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Determination of the required HLB values of some essential oils

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Abstract

The required HLB values of eucalyptus, lippia and peppermint essential oils were determined using droplet size analysis and turbidimetric method on emulsions prepared with emulsifier blends of varying HLB values. The percentage increase in mean droplet diameter and the degree of dispersion of droplet sizes were determined before and after centrifugation of the emulsions. The HLB value of the emulsion with the least dispersion ratio or the least percentage increase in mean droplet diameter was taken as the required HLB of the respective essential oil. The turbidimetric method was validated by the existence of correlation ($r = -0.958$) between the mean droplet diameter and the turbidity of the emulsions. The turbidity curve went through a maximum at the HLB value where the mean droplet diameter was least. Based on these methods, the required HLB values of eucalyptus, lippia and peppermint oils were determined as 9.8, 12.1 and 12.3, respectively ($P < 0.05$). Liquid paraffin was used as a reference standard and its required HLB fell within literature value. © 2002 Published by Elsevier Science B.V.

Keywords: Required HLB; Essential oils; Degree of dispersion of droplets; Turbidimetric method

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1. Introduction

Essential oils are used primarily as natural preservatives (Deans and Ritchie, 1987; Lund, 1994; Hammer et al., 1999), flavourants (Pharmaceutical Codex, 1979) and fragrances in cosmetic products (Balsam, 1972). More recently, studies on the medicinal activity of essential oils have been intensified. Some essential oils have been found useful as ectoparasitic (Oladimeji et al., 2000), insect repellent (Oyedele et al., 2000), and anti-diarrhoeal (Orafidiya et al., 2000) agents for which they are best formulated at their effective concentrations as emulsions. Orafidiya et al. (2001) have prepared emulsions of ocimum oil as topical antiseptic applications.

Stable emulsions, especially where synthetic surfactants are used, are best formulated with emulsifiers or combination of emulsifiers having HLB (hydrophile–lipophile balance) values close to that required of the oil phase (Aulton, 1995). The required HLB values of commonly used fixed and mineral oils have been determined but those of essential oils are yet to be studied. The HLB method as first described by Griffin (1949) has

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been subjected to criticism and its limitations have been enumerated (Elworthy and Florence, 1969; Body et al., 1972; Lin et al., 1977). The phase inversion temperature (PIT) method has provided a reliable alternative for the determination of HLB values (Shinoda, 1968). However, the PIT method may not be suitable for the determination of the required HLB value of essential oils because of their very low temperature stability. Hence, the Griffin method was used in this study for the determination of the required HLB value of eucalyptus, lippia and peppermint oils. The values obtained were confirmed using the turbidimetric method which has been reported for the determination of droplet size and size distribution of emulsions (Sherman, 1968). The required HLB value of liquid paraffin was similarly determined to validate these methods.

2. Materials and methods

2.1. Materials

Liquid paraffin B.P., eucalyptus oil B.P., peppermint oil B.P. (Williams Ransome and Sons PLC, England), propylene glycol (Halewood Chemicals Ltd., England), lippia oil—the essential oil was extracted by hydrodistillation of the leaves of *Lippia multiflora* Moldenke (Verbenaceae) using the British Pharmacopoeia method (British Pharmacopoeia, 1980).

2.2. Methods

2.2.1. Preparation of emulsions

The emulsifiers, Span 80 and Tween 80, at total blend concentration of 3% w/v were used for the liquid paraffin emulsions (Prinderre et al., 1998), while a blend concentration of 1% w/v was used for the essential oils (higher concentrations of the emulsifier blends produced stable emulsions of the essential oils over a wide range of HLB). Oil-in-water emulsions, 100 ml per sample, containing 20% v/v of liquid paraffin, eucalyptus, lippia and peppermint oils, respectively, were prepared using the bottle method (Ansel et al., 1995). The required amount of Span 80 was dissolved in the oil phase and that of the Tween 80 in the aqueous phase. The oil phase was added in five portions to the aqueous phase in a 200 ml screw-cap bottle, and shaken vigorously for 60 s after each addition. The emulsions were passed twice through a laboratory hand homogenizer (Gallenkamp). A series of seven emulsions with HLB values ranging from 4.3 to 15.0 were first prepared by blending together the emulsifiers in different ratios. A second set of emulsions was later prepared using smaller ratio intervals between the two most stable emulsions from the first series.

