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Nanoliposome for Treating Oil Spill on Water

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THE AIM of the present study is to replace chemical surfactant with an alternative substrate containing high level of phospholipids. Phosphotidylcholine (PC) is extracted from soya bean oil waste which is used to obtain non toxic nanoliposome. Nano associated PC/ Chol liposome with different specific surfactants were prepared using modified (REV) method. The results obtained of the dispersed part show that associated liposome with non- ionic and cationic type surfactant show high efficiencies, while with anionic surfactant the efficiency is low. Gas chromatography technique was applied to study the hydrocarbon composition of undispersed part for each type of nanoliposome,the results obtained show that the nanoliposome affected not only on saturated fraction but also on aromatic and iso-paraffin. From TEM and DLS results the particle size of associated liposome showed better distribution than PC/Chol., and the average particle size and zeta potential of all associated liposomes were both reduced for nano-tween liposome and nano-nonylphenol ethoxylate liposome

Keywords: Liposomes, Dispersant, Oil spill, Nano liposomes, Associated liposomes.

Introduction

Pollution of water in sea and ocean by crude oil and various refined products is increasingly important problem. The essential known cause of oil pollution is that arising from petroleum tanker accidents forming oil spills. Such oil spill is undesirable not only from an aesthetic stand point, but also because cohesive films of oil represent a barrier to the transfer of oxygen from atmosphere to marine life and serious hazard to marine. Physical recovery of the environment from an oil spill is not easy, so there is considerable incentive to quick clean up this area after a spill spreads over a wide area, but these efforts were expensive and themselves destructive. Several techniques have been used for solving the oil spill. One solution involves the use of strong chemical surfactants to disperse the oil in water. Such surfactants might present on ecological hazard and they are not entirely effective which increase the spreading of oil in water and not its removal, which cause the damaging of marine environment [1]. The surfactant molecules used to disperse oil spill can be classified according to their charge into ionic, nonionic.

Liposome is type of amphoteric or zwitterionic surfactants, which represent an interesting class in our study used as dispersant and can be manufactured by using safe ingredients obtained from natural sources, such as yolk, soya bean or milk [2] . Liposome is usually made of phospholipids which are molecules that have a head and a tail group [3]. Phospholipids mixtures are known as lecithin that occurs in a great variety of vegetable oil crops and egg yolk. They are natural zwitter- ionic surfactants. The main phospholipids in lecithin from soya bean, sunflower and egg yolk are considered as phosphotidyl choline (PC). It can be obtained from lecithin which can be extracted chemically (using hexane) or mechanically from soya bean oil waste. PC liposome is preferred to use as dispersant for oil spill, because it is available, economic, non-toxic and supply essential nutrients(C, H, P, N) for marine organisms and biodegradation process can occur.

The PC mixed with cholesterol (Chol) to increase the stability of liposome [4,5], and the complexation between PC /Chol and specific surfactants is a way to increase the long term stability of liposomes, as well as inhibit liposome

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fusion [6-8]. The most important parameters of nanoliposome characterization include visual appearance, size distribution, stability, Zeta potential, lamellarity and entrapment efficiency and transmission electron microscopy (TEM). Physico-chemical characteristics of nano-liposome with the properties of their constituents were studied to realize the mechanism of their formation.

Liposome is not widely used by industries due to its high production cost and use of expensive substances. The aim of the present study is to use waste soybean which is more economic in addition to the presence of high levels of natural phospholipids (PC) which is necessary for the production of liposome. Moreover, the use of waste contributes to decrease environmental pollution and increase the economic value of wastes. By applying nano associated liposome in the treatment of oil spill will enhance the dispersion process in addition to increase the biodegradation process.

Experimental

Tween 80, Triton X-100, nonylphenol Ethyloxalate, dodocane Sulphate and cationic aerosol were supplied by Sigma Aldrich chemical company.

Soybean, L-α phosphotidylcholine (PC) and cholesterol (99% pure) were also donation from Sigma Aldrich Chemical Company. All solvents used in this study were also purchased from Sigma Aldrich Chemical Company of analytical grade, chloroform and absolute ethanol, buffer saline ranges (PH 7, 7.4). Crude oil: An Egyptian heavy crude oil used for this study was acquired from western desert.

Physicochemical characteristics of the tested crude oil

1.Physicochemical characteristics of the crude oil; density, kinematic viscosity, pour point, sulfur content, wax content, molecular weight and asphaltene content were determined according to the ASTM [9], IP[10] and UOP[11] standard test methods.

Fractionation of crude oils into their components hydrocarbon types:

The crude oil and the undispersed crude oils were fractionated into their components, hydrocarbon types asphaltenes and maltene , which was separated into oil and resin using liquid chromatography. Oil was further separated into saturate, mono, di-and poly-aromatic by silica gel chromatography, (Fig.1, Table 1).

