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Comparative analysis of rosemary oil extraction and preparation of nanoemulsion using ultrasonic cavitation

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The present work deals with the studies of rosemary oil extraction methods by steam and hydro-distillation and analysis for functional groups, and components present. Rosemary oil contains sensitive volatile lipophilic compounds which can be easily degraded and vaporized during handling and processing. To protect these sensitive components from the environmental conditions and its utilization into the field of flavor, fragrance, and cosmetics formulations, nanoemulsions were prepared. The preparation of nanoemulsion requires high energy, and it was overcome by ultrasound which generates ultrasonic waves and helps in disruption of larger size droplets into smaller size droplets. On the other hand surfactant and its hydrophilic lipophilic balance (HLB) play an important role in the formation of a stable emulsion. Different ionic surfactants and its blends were used for the preparation. The result indicates that surfactant S_{80}+T_{20} and S_{20}+T_{20} (Span and Tween blends) gave stable emulsion and reached mean droplet diameter (MDD) of 85.41 and 84.33 nm respectively.

Keywords: Rosemary oil, distillation, nanoemulsion, surfactants, ultrasound cavitation.

Introduction

Rosemary is the medicinal plant belongs to the mint family "Lamiaceae", its botanical name is *Rosmarinus officinalis*[1,2]. Rosemary herbs have a wonderful taste and aroma; it also shows strong antioxidant[3–7] and antimicrobial activity[8,9]. Lot of research is going on the rosemary oil and its antioxidant extract because it can act as natural preservatives in food product, packaging material, and cosmetics. This plant is considered to be the most important source of both volatile and nonvolatile bioactive compounds[10,11]. The rosemary oil is usually extracted using conventional steam and hydro-distillation. The main volatile components of rosemary oil are α-pinene, β-pinene, camphene, 1,8-cineol, camphor, myrcene, borneol[11–13]. Preparation of nano-emulsion using rosemary oil has a wide application in formulations of pesticides and insecticides[14,15]. Rosemary oil contains volatile lipophilic compounds which can be easily degraded and vaporized during handling and processing of oil therefore it is necessary to formulate them to minimize the evaporation and protecting it from high temperature, oxidation, and UV light. The non-ionic surfactant and its blend were used in the preparation of emulsion. Emulsions are thermodynamically unstable colloidal dispersions that consist of two immiscible liquids in which one liquid (minor component) is dispersed as a droplet into another component (major component) in the presence of surfactant[16]. The formation of emulsion included three steps that are droplet break up, adsorption of surfactants, and droplet re-coalescence. In emulsification, the role of surfactant was to decreases the interfacial tension which reduces the resistance for droplet disruption and helped in the process of droplet break-up. The strengthening of the newly formed film of surfactants imparts stability to the emulsion[17–20]. Ultrasonic assisted emulsification method was used for the preparation of oil in water nanoemulsion. Ultrasonication is categorized as the high energy methods as it require the intense mechanical forces for the disruption of droplets into smaller size droplets. Ultrasonic emulsification process occurred through the cavitation phenomenon[21,22]. Cavitation is the generation, subsequent growth and collapse of micro-bubbles occurs at the interface of two

immiscible phases (dispersed and continuous) under the influence of high-intensity acoustic field. The collapse of micro-bubbles produces shock waves which is responsible for producing a high turbulence that is resulting into high-velocity jets in liquid causing generation of intense shear forces in the liquid. The higher intense shear forces cause the disruption of the dispersed phase into the continuous phase. The cavitation phenomenon occurred through the acoustic waves and emulsification is carried out by acoustic cavitation[23]. The objective of this research is to study the extraction techniques of rosemary oil by using steam and hydro-distillation and, its nanoemulsion synthesis by using advance ultrasonication technique.

