Effect of pH on the Stability of Water in Heavy Crude Oil Emulsions Stabilized by Saponin and Rhamnolipid Biosurfactants

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ABSTRACT: *Biosurfactants are used widely in the oil and petroleum industries such as the production, transportation, and storage process of oil. This application of biosurfactants is due to the biodegradability of these biomaterials. This study investigated the effect of pH on stabilized water-in-oil emulsions using rhamnolipid and saponin biosurfactants. The phase separation was determined by measuring the volume of the water phase separated. The obtained results showed the introduction of HCl significantly affects the stability of emulsions stabilized by rhamnolipid and saponin but had no significant effect on the mixture of biosurfactant contents because of the synergistic effect. Also, the results showed that the maximum separation yield for rhamnolipid, saponin, and mixed biosurfactants at pH=1 and after 1 hour of sonication was 61, 57, and 20%, respectively. The water droplet sizes of the emulsion after sonication and before phase separation at pH of 5, 3, and 1 were around 100, 200, and 1000 μm for 40–60% of w/o emulsion stabilized by the mixture of rhamnolipid and saponin biosurfactants using DLS analysis. The results demonstrate that at low pHs the dramatic instability of w/o emulsion was obtained and significantly increased the rate of the Ostwald ripening process and coalescence rate of water droplets.*

KEYWORDS: *Emulsification, Hydrophilic-Lipophilic Balance, pH effect, Rhamnolipid, Saponin.*

INTRODUCTION

Emulsions are a commonly stable mixture of two or more immiscible liquids, such as oil and water. In general, emulsions can be divided into oil dispersed in water (o/w) or water dispersed in oil (w/o) [1, 2]. There can also be more complex systems, such as oil in water in oil (o/w/o). Surfactants are compounds that are used in the emulsification process to stabilize the suspension of small droplets and prevent them from instability, coalescing, or coming together to grow larger droplets due to the reduction of surface tension[3-5]. Biosurfactants are compounds that are produced from various types of plants and microorganisms and also have many advantages over commonly used commercial surfactants such as reduced surface tension, higher biodegradability, lower toxicity, preferred environmental compatibility, and high selectivity [6]. Also, owing to the biological production of these materials, there are many applications, especially in the oil and petroleum industry, such as the cleaning up of heavy oil tanks and heavy metals removal from contaminated soils [7]. Impurity pollutants from oilfields affect the

ecosystem and aquatic life in the sea. These materials are useful in washing the contaminated soil around the oilfields. Deposition of inorganic materials such salts in the oil production storage tanks is one of the major problems in the oil production process from wells resulted in corrosion and scaling in the near wellbore region during emulsification process [8]. These emulsions are undesirable and require low salinity water injection, demulsification and desalination to remove the dispersed water and associated inorganic salts in order to reduce the corrosion in equipments [9]. The chemical characteristic of an o/w emulsion is different from that of a w/o emulsion. The w/o emulsions are the basis of oil-based products and are effectively used in different industries such as cosmetics, oil, food, and pharmaceutical industries [10, 11]. Crude oil emulsions are formed when oil and brine come into contact with each other, with the influence of adequate mixing, and in the presence of an emulsifying agent (emulsifier). The mixing amount and the presence of an emulsifier are critical to the formation of an emulsion [12]. Among the factors that affect the formation and stability of the emulsion, it can be mentioned water concentration, surfactant concentration, mixing speed, salt concentration, chemical concentration, pH, and temperature. Furthermore, the change in droplet size and available interfacial area for any possible chemical reactions are important parameters that affect the stability and rheological characteristics of the emulsions [13]. The demulsification process has been proven to be a good method to break emulsions [14]. This process is one of the most important processes in many industries, such as enhanced oil recovery, heavy oil reservoir cleanup, and heavy crude oil separation [14-16]. Also, the emulsification process has a significant effect on the quality and yield of petroleum industry products [17]. Due to the separation of water and oil processes, various parameters such as temperature, pH, and salinity can affect the emulsification process [18]. It is very important to investigate the effect of key parameters such as temperature, salinity, pH, and water content simultaneously during the demulsification process. One of the most important methods to study the effect of different parameters on the efficiency and yield of the demulsification process is Response Surface Methodology (RSM) [19]. This method used for optimization of the process parameters and identifying the optimal conditions [20]. There are many studies about the effect of temperature,