Determination of the efficiency of oil spill dispersants

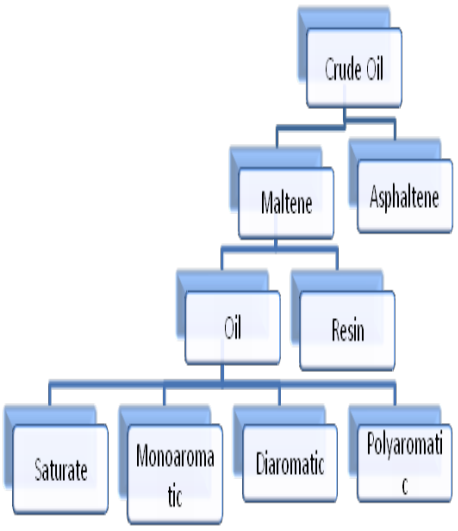

Fig. 1. Scheme of fractionation of the crude oil into its hydrocarbons types and classes.

TABLE 1. Refractive index (R.I.) of hydrocarbons.

Hydrocarbon	R.I. at 20°C
Saturate	<1.48
Monoaromatics	1.48-1.53
Diaromatic	1.53-1.59
Polyaromatic	>1.59
Resin	Dark

A definite amount of sea water sample (250ml) is transferred to a separating funnel and maintained at required temperature, and then (5ml) of used crude oil was added to the water surface and left for one minute. Then a known amount of dispersant was added. After a time of 2.5 minutes from the addition of oil, the oil was shacked for two minutes, then 50 ml of the oily water was drown in a 50 ml measuring cylinder in a time not exceeding 10 seconds. Then, the oily water was transferred from the measuring cylinder to separating funnel.

The measuring cylinder was washed twice with 10 cm^3 of chloroform. The washing was added to the separating funnel, and shacked for one minute. The phases were allowed to

separate completely and run off the chloroform layer through a Whatman filter paper containing anhydrous sodium sulfate. The chloroform extract was repeated twice more using 20ml chloroform. The dried extracts and washings were combined; in 100 cm^3 volumetric flask to the mark, stoppered and mixed well. Calibration, of the used crude oil is made at different concentrations (0.2, 0.5, 0.7 and 0.9g) in chloroform. The absorbance of each solution is measured at 580nm. The calibration curve (absorbance against concentration) is drawn.

The absorption of the chloroform extract was measured against a chloroform blank at 580nm in glass cells of 5 mm path length. Using the calibration curve the weight of the oil; contained in the 50 ml oily water sample was calculated. The efficiency index E, was calculated from the equation.

$$E = \frac{\text{Weight of oil in 50 ml sample of oily water} \times 500}{\text{Total weight of added oil to 250 ml}} \%$$

Preparation of liposome by reverse phase evaporation

Liposome was prepared by modified reverse phase evaporation (mREV) [7,8]

Using a mixture of chloroform: ethanol (2:1) to dissolve liposome PC/Chol (2:1). Solvent was evaporated at 50-60^{o}C under aspirate vacuum. The temperature was decreased gradually to 30^{o}C until the formation of thin film layer at the wall of the used flask. Gentle sonication at ambient temperature was applied for 20min until the lipid film was completely hydrated. Liposomes with different molar proportions of PC/Chol (1:0, 1:1, and 2:1) were studied.

Preparation of polymer associated liposome [12]

The complex between polymers and liposomes has been studied as a way to increase the long-term stability of liposomes. To increase circulation time, as well as to prevent liposome fusion, hydrophilic polymer is introduced onto the head groups of phospholipids, or water soluble polymer containing several hydrophobic groups is used. The polymer was dissolved in deionized water and added to liposome suspension previously prepared. A ratio (2:1) of PC/Chol. is used in all samples, the mixture was sonicated using high speed for 10 min.

Preparation of nanoliposome

Modified reverse phase evaporation (mREV) technique was used to prepare nanoliposome. The PC-Cholesterol liposome and polymer associated PC/Chol liposome suspension were sonicated by probe-type sonicator at pulse on for 5 seconds and pulse off 10 seconds for 3 min [13]. In the final step, the nanoliposome sample was stored in sterilized bottle in the dark at room temperature.

Characterization of liposome

Gas Chromatography (GC)

Extracted saturates from crude oil have been studied by computerized GC monitored apparatus (Agilent 6890) plus according to the standard test method IP 318/ 75.

Transition electron microscopy (TEM)

The morphology of nanoliposome was studied by TEM. Each sample was fixed on cupper-coated carbon grid. Images were acquired using a transmission electron microscope (JEOL JEM-2100, HRTEM) operated at an accelerating voltage 80KV to avoid fusion of liposome.

Dynamic light scattering and Zeta potential

The liposome mean diameter, size distribution and zeta potential were measured with dynamic light scattering (DLS), NANO "ZS''Malvern Zetasier equipment (Worcestershire,UK, at 25^{o}C, using a He-Ne laser of 633nm, size range (0.3nm -10 μm), Zeta potential range (3.8nm - 100 μm) [14, 15].