Experimental

The dried rosemary leaves were purchased from Excellent Spice shop Crawford market, Mumbai (MS), India. The surfactant Sorbitan monoleate (Span 80), Sorbitan monoolaurate (Span 20) and Polysorbate 80 (Tween 20) were ordered from Thomas Beaker, Mumbai (MS), India. Hydro-distillation is performed using Clevenger type of apparatus. Functional group analysis of rosemary oil was performed using Fourier transfer infrared spectroscopy (FTIR, Shimadzu). The components present in the essential oil were analyzed using Gas Chromatograpy-Mass spectrometry (GC-MS). An instrument equipped with capillary column DB-5 ms, 30 m×0.25 mm i.d., film thickness 0.25 µm was used. The column temperature was at 50°C and increased up to 280°C at a rate of 5°C/min. Helium as carrier gas was used in the system with a rate of 1 ml/min. Emulsions were prepared using Span 80 (S_{80}, HLB 4.3), Span 20 (S_{20}, HLB 8.6) and combination of Span 80, Span 20, Tween 20 (T_{20}, HLB 16.7). Total of four emulsions were prepared by changing the proportion between surfactants in order to find appropriate HLB value for rosemary oil as shown in Table 1. The HLB of the mixture was calculated according to: $HLB = x_A \cdot HLB_A + x_B \cdot HLB_B$ where x_A and x_B are the weight fractions of each surfactant[10]. Nanoemulsion was prepared by ultrasound probe under ultrasonic irradiation of 20 KHz with a maximum output of 750 W. The time and amplitude was set at 30 min and 35% respectively. Mean droplet diameter (MDD) of the emulsion was measured by the Zetasizer Nano ZS (Malvern instrument UK) equipped with dynamic light scattering technology. Surface tension of the emulsion was determined by the Kruss Tensiometer.

Table 1. Surfactant and HLB value

Emulsion using surfactant	S_{80} (wt%)	S_{20} (wt%)	T_{20} (wt%)	Total (wt%)	HLB
1	5	–	–	5	4.30
2	2.5	–	2.5	5	10.5
3	–	2.5	2.5	5	12.65
4	–	5	–	5	8.60

Results and discussion

Yield of rosemary oil: Yield and composition of rosemary oil is dependent on the extraction time. The yield was calculated using following eq. (1).

$$\text{Oil yield \%} = (m_{HE}/m_S)\times 100 \quad (1)$$

where, m_{HE} = essential oil mass (g), m_S = dry vegetal matter mass (g).

The yield of rosemary oil from steam and hydro-distillation was 2.11% and 2.0% respectively and it was observed that the yield of the oil increases with time. The relation of yield with time is shown in Fig. 1. The optimum time required for maximum yield was 10 h for both the cases.

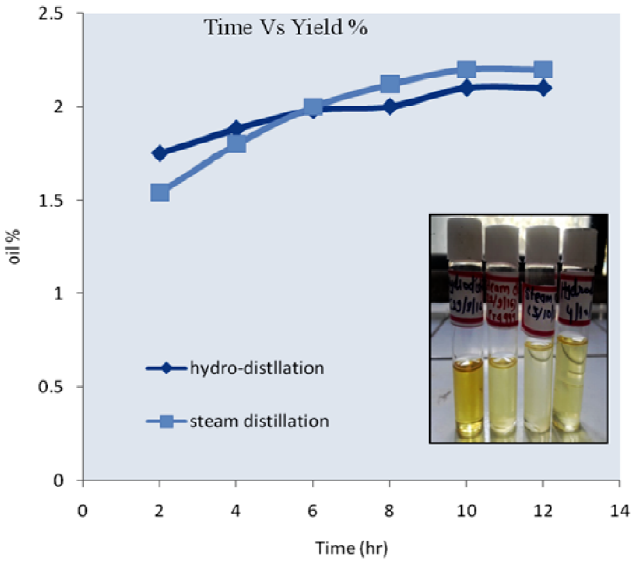


Fig. 1. Oil yield vs time.

FTIR: The FTIR results showed the presence of different type of functional groups on the basis of % of absorbance of UV-rays (wavelength range 4000–1000 cm^{-1}). Typical bands observed were at a range of 1743.65 and 1745.58 cm^{-1} (i.e. acid, C=O, stretching), 983.7 cm^{-1} (alkenes, =C-H, bending), 1051.2 and 1053.13 cm^{-1} (alcohol, C-O, stretching),

2962.66 and 2964.59 (alkane, C-H, stretching), 1215.15 cm^{-1} (ether, C-O, stretching). Fourier transfer infrared spectroscopy (FTIR) results of hydrodistilled and steam distilled oil were illustrated in Figs. 2 and 3.