salt concentration, and pH on the emulsification process of w/o emulsions [21]. *Bernard et al.* [18] showed that using nanocomposite microgel particles, at a pH 3-4 and salt concentration 0.24 mol/kg, the o/w emulsions were unstable. In another study, *Moradi et al.*[22, 23] investigated the effect of salinity on the stability of the w/o emulsions and determined the size distribution of the water droplet. The saponin and rhamnolipid used in this study are very sensitive at high temperatures and high salinity conditions. Considering the different HLBs of saponin and rhamnolipid, it seems to be a good idea to use the synergistic effect of these biosurfactants in crude oil emulsions. This research first investigated the synergistic effect of biosurfactants on emulsion stability and droplet size determination of water in oil emulsion. After this process, the effect of pH on the stability of water in heavy crude oil in an emulsion prepared by saponin and rhamnolipid was studied.

EXPERIMENTAL SECTION
Biosurfactants

To produce the saponin biosurfactant, 200 g of Glycyrrhizaglabra plant was defatted using 750 mL of *n*-hexane/petroleum ether mixture. In the next step, the Glycyrrhizaglabra plant residue was extracted using 750 mL of ethanol/ethanol mixture. The extracted solvents were concentrated in a vacuum and suspended in 60 mL of presaturated n-butanol 90% and phase separation was carried out with n-butanol. Then, diethyl ether was added to the n-butanol partition to precipitate the saponin. The extraction temperature was 60°C. All chemicals, such as petroleum ether, n-hexane, ethanol, methanol, n-butanol, and diethyl ether were purchased from Merck (Darmstadt, Germany) [24]. Rhamnolipid biosurfactant was produced from *pseudomonas aeroginosa* bacteria. After preparing the main culture medium and mineral salts, 10 to 15 mL of pre-culture prepared in lactose broth containing *Pseudomonas aeruginosa ATCC28793* were added to 200 mL of culture medium. Several drops of mineral compounds were also added to the original culture medium before starting the process. The carbon source, the original culture medium, and the minerals were autoclaved for 15 min at 120°C before the process began.

Production of rhamnolipid was performed in a 250 mL Erlenmeyer flask containing 200 mL of the original culture

Table 1: Biosurfactants were used throughout the investigation

Biosurfactants	Appearance	Mw	CMC (mgL^{-1})	HLB
Rhamnolipid	Brown viscose liquid	650	180	9.5
Saponin	Yellow solid	823	150	14.5

Table 2: Physical properties of heavy crude oil from TNIOC

Experiment	Value	Test method
Specific gravity(gcm^{-3})	0.94	ASTM D 4052
API	19.5	ASTM D 1288
Water content (vol %)	<0.05	ASTM D 4006
Pour point (ºC)	-15	ASTM D 5663
Viscosity at 20ºC(mPas)	2270	ASTM D 445
Asphaltene (wt%)	16.6	ASTM D 3381
Resin (wt%)	3.8	ASTM D 1996

medium, which was incubated in a shaker incubator at 150 rpm at 37°C for 7 days [25]. After the incubation process, the culture medium was centrifuged at 3450 rpm for 45 min to remove the biomass produced by the microorganisms. In the next step, it was separated by adding the same amount of n-hexane as the residual carbon source, or sunflower oil. In this step, the aqueous phase containing rhamnolipid was obtained. The pH of the obtained aqueous phase was adjusted to 2.5 to 3 using 1 M H$_3$PO$_4$ solution. Then, by adding equal amounts of ethyl acetate to the aqueous phase of the extraction process, the mixture of the aqueous phase and ethyl acetate was centrifuged at 3450 rpm for 45 minutes to obtain the aqueous rhamnolipid into the ethyl acetate phase. The extraction was evaporated by vacuum evaporation of ethyl acetate to produce the rhamnolipid biosurfactant [26]. The specification of these biosurfactants is shown in Table 1.

Emulsification and demulsification methodology

In this study, heavy crude oil from Tehran National Iranian Oil Company (TNIOC) was used as continuous phase. The physical properties of this heavy crude oil are summarized in Table 2.

Bottle test is the traditional method used to assess the stability of oil emulsion [27]. The bottle test is a simple assessment of the rate of emulsion separation by visual observation of the separated volume of water with time. Test tubes were used for the emulsion preparation. The emulsion composition was 6mL of heavy crude oil and 4mL of the aqueous phase. The aqueous phase contains 2 w/v% of saponin and rhamnolipid biosurfactants according to CMC value.