Results and Discussion

Physico-chemical characterization of crude oil

An Egyptian heavy crude oil was used for this study. The Egyptian heavy crude has low API gravity,15.16 , high, viscosity 141.23cSt, pour point 20^{o}C, low wax content 2.6 wt%, and high asphaltene 8.8 wt% as shown in Table 2

The resistance of the emulsion to disperse either naturally or chemically appears to be a function of asphaltene content. Its characterization was close to the state of weathered crude oil after a spill. The crude oil was separated into its components using n-heptane for deasphaltining followed by alumina column chromatography .The oil of the crude oil was further separated into its hydrocarbon types by applying the silica gel column chromatography (Table 1).

TABLE 2. physico-chemical properties of the studied crude oil.

Experiment	Method	Result
Density g/ml, at 15.56 °C Specific gravity API gravity	ASTMD-1298	0.9639 0.9648 15.16
Kinematic Viscosity, cSt at 60°C	ASTM D-445	141.23
Pour point, °C	ASTM D-97	20
Total Sulphur, wt.%	ASTM D-4294	3.2
Molecular weight	ASTMD-4294	249.37
Wax Content, wt.%	UOP-64	2.6
Asphaltene Content , wt.%	IP-143	8.8
Resin, wt%		27.06
Saturate,wt%		29.02
Aromatcs, wt%: Mono-aromatic Di-aromatic Poly-aromatic		35.10 20.3 8.7 6.1

Gas chromatography analysis of crude oil sample

The hydrocarbon distribution of the saturated fraction of the crude oil as given in Fig. 2 showed that the percentage of paraffinic hydrocarbons increased up to carbon number C_{18} followed by continuous decrease up to C_{40}. This trend is characteristic for all saturated fractions of crude oil.

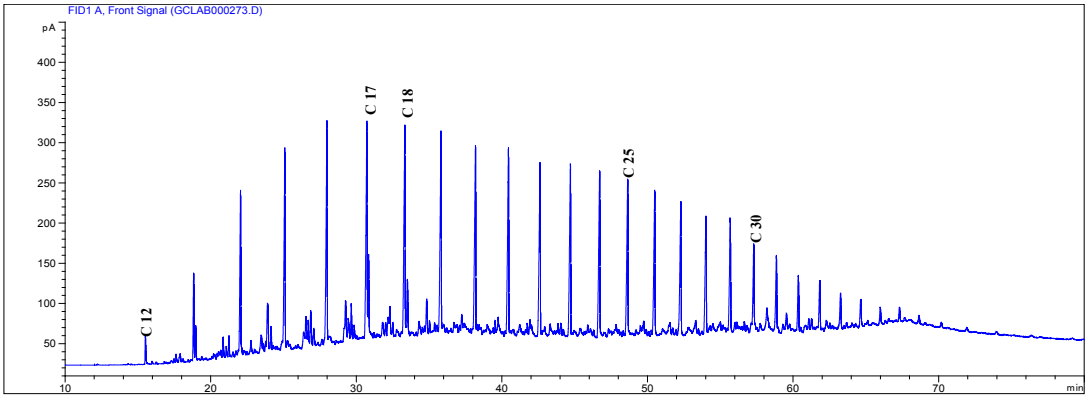

Fig. 2. GC of crude oil.

Dispersants liposome

The influence of cholesterol (Chol)

The soya bean phosphotidylcholine showed low dispersion and high tendency to aggregate so, Chol. was added with different molar ratio to increase stability of dispersion process due to increase stability of liposome formed which depend on head group composition of lipid and Chol [16]. Addition of Cholesterol to PC results in the formation of small droplets which stay dispersed for extended periods and the ratio 2:1 PC/Chol give the best efficiency on additional stabilizing effect. As the addition of Cholesterol to PC structure leads to the weakness of Vander Waal's interactions between hydrocarbons chains of fatty acid and prevent liposome crystallization which agree with Socaciu who found that Cholesterol affect on the mobility of acyl chains of phospholipids PC/Chol [17] .

Polymer associated liposomes

Polymer associated Pc/Chol liposomes containing different specific surfactants using 1:1 (v/v) ratio (tween 80, triton X-100, aeresol, dodocane sulfate, nonylphenol ethyloxalate) were prepared. It was found that the complex formed between polymers and liposome increase long

term stability of liposome structure as well as inhibit liposome fusion which gave good result in dispersing oil spill.

Liposomes efficiencies

The effect of temperature on efficiency of liposomes

The effect of temperature on efficiency of liposome was performed separately on each of the dispersants combination liposomes. It is clear that percentage of efficiencies increased with increasing temperature from 10°C to 30 °C but decrease at 40°C, in case of dispersant associated liposome. The dispersant effectiveness value of tween80 associated liposome at (10, 30, 40°C) were (35.52, 75.87 and 70.48%), as shown in Table 3. While in case of nano-tween80 associated liposome, the percentage of effectiveness will increase with temperature to (46.71, 90.44 and 95.15%). This is due to increase in stability of nano-scale liposome which is also evident from Tables 3, 4.