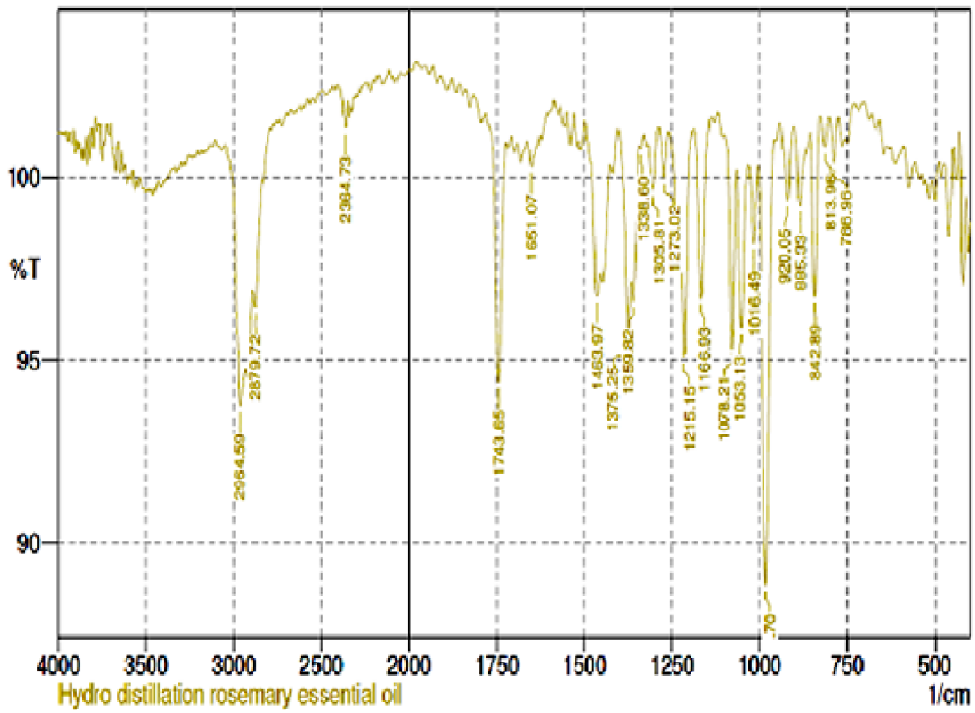


Fig. 2. FTIR result of hydro-distillation.

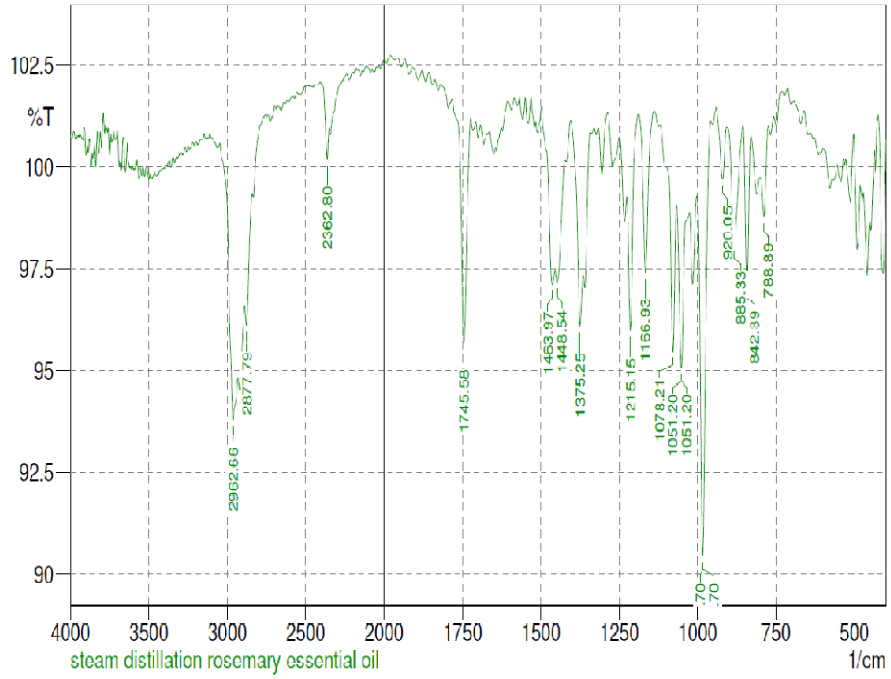


Fig. 3. FTIR result of steam.