To evaluate the effect of pH on the stability of emulsions, the test tubes were used. Some studies exhibited that the emulsions produced with saponin and rhamnolipid are strongly stable in the temperature range of 25-60°C and 3M salinity concentration[28]. Therefore, in this study, only the effect of pH on emulsification and demulsification of crude oil emulsions was investigated. To evaluate the pH stability, the pH value of the test tubes (containing 10 mL of biosurfactants and heavy crude oil) were separately adjusted to 1, 3, and 5 by adding the required amount of 1N HCl. The mixtures were sonicated (Sonopuls HD 3200, 15 Watt, Bandelin Electronic, Germany) for different times and then allowed to settle down for 24 h. All experiments were performed at room temperature. The volume fraction of phase separation was defined as Emulsification Index (E24). The E24 index is determined using Eq. (1) [29].

$$E24 = \frac{\text{height of emulsion layer in the test tube}}{\text{total height of the test tube}} \times 100 \qquad (1)$$

Comparing the effect of studied synthesis biosurfactants with chemical surfactants

To investigate the effect of synthesis surfactants on the stability of prepared emulsion in section 2.2, Tween 80 and Span 80 were used as chemical surfactants to compare the saponin and rhamnolipid biosurfactants on the E24 index under the same condition of experiments.

Droplet size determination

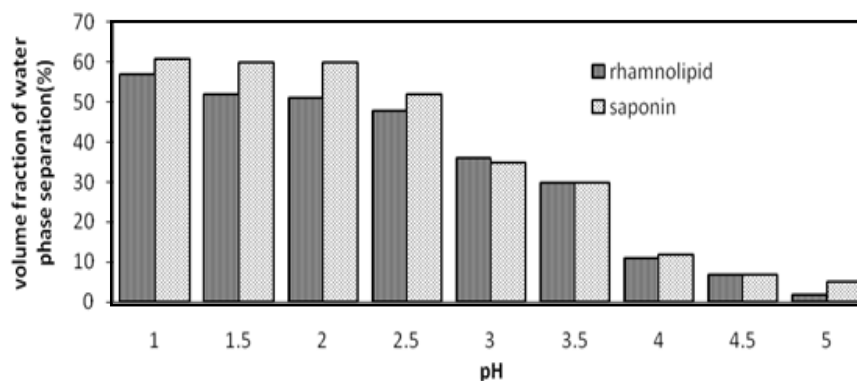The mean droplet size of prepared w/o emulsions at different pH was measured at 25°C using Dynamic Light

Fig. 1: Water phase separation at different pH (6 mL of oil and 4 mL of aqueous phase containing 2 w/v % of saponin and rhamnolipid biosurfactants)
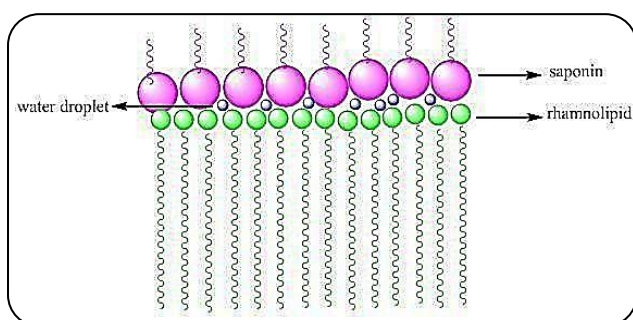

Fig 2: Schematic of the synergistic effect of two biosurfactants with different HLB amounts

Scatting (DLS) (NanotracWave, MicrotracSandiego, CA). An optical microscope(Zenith Microlab 1000B, U.K) was used to observe the dispersion of water droplets in the oil phase.

RESULTS AND DISCUSSION
Effect of pH

The E24 was 100% for the w/o emulsion without HCl addition at pH 5.5 and there is no phase separation in the w/o emulsion. The volume fraction of phase separation after sonication increased with the addition of HCl and the decrease in pH. Fig. 1 shows the phase separation of the w/o emulsion as the pH decreases from 5.5 to 1.