Results indicate that the higher efficiencies of nano-liposomes structure have higher amount of each asphalt and resin as in case of applying nano-tween80 liposome, nano-nonylphenol ethyloxalate liposome, nano-aresol liposome and the lower content of the saturate and aromatic fraction. The low value of aromatic contents is observed by using nano-nonylphenol ethyloxalate liposome (11.67%) which have high efficiency while high value of aromatic content in case of using nano-dodocane sulphate liposome which has lowest efficiency (29.6) (Table 5).

Gray and Clement [18] reported that as the dispersion and biodegradation rate increase, the number of polar compounds increase, which is

TABLE 3. Efficiency percent of polymer associated liposome at different temperatures.

Samples	Efficiency @ 10°C	Efficiency @ 30°C	Efficiency @ 40°C
Tween 80	35.52	75.87	70.48
Triton X-100	34.87	70.83	65.44
Aerosol	40.75	77.17	75.97
Nonylphenol Ethyloxalate	30.62	80.77	77.91
Dodocane Sulphate	8.71	12.32	6.19

TABLE 4. Efficiency percent of nanoliposomes at different temperatures.

Nano-Samples	Efficiency @10°C	Efficiency @30°C	Efficiency @40°C
Nano-tween 80 liposome	46.71	90.44	95.15
Nano-triton X-100 liposome	35.43	73.88	78.52
Nano-aerosol liposome	40.52	88.84	98.22
Nano-nonylphenol Ethyloxalate liposome	67.33	95.73	96.34
Nano-dodocane Sulphate liposome	10.65	32.44	35.83

TABLE 5. Hydrocarbon component of the undispersed crude oil by using nano-liposome.

Sample	Asphaltene, wt.,%	Maltene, wt.,%	Resin, wt.,%	Oil, wt.,%	Saturate, wt.,%	Aromatic, wt.,%
Crude oil	8.8	91.18	27.06	64.12	29.02	35.1
Nano-tween80 liposome	29.54	70.5	50.35	20.20	8.1	12.05
Nano-tritonX-100 liposome	16.45	84.53	49.72	33.83	12.03	21.8
Nano-aresol liposome	19.29	80.7	56.88	23.82	10.2	13.8
Nano-nonylphenol ethyl oxalate liposome	23.96	76.04	59.67	19.37	7.7	11.67
Nano-dodocane sulphate Liposome	12.2	87.8	37.8	50.0	20.4	29.6

due to its higher resistance to dispersant and biodegradation. The highest value of resin and asphaltene by using nanotween – liposome (50.35, 29.54) and nano -nonylphenol-ethyloxalate-liposome (59.67, 23.96 %) which have high efficiencies, while the lowest value with nano-dodocanesulfate- liposome (37.8, 12.2) which has the lowest efficiency.

Characterization of Liposome

Gas chromatography analysis of saturate fraction

The saturate separated from the undispersed crude oil after exposure with natural dispersant liposome was subjected to gas chromatographic (Fig. 3A-E). It was found that the gas chromatographic pattern of crude oil before treatment (Fig. 2) is distorted and the fingerprints are altered or in some cases, it may be drastically changed by dispersion processes using different dispersants liposome. There is a change in GC profiles such as reduction in peak heights or disappearance of number of n-paraffin and iso-paraffin peaks. By using nano-nonylphenol ethyloxalate liposome which has high efficiency (95.73%) shows a chromatogram somewhat similar to that of nanotween80-liposome as shown in Fig. 3A. It affects clearly on all the "n" and "iso" paraffin. The high molecular weight hydrocarbon paraffin has nearly diminished or disappeared with a great reduction of peaks area of the middle carbon numbers.

Dispersants liposome associated with nano-tween 80 or nano -nonylphenol ethyloxalate have nearest high efficiencies but have different behavior. The both have higher efficiencies on the iso-paraffin (which resist dispersion process), it disappeared nearly to 100% as shown in Fig.3B. By using nano-triton X-100 associated with liposome, special pattern of gas chromatogram is obtained as shown in Fig.3C. It can effect on the lower molecular weight "n" and "iso" alkanes as shown in Fig.3C. Figure 3D shows the gas chromatogram of undispersed crude oil by using nano-aerosol liposome. General change can be observed by disappearing of n- and iso-paraffin and high reduction in the percent of n-paraffin and complete disappearance of high molecular weight n-alkanes. In case of low efficiencies liposome as in case of nano-dodocane sulphate, we can observe the general decrease in percent of n-paraffin and relative decrease in peak areas of low molecular weight of n-alkane than its higher molecular weight (Fig.3E). This is due to lower efficiencies.

On studying the effect of different dispersants liposome in dispersing heavy crude oil, the use of natural soybean liposome and associated liposome are suitable for dispersion and biodegradation processes than the use of chemical dispersants because liposome structure supply microorganism with suitable nutrient thus increase biodegradation. This is appearing from the absence of most high molecular weight n- paraffin and iso-paraffin [19].