GC-MS analysis: The identification of the components of the essential oil extracted by both steam and hydro-distillations was carried out by comparison of their retention time and mass spectra library search. The mass spectrum produced by a certain component is considered as a fingerprint for that particular molecule. The results are presented in Table 2. Total 25 components were detected and the main components present in the essential oil are camphor, α-pinene, β-pinene, cineol, myrcene, camphene, borneol, α-terpineol, bornyl acetate. The relative content of each component were calculated based on GC peak area.

Table 2. Chemical composition of RO extracted by steam distillation and hydro-distillation

Sr. no.	Compound	Retention time (min)	Relative content	
			Hydro	Steam
1.	α-Thujene	6.81	0.3	0.1
2.	α-Pinene	7.64	9.8	9.23
3.	Camphene	8.22	6.3	7.8
4.	β-Pinene	9.01	16.8	5.4
5.	β-Myrcene	9.36	2.5	3.4
6.	Cinenol	10.73	10.8	12.9
7.	Terpinene	11.62	1.1	1.8
8.	Terpineol	12.52	2.4	2.8
9.	β-Linalool	13.09	0.9	1.68
10.	Camphor	14.56	32.2	32.8
11.	Barneol	15.30	3.9	5.2
12.	*cis*-Verbenol	16.59	1.8	1.92
13.	Pulegone	16.88	1.4	Tr
14.	Thymol	17.23	2.1	Tr
15.	*cis*-Ocimene	17.76	Tr	–
16.	Bornyl acetate	18.54	Tr	0.9
17.	Copaene	20.22	Tr	0.4
18.	Caryophyllene	21..62	1.4	4.9
19.	α-Caryophyllene	22.15	Tr	1.5
20.	*trans*-Gerany acetate	22.79	0.4	0.8
21.	Caryophyllene oxide	23.47	0.2	0.6
22.	Napthalene	24.12	0.1	Tr
23.	β-Pinene oxide	24.98	0.9	–
24.	Limonene-6-ol	25.17	Tr	–
25.	Falcarinol	26.88	Tr	–

Tr, trace < 0.05%.