2.2.2. Droplet size analysis

The droplet size of the emulsions was measured with an optical microscope (Lenca Galen III) fitted with X40 objective and a standardized X10 eyepiece micrometer scale 24 h after preparation. Sampling was carried out after a gentle rotation of the emulsion container in order to obtain an even dispersion of the droplets. The emulsion was diluted one in 100 using aqueous propylene glycol (Levius and Drommond, 1953). The emulsion slide was prepared and examined to ensure uniform distribution of the droplets, after which not less than 1500 droplets were measured covering at least eight fields of view. The geometric mean droplet diameter (M) was calculated using the formula suggested by Smith and Jordan (1964) for polydisperse systems:

$$\text{Log}\ M = \frac{\sum_i n_i \log x_i}{\sum_i n_i}.$$

The geometric standard deviation (δg) was calculated as:

$$Log\ \delta g = \frac{\sqrt{\sum_i n_i(\log X_i - \log M)}}{\sum_i n_i},$$

where n_i is the number of droplets whose diameter lie in an interval of which the mid-point is X_i.

2.2.3. Effect of centrifugation on droplet size distribution of emulsions

Triplicate samples of 2.5 ml each of the emulsion preparations were withdrawn into glass tubes with closely fitting caps and subjected to centrifugation at 10,000 r.p.m for 10 min using Harrier 15/80 centrifuge (Sanyo Gallenkamp). Emulsion samples with no visible oil separation was allowed to stand for 24 h after which the tubes were gently rolled over for 60 s to ensure redispersibility of the droplets. The emulsions were then analyzed for droplet size following the method described above. The percentage increase in the mean droplet diameter of each emulsion formulation was calculated thus:

$$x\% = 100\,\frac{(x_2 - x_1)}{x_1},$$

where x is the difference in the mean droplet diameter of the centrifuged emulsion x_2, and the uncentrifuged emulsion x_1.

2.2.4. Turbidimetric method

The creaming rate of an emulsion as defined by Stokes' law gives only the rate of creaming of a single droplet. However, in a polydisperse system consisting of n_i droplets of radius r_i, the mass creaming rate ($\bar{u}$), has been defined as (Bechner, 1965):

$$\bar{u} = \sum_i \frac{8\pi}{27\eta V} g n_i r_i^5 (d_i - d_2),$$

where V is the total volume of the disperse phase; η is the viscosity of the emulsion; g is the acceleration due to gravity and $d_1 - d_2$ is the density difference between the dispersed and the continuous phases.

This equation suggests that the degree of separation of an emulsion is a function of both the droplet size and size distribution, all other factors being kept constant. Since larger droplets cream rapidly, an emulsifier blend giving the smallest droplet size should produce the most stable emulsion. The degree of stability of an emulsion can, therefore, be determined by turbidimetric method, which is a measure of the reduction of light directly transmitted through the emulsion, particularly through the creamed aqueous layer.

Triplicate samples of 5 ml of the emulsion preparations were withdrawn into colourless glass ubes of 11.5 mm inner diameter, with flat base and close fitting caps. The tubes were stored undisturbed at room temperature (28 °C). On the 7th day, 0.5 ml sample was gently withdrawn from the base of the tube using a 1 ml syringe with the tip of the needle touching the base of the tube. The sample was diluted to 25 ml with distilled water and the percentage transmission (%T) was measured at 600 nm (previously determined for distilled water used as the blank control) with a colorimeter (Jenway 6051). For liquid paraffin emulsions, additional test was carried out on the 14th day.

With the blank control set at 100% transmission, the turbidity of the diluted emulsion was calculated as:

$$\text{Turbidity} = 100 - \%T.$$

The results obtained were average of three determinations.

2.2.5. Degree of creaming

A 10 ml emulsion sample was poured into a stoppered 10 ml-graduated cylinder immediately after preparation. The volume ratio of the separated aqueous phase to the total volume of the emulsion was determined at room temperature (28 °C) as a function of time over 28 days. The values obtained were average of three determinations.