Transition electron microscopy TEM

Transmission electron microscopy (TEM) is often used to determine size and morphological characterization of liposome [20]. Many TEM studies on liposome also indicated that the particle size, vesicle shape and lamellarity of liposome may be different due to the process of preparation [21-25]. This study, was focused only on thin film method by using modified reverse phase evaporation (mREV) to produce nanoliposome particles of much smaller unilamellar [26]. Figure 4 shows TEM micrographs of nano-liposome and nanosurfactant associated liposome. It is clear that all vesicles have similar geometric (spherical in shape) and differ only in size. The average size of PC/Chol vesicles was <100 nm while for polymer associated lyposomes were <100 nm. The reduction of size of particle is due to repulsion force from charges on particles of liposome , this is in agreement with result obtained by Eun-Chul [23]. The tendency of liposome to aggregate was observed in Fig.4A this may be due to less in repulsion force.

TEM images do not lead themselves readily to evaluation of the size distribution of the particle of liposome. To address these issues, we also characterized by dynamic light scattering (DLS). Because DLS collects scattering information of particles, it is a simple matter to obtain information on of particle size distribution.

Zeta potential and differential light scattering:

Zeta potential is the charge at particles mobile surface and is used to determine the degree of flocculation or deflocculating in nano-systems and to make prediction regarding the stability of the colloidal dispersion. Its nature may be negative or positive depending on type of surfactants and lipid structure. A high zeta potential (+,-) indicates that the system shall be deflocculated as for aggregation particles have to overcome electrostatic energy barriers [27]. The values measured with Zetasizes Nano Zs were presented as the mean particle

diameter, Z-average (n.m) and the polydispersity index (PDI) values presented concurrently as the index, the uniformity of the liposome particle size distribution was evaluated. The PDI indicates that the particles were within nano-range mostly with a variable particle distribution patterns.

If the particles have a sufficiency high repulsion, the dispersion will resist aggregation and the colloidal system will be stable and this done by PC/Chol liposome (Fig.4A) and if a repulsion inefficiently, the particle in a dispersion many adhere to one another and form aggregates and successively increase size. The soyabean phosphotidylcholine showed lower dispersed and higher tendency to aggregate .when we added Chol with molar ratio (2:1), The PC/Chol liposome has increased stability in suspension and has negative charge which reduces aggregation which noticed clearly from Fig.4A and this is agreement with Lian and Lee [28]. It was noted that it contains highly negative surface charge (-14 mV) this is due to negative charge on phosphate and choline groups and the average particle size was 130nm.

