molecular structure of the membrane, which results in colloid-osmotic swelling of the erythrocyte and ultimately cellular rupture. Micelle production from surfactant molecules and membrane phospholipids leads to an increase in membrane perme-

Table 5. Hemolysis induced by Brijs 52, 56, 72 and 92 after 30 min at 37°C (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.000	0.000
0.01	0.30	0.153	0.10	0.173	0.12	0.100	0.14	0.153
0.05	0.40	0.153	0.20	0.153	0.12	0.058	0.00	0.058
0.1	0.61	0.153	0.10	0.100	0.12	0.208	0.14	0.100
0.14	0.81	0.361	0.10	0.100	0.25	0.153	0.14	0.200
0.2	1.01	0.289	0.71	0.115	0.37	0.252	1.70	0.153
0.3	9.00	0.529	0.92	0.208	0.62	0.153	2.84	0.351
0.4	74.72	0.569	1.02	0.100	0.37	0.153	3.40	0.058
0.6	97.47	0.643	1.33	0.153	0.62	0.058	4.40	0.265
1	98.08	0.252	1.73	0.153	1.00	0.252	5.53	0.173
2	98.48	0.666	2.96	0.153	1.50	0.200	5.82	0.208
4	99.80	0.379	5.31	0.265	4.99	0.173	5.82	0.379
6	99.49	0.757	9.47	0.265	5.61	0.100	5.96	0.153
10	99.19	0.961	10.20	0.252	6.48	0.153	5.96	0.153

Table 6. Hemolysis induced by Brijs 52, 56, 72 and 92 after 45 min at 37 °C (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD	H (%) (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	0.81	0.361	0.00	0.100	0.13	0.173	0.14	0.265
0.05	0.81	0.265	0.00	0.115	0.13	0.208	0.14	0.153
0.1	0.91	0.058	0.00	0.100	0.13	0.058	0.14	0.100
0.14	1.01	0.208	0.10	0.153	0.26	0.252	0.29	0.115
0.2	1.31	0.200	0.10	0.000	0.26	0.100	1.72	0.208
0.3	10.88	0.513	0.21	0.115	1.03	0.153	8.33	0.361
0.4	95.77	1.308	0.21	0.058	1.16	0.252	8.33	0.265
0.6	97.18	0.416	0.51	0.252	1.03	0.153	8.33	0.265
1	98.39	0.757	2.05	0.321	1.03	0.400	8.91	0.361
2	99.30	2.050	4.72	0.231	1.16	0.306	8.76	0.153
4	98.39	0.436	5.74	0.153	5.14	0.458	8.76	0.153
6	99.60	0.656	9.13	0.306	5.91	0.173	9.05	0.265
10	99.30	0.702	10.15	0.321	7.58	0.451	9.48	0.058

ability and colloid-osmotic lysis of the erythrocyte. The above mechanism highly depends on surfactant concentration and temperature and by increasing in these factors, the level of haemolysis increases. These effects support the idea of surfactant usage as an absorption enhancer (Zaslavsky et al., 1987; Kleszczynska et al., 2005).

Haemolysis is due to the destruction of red blood cells which resulted from the lysis of membrane lipid bilayer emulsion and cellular membrane destruction. As this haemolysis relates to the concentration and potency of surfactants, the model can be used for the evaluation of surfactants potency. Biological membranes consist of a lipid bilayer which surrounds the whole cell surface and the proteins. Lipid bilayer structure is stabilized by non-covalent bonds among acyl groups and ionic bonds between polar heads and aqua. In non-ionic surfactants, the interaction with biological membranes needs hydrophobic interaction between alkyl chains of surfactant and lipoprotein parts of membrane (Swenson and Curatolo, 1992).

In this study, the hemolytic effects of surfactants increased as temperature increased. Note that the liquid characteristic and fluidity of the bilayer lipid is one of its

Table 7. Emulsification index at different concentration of Brijs 52, 56, 72 and 92 (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	E_{24} (Mean)	±SD	E_{24} (Mean)	±SD	E_{24} (Mean)	±SD	E_{24} (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.05	9.09	0.658	0.00	0.000	0.00	0.000	0.00	0.000
0.1	38.74	0.599	0.00	0.000	0.00	0.000	0.00	0.000
0.14	53.17	0.687	0.00	0.000	0.00	0.000	0.00	0.000
0.2	53.57	0.000	0.00	0.000	0.00	0.000	1.18	0.000
0.3	53.75	0.318	0.00	0.000	0.00	0.000	1.18	0.006
0.4	53.97	0.687	0.00	0.000	0.00	0.000	1.18	0.006
0.6	53.57	0.000	4.74	0.029	1.19	0.000	1.18	0.006
1	54.55	0.370	5.77	0.306	1.19	0.006	1.19	0.010
2	55.16	0.687	6.30	0.662	1.19	0.006	1.19	0.006
4	60.30	0.612	6.37	0.668	1.19	0.000	1.19	0.006
6	62.20	0.260	6.39	0.647	1.20	0.006	1.19	0.000
10	63.14	0.681	6.74	0.625	1.19	0.006	1.19	0.006

Table 8. Foam formation activity at different concentration of Brijs 52, 56, 72 and 92 (n = 6).