3. Results

3.1. Droplet size analysis

Plots of the mean droplet diameters of uncentrifuged and centrifuged liquid paraffin emulsion against HLB values are depicted in Fig. 1. The point of inflection which corresponds to the smallest mean droplet diameter (2.019 ± 1.331 μ) was attained at HLB 11.8 for the uncentrifuged emulsions. Centrifugation at 10,000 rpm for 10 min did not induce cracking in any of the emulsion samples. However, there was a significant difference ($P < 0.05$) in the mean droplet diameters of the emulsions. The smallest mean diameter of the centrifuged emulsion (2.105 ± 1.44 μ) was attained at HLB 12.0, while the uncentrifuged emulsion had a mean diameter of 2.041 ± 1.349 μ

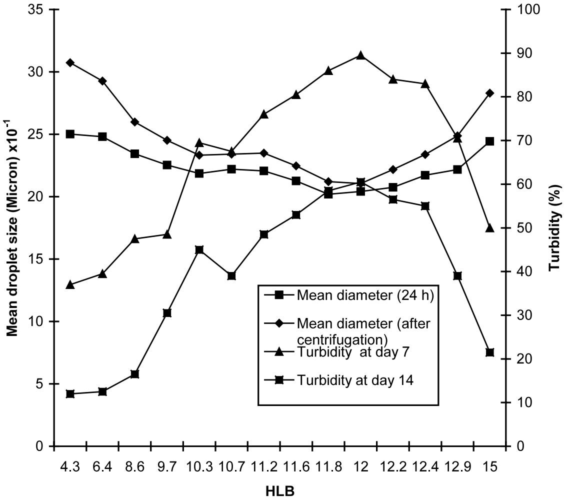

Fig. 1. Curves of mean droplet diameter and turbidity of liquid paraffin emulsions versus HLB.

at the same HLB value. The percentage increase in the mean diameter was 3.4% at HLB 12.0 as against the 5.0% obtained at HLB 11.8. The highest value of 22.8% was recorded at HLB 4.3. Thus, analysis of the droplet size of the uncentrifuged and centrifuged liquid paraffin emulsions indicated required HLB values of 11.8 and 12.0, respectively for liquid paraffin.

The lowest mean droplet diameters for eucalyptus oil, lippia oil and peppermint oil emulsions were obtained at HLB 9.7 (1.851 ± 1.187 μ), 12.0 (2.028 ± 1.348 μ) and 12.2 (2.177 ± 1.463 μ), respectively. While emulsions at the two extremes of HLB range cracked on centrifugation, the smallest mean diameters were obtained at the same HLB values as recorded for the uncentrifuged emulsions.

The influence of HLB on percentage increase in the mean droplet diameters of the emulsions of the three essential oils after centrifugation is illustrated in Fig. 2. The trough of the curve occurred at HLB 9.9, 12.4 and 12.4 for eucalyptus, lippia and peppermint oils, respectively. These values were not significantly different ($P > 0.05$) from the values obtained from the droplet size analysis of the essential oil emulsions.

Using the geometric standard deviation values as the measure of dispersion (Smith and Jordan, 1964), there was a significant difference ($P < 0.001$) between the degree of dispersion of the droplet size of the centrifuged and uncentrifuged emulsions (Table 1). The dispersion ratio for each emulsion sample was calculated as the ratio of the geometric standard deviation of the droplet size distribution of the centrifuged emulsion to that of