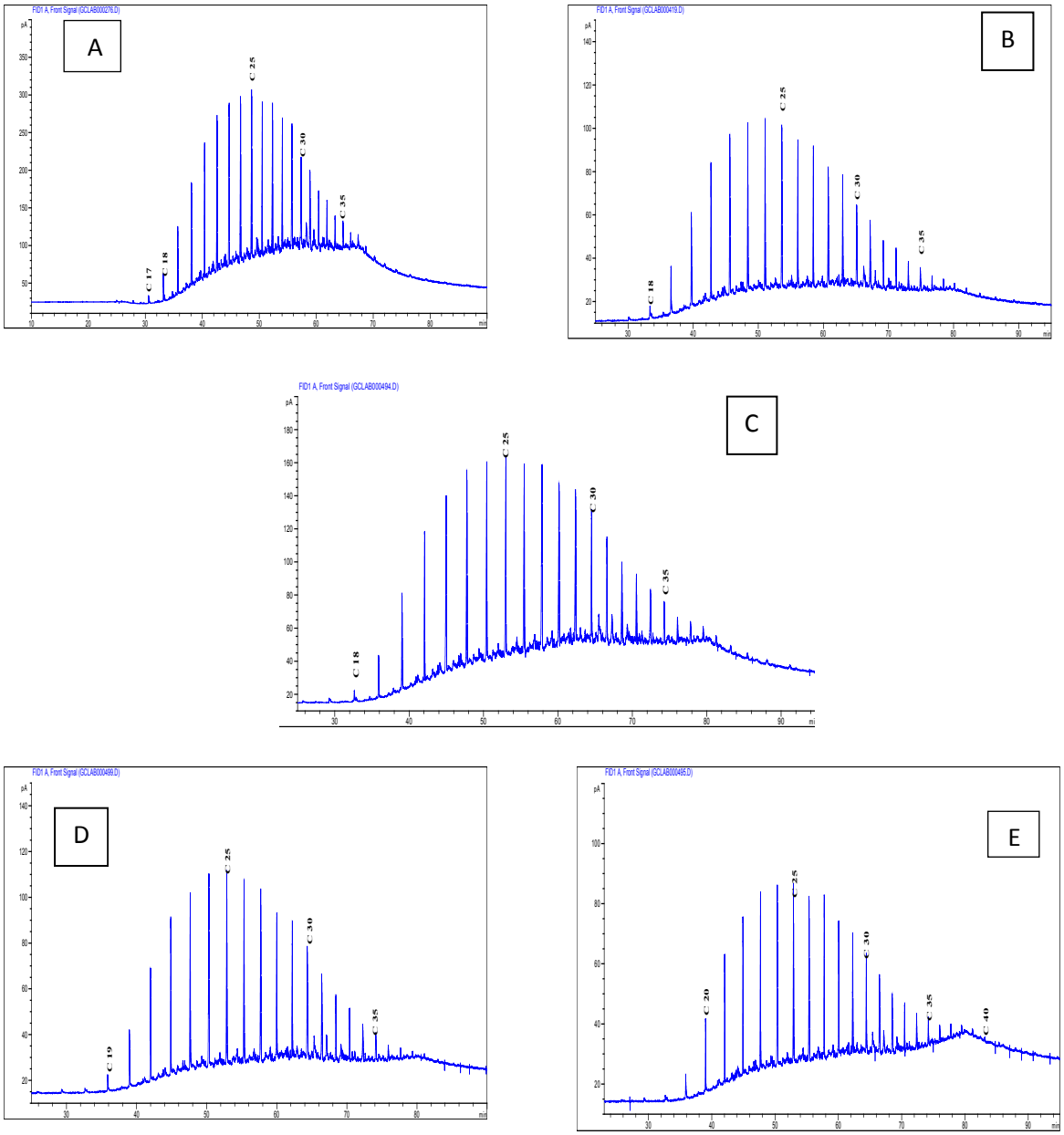

Fig. 3. GC of saturated fraction of undispersed crude oil treated by (A) nano-nonylphenol ethyloxalate liposome (B) nano-tween 80 liposome (C) by nano-tritonX-100 liposome (D) nano-aerosol liposome (E) nano-dodocane sulphate liposome.

The particle sizes of all nano-associated liposome used was less than (100nm). Therefore, the nano-associated-liposomes show relative high stability and good dispersion quality. Their particle size distribution of nano-surfactants liposomes showed better distribution than PC/Chol. Furthermore, the liposomes, which were prepared by mREV method, appeared better spherical shape and mono-dispersed size distribution and have average particle size smaller than control as shown in Table 6. The surfactant of different type associated with liposomes give different effect.