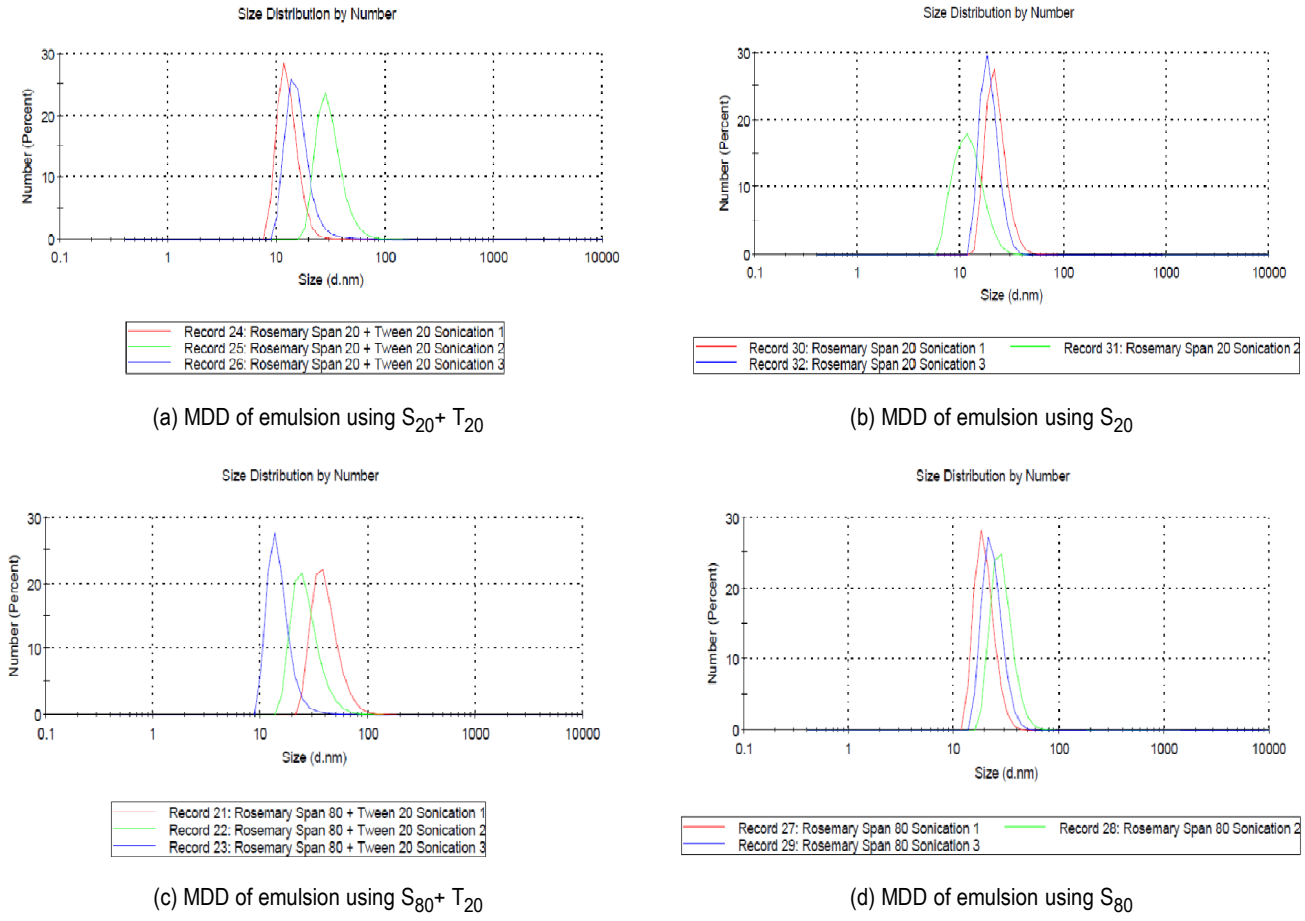
Mean droplet size: The samples of emulsions were diluted 1:100 with deionized water before the measurement studies. Minimum droplet diameter of emulsion is related to the HLB. The emulsion is having greater stability when it is formulated with the surfactants mixture having HLB values nearest to the required HLB value. The lowest mean droplet diameter for emulsion was found to be 84.33 nm for HLB of 12.65 with the blend of S_{20}+T_{20}. The MDD of other emulsions were 85.41 nm for S_{80}+T_{20}, 121.1 nm for S_{80}, and 116 nm for S_{20}. The size distribution for all emulsion is shown in Fig. 4.

Effect of surfactant concentration and HLB on rosemary essential oil: Different concentration of surfactant and their blends were taken to find the optimum HLB for rosemary oil. The surfactants and oil percentage in the emulsion was kept fixed at 5% each. All the emulsion found to be initially stable at normal temperature conditions. The emulsion was little transparent with neutral pH. Different surfactants were used in order to select the best emulsifier, in this sense blend of S_{80}+T_{20} and S_{20}+ T_{20} showed excellent results. The relation of HLB and MDD (nm) is illustrated in Fig. 5.

Surface tension: In order to measure the surface tension, the plate method was selected[24]. Water and air were set as liquid and gas phase. 35 ml of sample which contains 5% by weight of each surfactants (Tween and Span) were used for each measurement. The maximum time and detection speed were set at 60 s and 6 mm/min respectively. It was found that the average surface tension of S_{80}+T_{20} and S_{20}+T_{20} blend was found to be 30.687 mN/m and 29.072 mN/m respectively. The relation of surface tension with respect to time is shown in Fig. 6.

Emulsion stability: The emulsions were prepared and stored at normal atmospheric conditions in glass bottle. The temperature, pH, and electrical conductivity all affect the emulsion stability. To perform the thermal stress emulsion were heated in a thermostat at a temperature ranging from 40°C to 80°C. The temperature was increased by 5°C in every 5 min and the nanoemulsions were centrifuged at 3000 rpm for 10 min in each rotation to accelerate possible instability. The pH values of the emulsions were simply measured using pH meter during different time intervals.