As shown in Fig., at pH 1, the maximum phase separation of was 61% stabilized by saponin biosurfactant. At pH below 1, due to the destruction of the biosurfactant structures, the w/o emulsion is not formed and the oil and water phases are separated. The mechanism of water phase separation is consistent with the significant increase in the coalescence occurring between dispersed oil droplets. The water phase separation includes two processes, coalescence and Ostwald ripening theory [30, 31]. Considering that the high Hydrophilic-Lipophilic Balance (HLB) emulsifiers are not

suitable for thew/o emulsions, in saponin-stabilized emulsions with the high HLB compared to rhamnolipid-stabilized emulsions, the water phase in the emulsion increases with the addition of acid which leads to an increase in the coalescence. As a result, the rate of the Ostwald ripening mechanism and phase separation increases. The anionic biosurfactants used in this study (saponin and rhamnolipid) at high pH (8-13) resulted in the production of homogeneous emulsions due to the increase in the number of OH ions and the interaction between (OH) anionic biosurfactants which seems to be stable. Also, *Morales et al.*[32] showed the emulsions produced using saponin biosurfactant were strongly stable ata pH range of 4-10 during 5 days. According to the HLB of saponin and rhamnolipid, the amount of OH ions in rhamnolipid biosurfactant is lower than saponin [33] therefore the phase separation at the same pH in the emulsion stabilized by rhamnolipid was low. Although some surfactants such as cationic and nonionic synthetic surfactants show more stability of emulsions at acidic pH, the crude oil emulsions produced by saponin and rhamnolipid biosurfactants as two anionic biosurfactants are unstable at low pH. Due to the fact at low pH, the hydrogen bound between the OH^- of biosurfactants and H^+ inhibits the micelle formation of biosurfactants resulting in decreasing the stability of emulsions.[34]

Synergistic effect of biosurfactants

Fig. 2 schematically shows the synergistic effect of two biosurfactants with different HLB values.

According to this schematic and Fig. 3, in the test tube containing 6mL of heavy oil and 4 mL of biosurfactant solution, the water phase separation of emulsion stabilized

Table 3: Water phase separation at different pH (6 mL of oil and 4 mL of aqueous phase containing 2 w/v % of saponin and rhamnolipid biosurfactants)

Surfactants	pH=1	pH=2	pH=3	pH=4	pH=5
Saponin	61%	60%	35%	12%	5%
Rhamnolipid	57%	51%	36%	11%	2%
Mixed	44%	40%	9%	2%	2%
Tween 80	58%	55%	40%	10%	6%
Span 80	61%	56%	41%	10%	5%

Table 4: Reduction in interfacial tension of emulsions (initial interfacial tension= 39 mNm^{-1}, 6mL of oil and 4mL of individual surfactants at 2 w/v %))

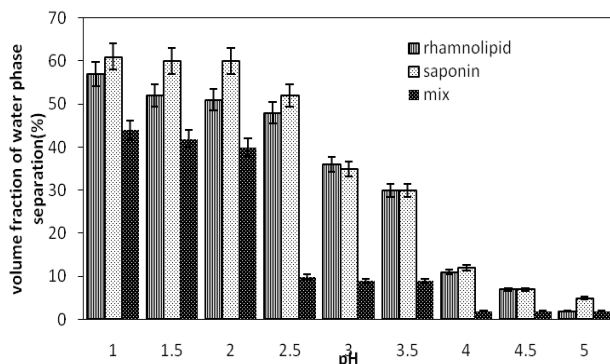| Surfactants	pH=1	pH=3	pH=5
Saponin	71%	61%	35%
Rhamnolipid	43%	28%	23%
Mixed biosurfactants	28%	23%	12%

Fig 3: Water phase separation at different pH (6 mL of oil and 4 mL of aqueous phase containing 2 w/v % of saponin and rhamnolipid biosurfactants
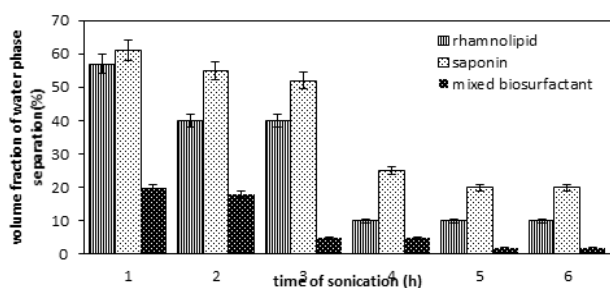

Fig 4: Water phase separation with time

by the mixture of rhamnolipid (50 %) and saponin (50 %) was lower than individual saponin and rhamnolipid owing to different HLB values and synergistic effect of saponin and rhamnolipid in the emulsion.