Conc. (mM)	Surfactant							
	Brij 56		Brij 52		Brij 72		Brij 92	
	F_{h24} (Mean)	±SD	F_{h24} (Mean)	±SD	F_{h24} (Mean)	±SD	F_{h24} (Mean)	±SD
0	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000
0.01	2.33	0.289	0.50	0.000	0.67	0.289	0.00	0.000
0.04	10.50	0.500	2.50	0.000	3.33	0.289	1.67	0.289
0.08	15.00	0.500	4.00	0.500	5.00	0.500	1.83	0.289
0.12	15.83	0.289	4.33	0.289	6.00	0.500	2.33	0.289
0.16	17.83	0.289	6.00	0.000	9.17	0.289	2.33	0.289
0.2	20.50	0.500	6.33	0.289	9.33	0.289	2.50	0.000
0.4	19.00	0.000	7.50	0.000	14.00	0.500	3.00	0.500
0.6	20.33	0.289	9.33	0.289	14.50	0.500	3.00	0.000
0.8	20.00	0.500	9.67	0.289	15.83	0.289	3.17	0.289
1	19.83	0.577	10.00	0.000	16.83	0.289	3.33	0.289

special features. Therefore, some parts of the membrane can easily move throughout the surface. This characteristic is due to membrane phospholipids which covert to jelly in temperatures lower than the physiologic temperature. This conversion of phospholipids helps in more stabilized and regular membranes and increases their resistance. As a result, the amount of haemolysis at 37°C is more than at 25°C. The reason is that with the increase in temperature, the membrane fluidity, and accordingly its permeability, increases (Zaslavsky et al., 1987).

In solutions with higher concentration of surfactants, haemolysis amounts were more. This result can be easily described by Fick's law which states that, the diffusion flux from a membrane is proportional to the concentration difference between both sides (Muranishi, 1990). In other words, the concentration of intra-membrane surfactant is related to its extra-membrane concentration and by increasing the latter concentration, the first one increases until it reaches a specific concentration which leads to membrane destruction and hemolytic effects (Zaslavsky et al., 1987).

The first step in surfactant-membrane interaction is membrane saturation with surfactant's monomers; following this, process cellular lysis is possible. The onset is followed by destruction and deconstruction of surfactant-protein-lipid complexes and surfactant-lipid mixture micelles. Adding more surfactant enriches the surfactant-protein-lipid complexes and more mixture micelle pro-duction. At the extremity and in cmc, the amount of protein-surfactant complexes, mixture micelles and surfactant's micelles

Table 9. Comparison of different Brijs based on of their properties.

Properties of Brijs	Different kind of Brijs
HLB	B56>B52>B72=B92
Alkane chain length	B72=B92>B52=B56
Weight of hydrophile/Weight of liphophile	B56>B52>B72=B92
% Hemolysis (37°C)	B56> B52> B92>B72
% Hemolysis (25°C)	B56> B52> B92>B72
Foam formation activity	B56>B72>B52>B92
Emulsification index	B56>B52>B72=B92

become balanced with free surfactant (Schott, 1995). Our results showed that the hemolytic effects of surfactants increase as the latency of incubation and the amount of contact duration with erythrocytes increase. It is reported that the more the contact duration of erythrocytes with a solution, including a surface active agent solution, the more the amount of cellular lysis (Tragner and Csordas, 1987).

Adherence of surface active agents to erythrocyte's membrane which is followed by their entrance leads to an alteration of the molecular structure of the membrane, osmotic-colloid swelling and erythrocyte membrane rupture. The above mechanism depends on surfactant concentration, temperature and duration of contact with erythrocyte; and by increasing these factors, membrane permeability and haemolysis, that happen due to micelle production from surfactant and membrane phospholipids bilayer increases (Araki and Rifkind, 1981).

Another aspect of this study was to evaluate the membrane toxicity of surfactants. As the agents or any other substance which have the ability to destroy the erythrocytes membrane can have similar effects on other cell membranes, evaluating erythrocytes membrane stability is a proper criterion for the determination of surfactant toxicity. Further, haemolysis was observed by increasing the incubation period and temperature. In 1 mM and temperature of 37°C, Brij 56 caused 99.39% of erythrocytes destruction, while, 52, 72 and 92 caused 56.31, 1.83, 1.33, 0.98 and 2.15% of destruction, respectively. Moreover, Brij 56 induced almost 100% haemolysis while Brij showed the least destructive effects, respectively. The hemolytic activity of surfactants in this study increase in higher concentrations and at the critical concentration for micelle formation reached its utmost, and after this point remained steady. Hence, the ability to increase membrane permeability and after its osmotic cellular lysis are due to mixture micelle formation in the bilayer membrane. Evaluating the erythrocyte haemolysis showed that Brij 72 had lower destruction level and less toxicity on cellular membranes. Erythrocyte haemolysis method is used to evaluate surfactant and cellular membrane interactions, enhancing activity and emul-sifying ability. Accordingly, Brij 72 with lower toxicity should be preferred for use as surface active agent and more studies on its enhancing abilities and formulatory properties are required. We showed that Brij 56 with low hydrophobic and high hydrophilic properties has more capability for membrane destruction, while Brij 72 with lower hydrophobic properties has less destruction capability. The haemolytic effects of some non-ionic surface active agents was evaluated and reported. In a series of surfactants from one family, the ones with the higher hydrophobic contents and lower hydrophile-lipophile balance (HLB) had lower haemolysis (Schott, 1995).

According to the results (Table 9), it can be concluded that higher content of the hydrophobic part may lead to a reduction in permeability and hemolytic effects. Another potential property of surface active agents is their ability in induction and stabilizing emulsions. Emulsifying index has a direct relationship with surface tension and agent ability in micelle production. In this study, increasing the concentration of all surfactants leads to an increase in emulsion stability; however, this trend was not the same in all surfactants (Table 7). This effect started in low concentration (0.01 mM) and reached its maximum at 10 mM. According to hemolytic data and emulsifying index, Brij 72 had the least toxicity and the best properties for emulsification to be used in formulations. Foaming ability of surfactants is a property which may help prove the existence of surfactants in a solution. Furthermore, this ability can be used in order to compare the detergency properties of detergents with high ability of foam production.

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