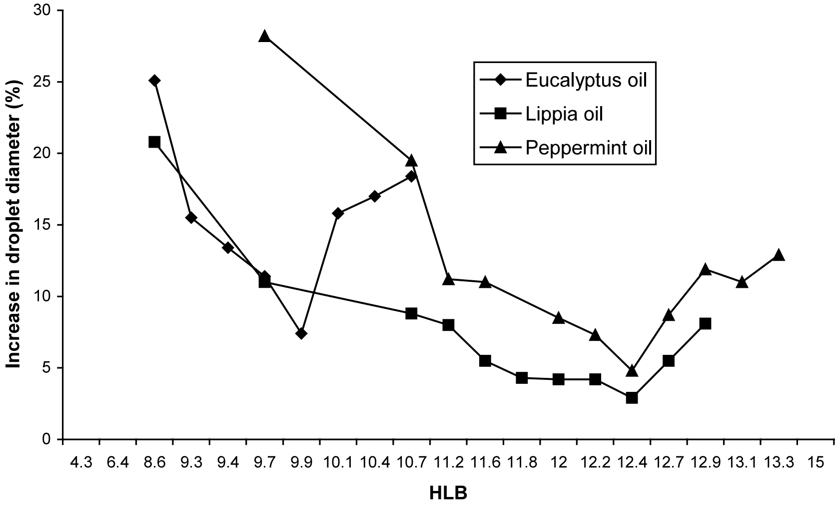

Fig. 2. Influence of HLB on percentage increase in mean droplet diameter of centrifuged eucalyptus, lippia and peppermint oils emulsions.

Table 1
The t-test of significance between the degree of dispersion of centrifuged and uncentrifuged emulsions of eucalyptus, lippia and peppermint oils at varied HLB values

Test samples	Calculated t-value	Critical t-value at $P<0.001$
Eucalyptus oil emulsion	15.629	5.405 (7)
Lippia oil emulsion	7.466	4.437 (11)
Peppermint oil emulsion	5.912	4.437 (11)

Figures in parenthesis are degree of freedom (df).

the uncentrifuged emulsion. Ratios plotted against HLB (Fig. 3) showed a similar dependence on HLB as observed in Fig. 2. The lowest dispersion ratios were obtained at HLB 9.9, 12.0 and 12.4 for emulsions containing eucalyptus, lippia and peppermint oils, respectively.

3.2. Turbidity values

The turbidity values for liquid paraffin emulsion at varied HLB values are illustrated in Fig. 1. The turbidity values went through a maximum at the same HLB value at which the mean droplet diameter was minimal. The correlation coefficients between the turbidity values and the mean droplet diameters of liquid paraffin emulsions were -0.958 ($100r^2 = 91.8$) and -0.965 ($100r^2 = 93.1$), obtained at the 7th and 14th day, respectively. The degree of turbidity peaked at HLB 12.0, thus defining the required HLB of the liquid paraffin.

The turbidity values of the emulsions of the essential oils measured at varied HLB values on the 7th day are indicated in Fig. 4. The correlation coefficient values between the turbidity and the mean droplet diameters were -0.915 ($100r^2 = 83.7$), -0.869 ($100r^2 = 75.5$) and -0.886 ($100r^2 = 78.5$) for eucalyptus, lippia and peppermint oil emulsions, respectively. The curve peaked at HLB 9.9, 12.0 and 12.2 for eucalyptus, lippia and peppermint oil emulsions, respectively.