The mixed rhamnolipid and saponin were able to create a film around the dispersed phase and improved the reinforced interfacial film and consequently increased the rate of micelle formation. Rhamnolipids orientated themselves at the oil-in-water interface then the saponin by hydroxyl groups protruded into the aqueous phase, enabling them to form micelles by hydrogen bonds. According to our results mixed rhamnolipid and saponin reduced the unfavorable contacts between hydrocarbon chains and water molecules. This means that the rhamnolipid and saponin could arrange more compactly at the interface and have synergistic effect during demulsification process. The results of this study demonstrated that the optimal demulsification condition was at the water conent of 40%, temperature range of 25 - 60°C, pH=5 and salinity concentration up to 3M. Under this condidtion the lowest phase separation was obtained. Table 3 shows the comparison between performance of saponin and rhamnolipid biosurfactants and chemical surfactants in phase separation and emulsion stability prepared in the same condition of surfactant concentration and water–oil volume fraction at different pH. As shown in the table, the stability efficiency of the mixed biosurfactant system is better than individual biosurfactants (Tween 80 and Span 80).

According to the properties of the emulsion, with the increase in the sonication time after settling down the emulsion for 24 h, the volume fraction of the water phase decreased due to increasing the micelle formation rate of biosurfactants. Fig. 4 shows the water phase separation of the saponin-stabilized w/o emulsion, rhamnolipid-stabilized w/o emulsion, and a mixture of biosurfactants with increasing time at pH= 1 and biosurfactant concentration of 2w/v%.

of sonication (pH = 1, biosurfactants concentration = 2 w/v %)
Interfacial tension investigation

To investigate the interfacial activity of the prepared emulsion after the emulsification process, the change in Interfacial Tension (IT) of emulsions was evaluated by the Du Nouy Ring method. The interfacial tension of the emulsion was measured at the same condition as the emulsification process. The results are shown in Table 4.

The mixed biosurfactants system showed the least decrease in interfacial tension. The initial Interfacial Tension (IT_i) was 39 m/Nm and after measuring the final Interfacial Tension (IT_f) the percent of interfacial tension reduction was obtained using the following equation.
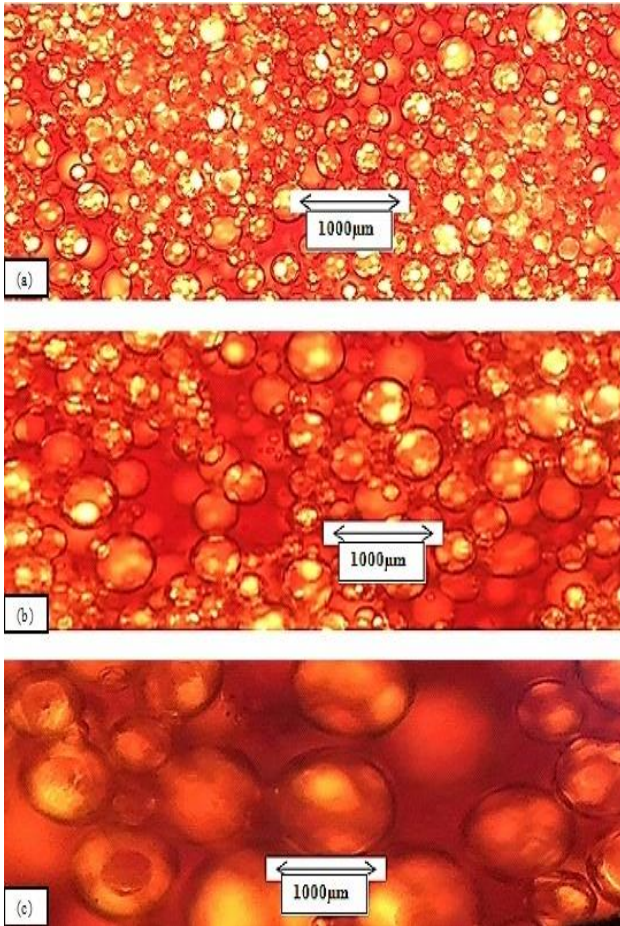
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Fig 5: Microscopic image of the water droplets in the oil phase at (a) pH = 5, (b) pH =3 and (c) pH = 1

Fig. 6: DLS analysis of the w/o emulsions at (a) pH = 5, (b) pH = 3 and (c) pH = 1

$$\% \ Reduction \ in \ interfacial \ tension = ((IT_f) - (IT_i)/(IT_i)) \times 100 \qquad (2)$$

Droplet size investigation

The image of the particle size distribution of the w/o emulsion in the presence of 2% w/v of the mixed biosurfactants at pH 1, 3, and 5 after sonication is shown in Fig. 5.