Stability testing using centrifuge: All the four samples were tested for centrifuge behavior and it was found that emulsion prepared using S_{80} and S_{20} (single surfactant) showed poor stability characteristics and the emulsions got separated into



(a) MDD of emulsion using S_{20}+ T_{20}

(b) MDD of emulsion using S_{20}

(c) MDD of emulsion using S_{80}+ T_{20}

(d) MDD of emulsion using S_{80}

Fig. 4. Size distributions.

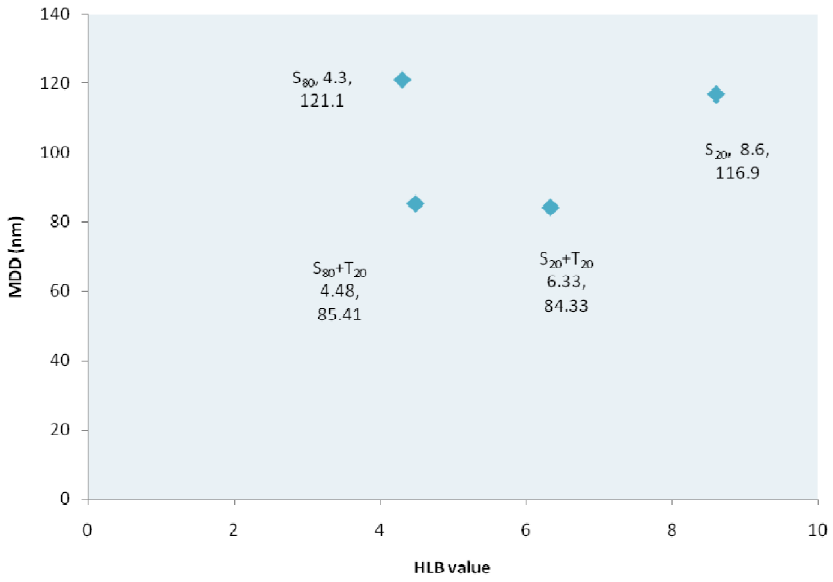


Fig. 5. HLB vs mean droplet diameter (nm).