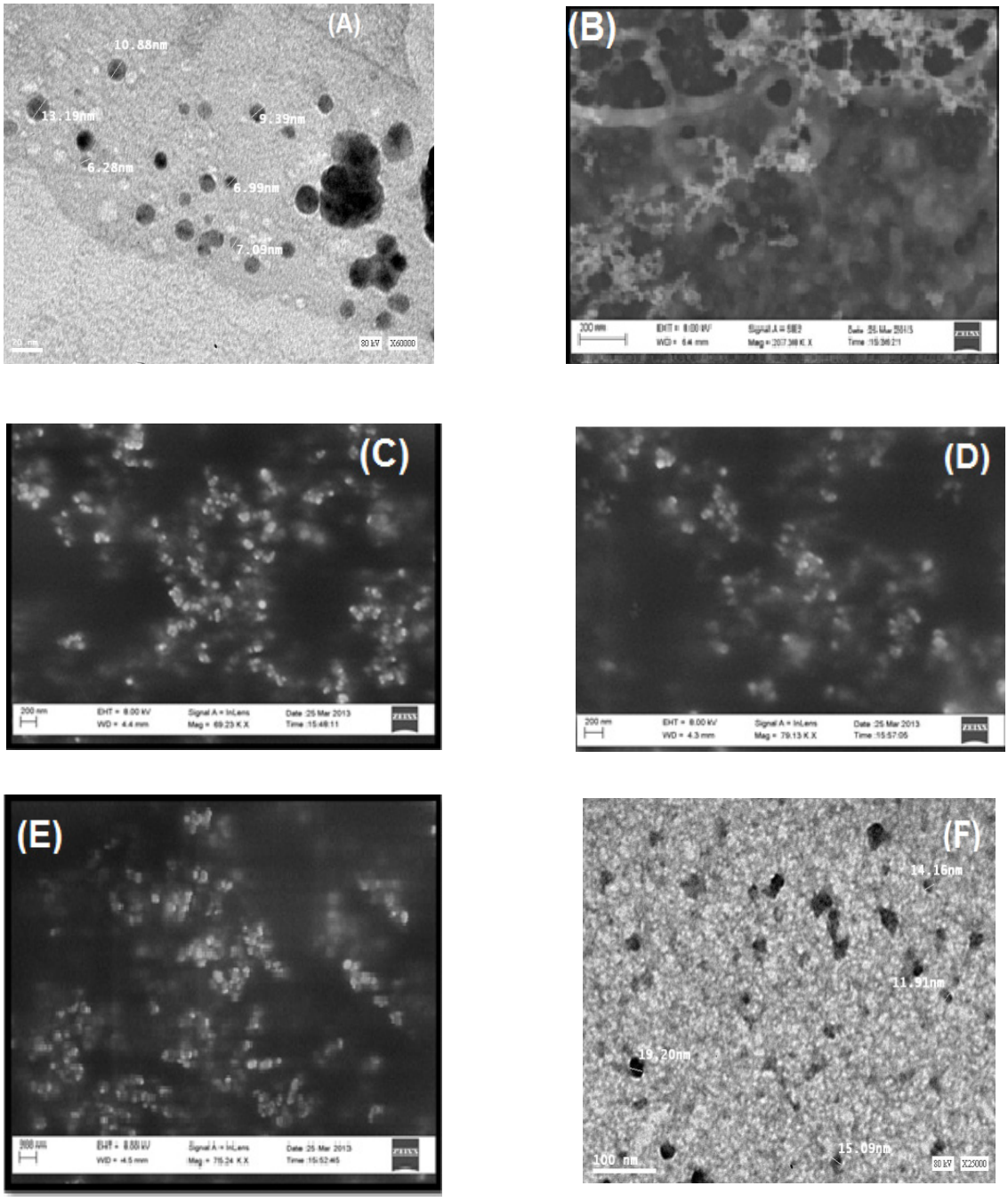

Fig. 4. TEM micrographs: (A) nanoliposome (B) nano-aeresol liposome (C) nano-tritonX-100 liposome (D) nano-nonylphenol ethyloxalate liposome (E) nano-tween80 liposome (F) nano-dodocane sulphate liposome.

Among nonionic surfactant used (nano-tween80 and nano-nonylphenol ethyloxalate associated with liposome) give maximum dispersion efficiencies (90.44%, 95.73%) with average particle size (14.40, 24.61 nm) respectively. With ionic surfactant associated with liposomes, the cationic give more efficiency than anionic *i.e.* the efficiency of cationic nano-aerosol liposome is (88.84%) while with nano-dodocane sulphate liposome is (32.44%). The average particle size and zeta potential of all associated liposomes were both reduced which were consistent with the result obtained from TEM and Zeta potential (Table 6).

When used nano-tween 80 liposome which has high dispersion efficiency (90.44%). The average particle size reduces from (130.9 to 14.40 nm) which help in dispersion process of oil spill and also zeta potential reduce from (-14 to -7.08) as shown in Table 6 which were consistent with results obtained