The average droplet size at pH of 1, 3, and 5 was about 300, 110, and 80 µm, respectively. As the pH of w/o emulsion decreased in the stabilized emulsion, the size of the water droplets grew and the emulsion separated [35, 36]. According to the microscopic image, the oil droplets at pH equal 3 and 5 were smaller than the water droplets at pH value 1. Also, Fig. 6 displays the DLS analysis of the w/o emulsions shown in Fig. 4. The mean water droplet size of the emulsion at pH 5, 3, and 1was about 80, 100, and 300 µm, respectively.
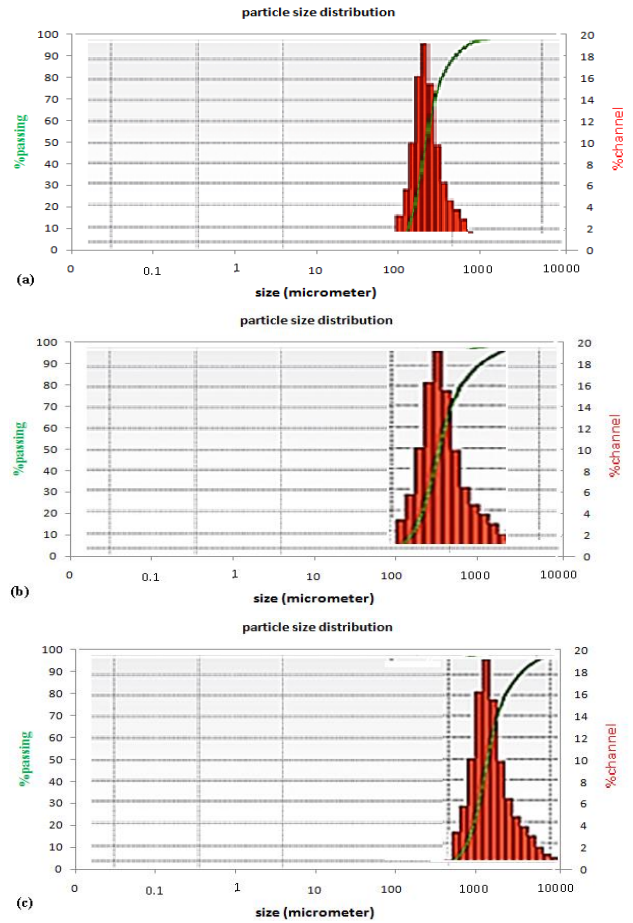
CONCLUSIONS

In this research, the effect of pH on stability of w/o emulsion was studied. Also, the synergistic effect of two new biosurfactants on the emulsification of crude oil was investigated. Rhamnolipid and saponin biosurfactants were used as emulsifiers to stabilize the emulsions. The results showed that the stability of the emulsion decreased with decreasing the pH. The maximum reduction in stability of emulsion was at pH = 1 after 1hr of sonication. The average droplet size at pH 1, 3, and 5 was about 300, 110, and 80 µm, respectively. The maximum water phase separation was 61% at the saponin concentration of 2w/v% and the maximum reduction of interfacial tension was 71% at the saponin concentration of 2 w/v %. Also, the results showed that the stability of the emulsion prepared by saponin biosurfactant was significantly affected by adding acid. Finally, the results obtained from the experiments exhibited an increasing coalescence rate of water droplets by decreasing the pH.

REFERENCES

[1] Martínez-Palou R., de Lourdes Mosqueira M., Zapata-Rendón B., Mar-Juárez E., et al., Transportation of Heavy and Extra-heavy Crude Oil by Pipeline: A Review, *Journal of Petroleum Science and Engineering,* **75(3-4)**: 274-282. (2011).

[2] Sjöblom, J., Aske N., Auflem I.H., Brandal Ø., et al., Our Current Understanding of Water-In-Crude Oil Emulsions.: Recent Characterization Techniques and High Pressure Performance, *Advances in Colloid and Interface Science*, **100**: 399-473 (2003).