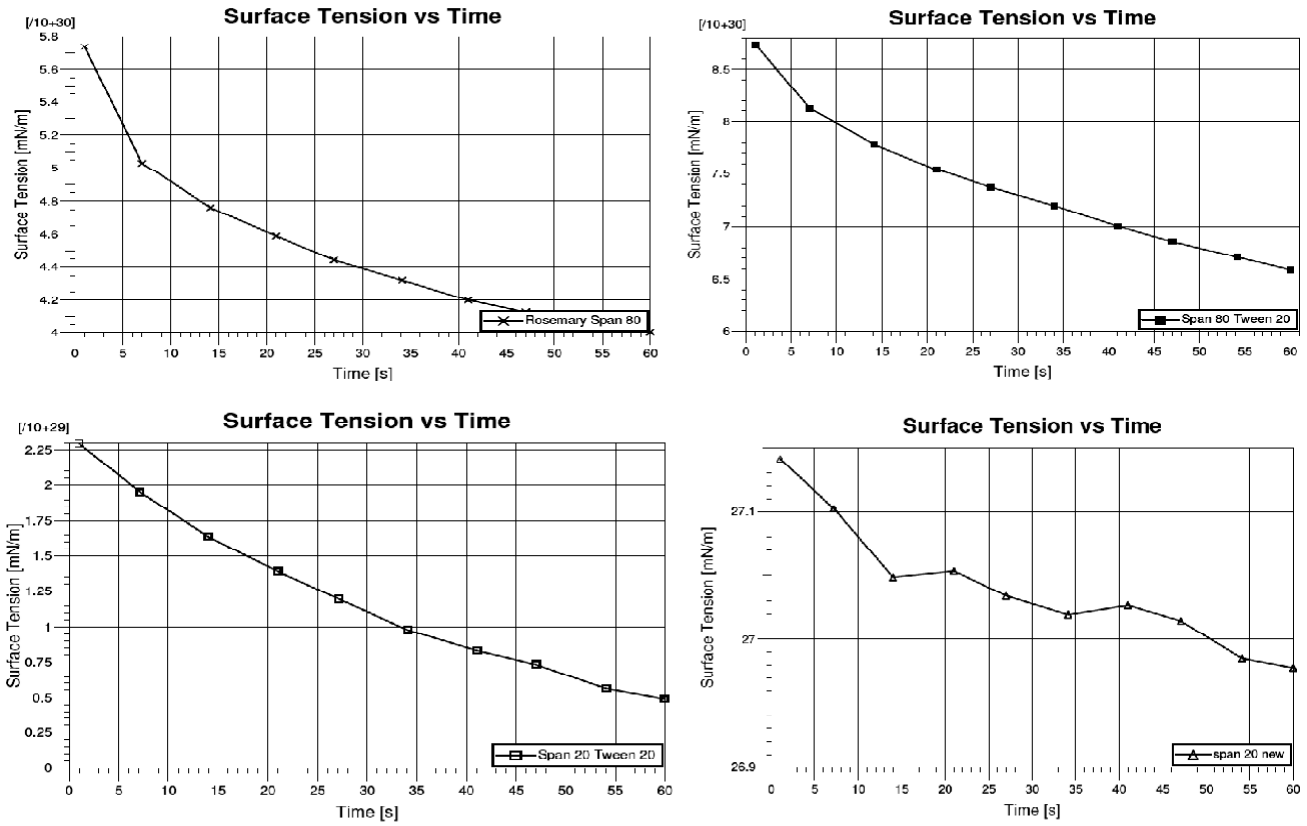


Fig. 6. Surface tension of (a) emulsion using S_{80}, (b) emulsion using S_{80}+ T_{20}, (c) SFT of emulsion using S_{20}+ T_{20} and (d) emulsion using S_{20}.

two layer and showed a little amount of creaming while other two emulsion with surfactant blends does not showed effect of creaming appearance.

pH measurement: pH monitoring is important for determining emulsion stability because changes in pH values indicate occurrence of chemical reaction that can alter the quality of the final product. All the emulsions showed the stable pH value.

Creaming Index (CI): 50 ml of emulsion samples were stored in a bottle for 30 days at ambient conditions. The susceptibility of the emulsions to creaming was ascertained by measuring the height of the boundary layer between the opaque droplet-rich layer at the top and the transparent or turbid droplet-depleted layer at the bottom of the bottle. Low creaming index indicated good homogenization. Creaming results are reported as CI = 100×(height of aqueous layer)/(height of total emulsion). None of the sample showed creaming when stored for 24 h therefore creaming index of all the samples was zero.

Table 3. Property of emulsions

Sr. No.	Emulsion using surfactant	HLB (nm)	MDD	SFT(Average) (mN/m)
1.	S_{80}	4.30	121.1	30.415
4.	S_{20}	8.60	116.7	27.005
2.	S_{80}+T_{20}	10.5	85.41	30.687
3.	S_{20}+T_{20}	12.65	84.33	29.072

Storage condition: The stability of the emulsions was measured at different time intervals (1, 30 and 60 days) by macroscopic analysis by color, visual aspect, phase separation, creaming and sedimentation. Macroscopic analyses of emulsion were carried out over the period of 2 months. After this period of storage, all the emulsion did not show any microscopic change in their appearance. But after 3 month of storage, the emulsion with S_{20} and S_{80} got separated into two layers, while other two emulsions with surfactant blends S_{20}+T_{20} and S_{80}+T_{20} did not show any changes.

Conclusions

Rosemary essential oil was extracted using hydro-distillation and steam distillation, of which steam distillation gave higher yield of 2.11% and the quality of oil, is excellent. Oveall 25 components were detected by GC-MS analysis and the major components were α-pinene, β-pinene, camphene, myrcene, cineol, etc. Ultrasound probe has proved the good method for emulsion preparation with surfactant blends of S_{20}+T_{20} and S_{80}+T_{20} were able to reach the require HLB value for rosemary oil. The lowest MDD (nm) was found to be 84.33 for an emulsion of S_{20}+T_{20}. The stability studies of the prepared emulsions were carried out and it is a function of sedimentation, creaming index, storage conditions, pH and time. The emulsions using blend of surfactants S_{80}+T_{20} and S_{20}+T_{20} were found to be most stable. The overall results indicated that the optimum blend of surfactants employed for preparing nanoemulsion resulted in stable formulation and these types of formulations can be used for delivery of fragrances and perfumes, personal care formulations, and pharmaceutical industries.

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