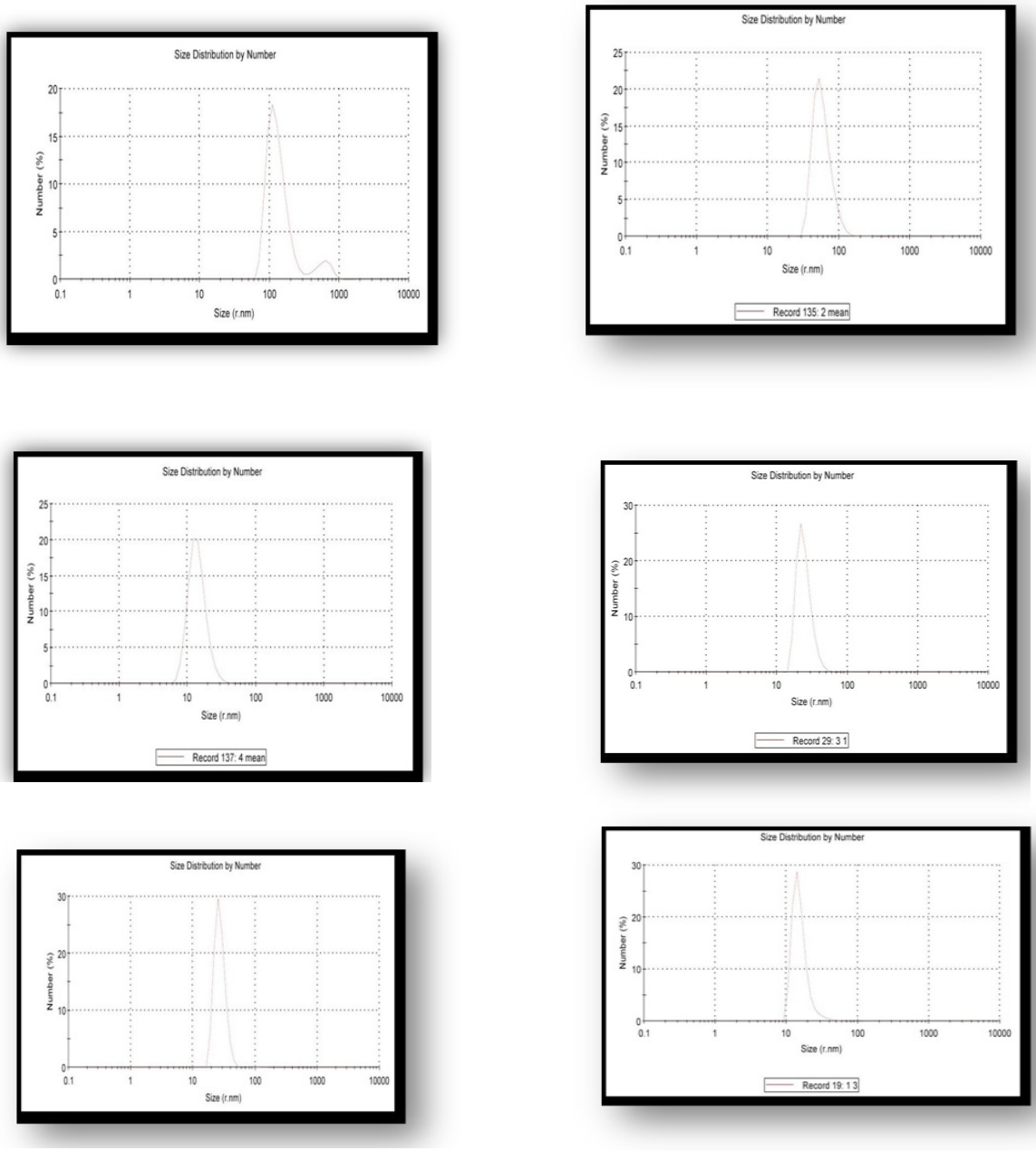

Fig. 5. Size distribution and zeta potential: (A)Nanoliposome (B)nano- tween80 liposome (C)Nano-tritonX-100 liposome (D)Nano-nonylphenol ethoxylate liposome (E)Nano-dodocyl sulphate liposome (F)Nano-aerosol liposome.

from TEM and efficiency is supported by the finding of Shimizu [29].

TABLE 6. The Zeta potential and particle size.

Sample	Z-average Diameter (nm)	Zeta potential (mV)	Polydispersity index (pdI)	Quality of Result
Nanoliposome	130.9	-14	0.588	Good
Nano-tween80 liposome	14.40	-7.08	0.366	Good
Nano-triton X-100 liposome	60.58	-6.60	0.626	Good
Nano-Aeorsol liposome	15.90	29	0.507	Good
Nano-nonylphenol ethyloxalate liposome	24.61	-6.57	0.700	Good
Nano-dodocane sulphate liposome	27.31	-60.4	0.435	Good

approximate the weathered state of crude oil after a spill occurs. The oil component of the crude oil was further separated into hydrocarbon types by applying the alumina column chromatography. Using (mREV) were produced nanoliposome from natural soybean phospholipids of (20-100 nm), in diameter, which were stable to storage at room temperature resisting aggregation or fusion for long time, and can be used in safely in treatment of oil spill. The best ratio is (2:1) PC/Chol as it gives the best efficiency on additional stabilizing effect.

The polymer associated liposome containing different surfactant(tween 80, triton X-100, Aeresol, dodocane Sulfate, nonylphenol ethyloxalate), with ratio (1:1) which gave good result in dispersing oil spill testing that is a way to increase the long-term stability of liposome, as well as inhibit liposome fusion. The percentage efficiency of liposome associated surfactant decrease with temperatures but with nano-scale increase with temperatures due to the increase in stability of nano-scale liposome. Results indicate that the higher efficiency of nano-liposome structure has higher amount of each asphalt and resin in undispersed part as in case of nanotween-liposome, nanononylphenol ethyloxalate liposome, nano-aresol liposome and the lower of the saturate and aromatic fraction.

From the TEM and DLS results noticed that the particle size distribution of nanosurfactants-liposomes showed better distribution than PC/Chol . Also, we found that the average particle size and zeta potential of all associated liposome were both reduced which were consistent with the result obtained from TEM and Zeta potential.

Conclusion

Our study was carried on a heavy crude oil. Its characterization was conducted to more closely

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نانوليبوسوم لمعالجة تسرب النفط على المياه

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والهدف من هذه الدراسة هو استبدال المشتتات الكيميائية بمواد بديلة تحتوي على مستوى عال من الفوسفوليبيدات، تم استخراج فوسفهوتديلكولين (PC) من نفايات فول الصويا التي استخدمت للحصول على نانوليبوسوم غير سامة. تم تحضير نانو مرتبط PC / Chol مع بعض مواد عوامل التوتر السطحي باستخدام طريقة (REV) المعدلة. النتائج التي تم الحصول عليها من الجزء المشتت تظهر أن الليبوسوم المرتبطة مع المواد المشتتة غير الأيونية والموجبة تظهر كفاءة عالية، في حين أن السطحي أنيوني كفاءة منخفضة. تم تطبيق تقنية كروماتوجرافيا الغاز لدراسة تركيبة الهيدروكربون للجزء غير المشتت لكل نوع من أنواع نانوليبوسوم تظهر أن نانوليبوسوم تأثر ليس فقط على الهيدروكربونات المشبعة ولكن أيضا على العطرية و إيسو-بارافين. من دراسة TEM و DLS أظهرت النتائج ان حجم الجسيمات المرتبطة أفضل توزيع من./ Chol PC، ومتوسط حجم الجسيمات وإمكانات زيتا لجميع الجسيمات الليبوسوم المرتبطة كلاهما انخفض ل نانو توين ليبوسوم و نانو-نونيلفينول إيثوكسيلات ليبوسوم