[3] Bouyer E., Mekhloufi G., Huang N., Rosilio V., et al., β-Lactoglobulin, Gum Arabic, and Xanthan Gum for Emulsifying Sweet Almond Oil: Formulation and Stabilization Mechanisms of Pharmaceutical Emulsions, *Colloids and Surfaces A: Physicochemical and Engineering Aspects.*, **433**: 77-87 (2013).

[4] Okushima S., Nisisako T., Torii T. Higuchi T., Controlled Production of Monodisperse Double Emulsions by Two-Step Droplet Breakup in Microfluidic Devices, *Langmuir*, **20(23)**: 9905-9908 (2004).

[5] Xu J.-H., Ge X.-H., Chen R. Luo G.-S., Microfluidic Preparation and Structure Evolution of Double Emulsions with two-Phase Cores, *RSC Advances*, **4(4)**: 1900-1906 (2014).

[6] Hajimohammadi R. Johari-Ahar S., Synergistic Effect of Saponin and Rhamnolipid Biosurfactants Systems on Foam Behavior, *Tenside Surfactants Detergents*,. **55(2)**: 121-126 (2018).

[7] Hajimohammadi R., Amani H., Soltani H., Synergistic Effect of Rhamnolipid and Saponin Biosurfactants on Removal of Heavy Metals from Oil Contaminated Soils, *Tenside Surfactants Detergents*, **57(2)**: 109-114 (2020).

[8] Olajire A.A., A Review of Oilfield Scale Management Technology for Oil and Gas   Production. *Journal of Petroleum Science and Engineering*, **135**: 723-737 (2015).

[9] Khormali A., Koochi M.R., Varfolomeev M.A., Ahmadi S., Experimental Study of the Low Salinity Water Injection Process in the Presence of Scale Inhibitor and Various Nanoparticles, *Journal of Petroleum Exploration and Production Technology*, 1-14 (2022).

[10] Talegaonkar S., Azeem A., Ahmad F.J., Khar R.K., et al., Microemulsions: A Novel Approach to Enhanced Drug Delivery, *Recent Patents on Drug Delivery & Formulation*, **2(3)**: 238-257. (2008).

[11] Kogan, A., Garti N., Microemulsions as Transdermal Drug Delivery Vehicles, *Advances in Colloid and Interface Science*, **123**: 369-385 (2006).

[12] Umar A.A., Saaid I.B.M., Sulaimon A.A., Pilus R.B.M., A Review of Petroleum Emulsions and Recent Progress on Water-In-Crude Oil Emulsions Stabilized by Natural Surfactants and Solids, *Journal of Petroleum Science and Engineering*, **165**: 673-690 (2018).

[13] Goodarzi F., Zendehboudi S., A Comprehensive Review on Emulsions and Emulsion Stability in Chemical and Energy Industries, *The Canadian Journal of Chemical Engineering*, **97(1)**: 281-309 (2019).

[14] Chu L.Y., Utada A.S., Shah R.K., Kim J.W., et al., Controllable Monodisperse Multiple Emulsions, *Angewandte Chemie,* **119(47)**: 9128-9132 (2007).

[15] Shetty C., Nikolov A., Wasan D., Bhattacharyya B., Demulsification of Water in Oil Emulsions Using Water Soluble Demulsifiers, *Journal of Dispersion Science and Technology*, **13(2)**: 121-133 (1992).

[16] Wu J., Xu Y., Dabros T., Hamza H., Effect of EO and PO Positions in Nonionic Surfactants on Surfactant Properties and Demulsification Performance, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **252(1)**: 79-85 (2005).

[17] Guzmán-Lucero D., Flores P., Rojo T., Martínez-Palou R., Ionic Liquids as Demulsifiers of Water-in-Crude Oil Emulsions: Study of the Microwave Effect, *Energy & fuels*, **24(6)**: 3610-3615 (2010).

[18] Fortuny M., Oliveira C.B., Melo R.L., Nele M., et al., Effect of Salinity, Temperature, Water Content, and pH on the Microwave Demulsification of Crude Oil Emulsions, *Energy & fuels*, **21(3)**: 1358-1364 (2007).

[19] Ahmadi S., Khormali A., Khoutoriansky F.M. Optimization of the Demulsification of Water-In-Heavy Crude Oil Emulsions Using Response Surface Methodology, *Fuel,* **323**: 124270 (2022).

[20] Roshan N., Ghader S., Rahimpour M.R., Application of the Response Surface Methodology for Modeling Demulsification of Crude Oil Emulsion Using a Demulsifier, *Journal of Dispersion Science and Technology*, **39(5)**: 700-710 (2018).

[21] Daaou M., Bendedouch D., Water pH and Surfactant Addition Effects on the Stability of an Algerian Crude Oil Emulsion, *Journal of Saudi Chemical Society*, **16(3)**: 333-337 (2012).

[22] Binks B.P., Murakami R., Armes S.P., Fujii S., Effects of pH and Salt Concentration on Oil-in-Water Emulsions Stabilized Solely by Nanocomposite Microgel Particles. *Langmuir*, **22(5)**: 2050-2057 (2006).

[23] Moradi M., Alvarado V. Huzurbazar S., Effect of Salinity on Water-In-Crude Oil Emulsion: Evaluation Through Drop-Size Distribution Proxy. *Energy & Fuels*, **25(1)**: 260-268 (2011).

[24] Kime B., Barminas J., Nkafamiya I. Akinterinwa A., Extraction and Evaluation of a Saponin-Based Surfactant from Balanites Aegyptiaca Plant as an Emulsifying Agent, *Int. J. Innov. Sci. Eng. Technol.*, **2**: 733-737 (2015).

[25] Ghorbani M., Hosseini M., Najafpour G. Hajimohammadi R., Synthesis and Characterization of Rhamnolipid Biosurfactant Produced by Pseudomonas Aeruginosa PTCC 1340 for Emulsification of Oil Sludge in Oil Storage Tank, *Arabian Journal for Science and Engineering*,: 1-8 (2021).

[26] Soberón-Chávez, G., F Lépine., Déziel E., Production of Rhamnolipids by Pseudomonas Aeruginosa, *Applied Microbiology and Biotechnology*, **68(6)**: 718-725 (2005).

[27] Raynel G., Marques D.S., Al-Khabaz S., Al-Thabet M., Oshinowo L., A New Method to Select Demulsifiers and Optimize Dosage at Wet Crude Oil Separation Facilities, *Oil & Gas Science and Technology–Revue d'IFP Energies Nouvelles*, **76**: 19 (2021).

[28] Udoh T., Vinogradov J., Experimental Investigations of Behaviour of Biosurfactants in Brine Solutions Relevant to Hydrocarbon Reservoirs, *Colloids and Interfaces,* **3(1)**: 24 (2019).

[29] Sadouk Z., Hacene H. Tazerouti A., Biosurfactants Production from Low Cost Substrate and Degradation of Diesel Oil by a Rhodococcus Strain, *Oil & Gas Science and Technology-Revue de l'IFP,* **63(6)**: 747-753 (2008).

[30] Jafari S.M., Assadpoor E., He Y. Bhandari B., Re-Coalescence of Emulsion Droplets During High-Energy Emulsification, *Food Hydrocolloids*, **22(7)**: 1191-1202 (2008).

[31] Jiao J., D Burgess.J., Ostwald Ripening of Water-In-Hydrocarbon Emulsions, *Journal of Colloid and Interface Science,* **264(2)**: 509-516 (2003).

[32] Sotomayor-Gerding D., Morales E., Rubilar M., Comparison between Quinoa and Quillaja saponins in the Formation, Stability and Digestibility of Astaxanthin-Canola Oil Emulsions. *Colloids and Interfaces*, **6(3)**: 43 (2022).

[33] Beattie J.K. Djerdjev A.M., The Pristine Oil/Water Interface: Surfactant-Free Hydroxide-Charged Emulsions, *Angewandte Chemie International Edition*, **43(27)**: 3568-3571 (2004).

[34] Tagavifar M., Jang S.H., Sharma H., Wang D., Chang L.Y., Mohanty K., Pope G.A., Effect of pH on Adsorption of Anionic Surfactants on Limestone: Experimental Study and Surface Complexation Modeling, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **538**: 549-558 (2018).

[35] Babapoor A., Hajimohammadi R., Jokar S.M., Paar M., Biosensor Design for Detection of Mercury in Contaminated Soil Using Rhamnolipid Biosurfactant and Luminescent Bacteria, *Journal of Chemistry*, **1**: 32-40 (2020).

[36] Touzouirt S., Hadjsadok A., Belhadji L., Ahmed Zaid T., Rheological Study of a Pickering Emulsion Stabilized by Algerian Clay Particles, *Iranian Journal of Chemistry and Chemical Engineering (IJCCE)*, **41(1)**: 346-351 (2022).