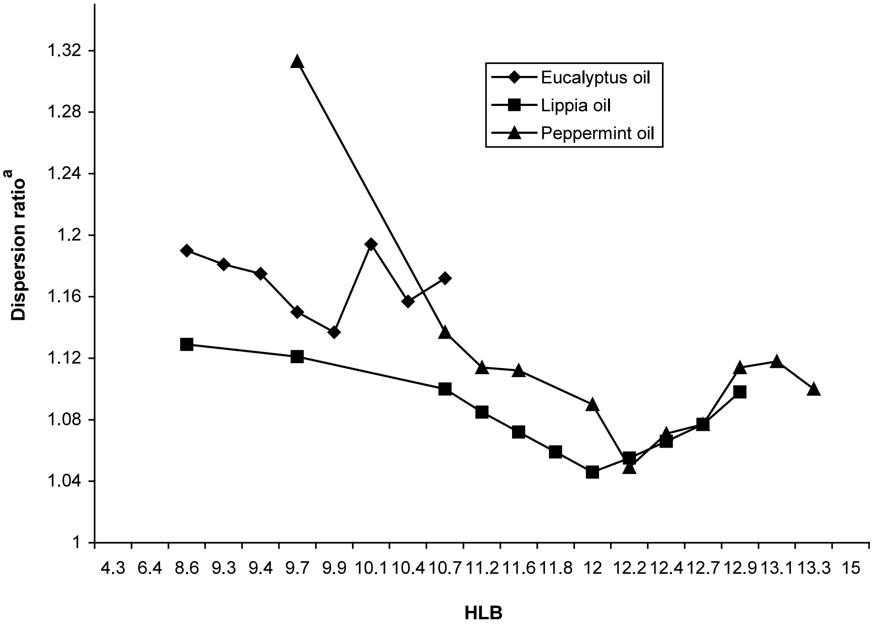

Fig. 3. Curves of dispersion ratio of droplet size distribution of centrifuged and uncentrifuged emulsions of eucalyptus, lippia and peppermint oils versus HLB. [a]Dispersion ratio = (geometric standard deviation of the droplet size distribution of centrifuged emulsion)/(geometric standard deviation of the droplet size distribution of uncentrifuged emulsion).

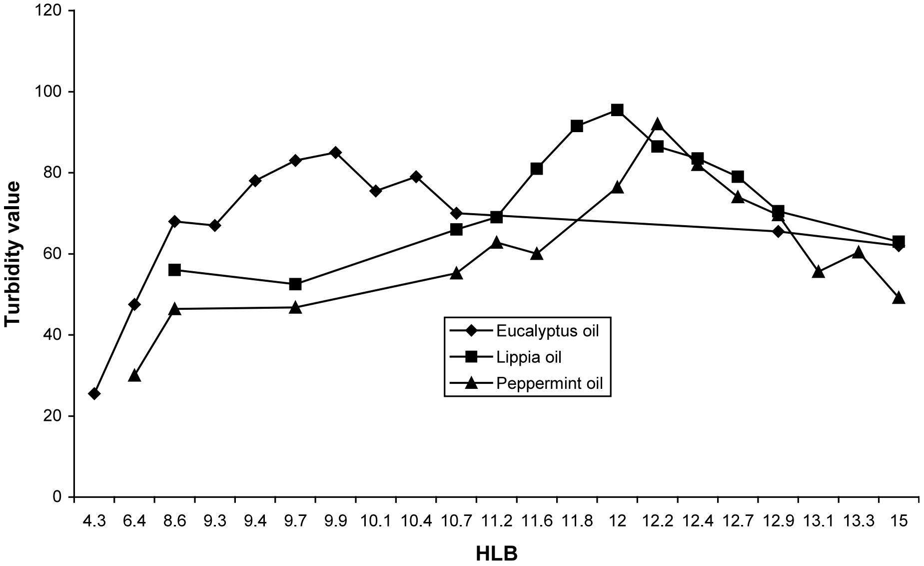

Fig. 4. Comparative curves of turbidity values of eucalyptus, lippia and peppermint oil emulsions versus HLB.

These values were within the range of HLB values obtained using the other methods discussed earlier.

The turbidity curves in Fig. 4 were analyzed by two linear regression equations, the intersection of which were used in defining the required HLB of the oils (Table 2). The required HLB values obtained from the equations for eucalyptus, lippia and peppermint oils were 9.7, 12.0 and 12.5, respectively. These were within the experimentally determined required HLB values of the essential oils. The turbidity values of liquid paraffin emulsion obtained on the 7th day were subjected to the same statistical analysis (Table 2) and required HLB value of 11.9 was obtained.

3.3. Degree of creaming

While the degree of creaming was studied for all the emulsions prepared within HLB 4.3 and 15.0, only the values for emulsions close to the required HLB of liquid paraffin are indicated in Table 3. Some of the emulsions did not cream within the first 14 days. The required HLB value of the liquid paraffin was found to lie within 11.6 and 12.2. Degree of creaming could not be used to determine the HLB of the essential oils, as most of the emulsions did not show a distinctive demarcation between the separated lower aqueous phase and the cream layer.

4. Discussion

Methods used in determination of the required HLB values of the oils in this study were based on the assumption that the droplet size of the emulsions would be smallest at the optimum HLB of

Table 2
Linear regression equations defining the required HLB of liquid paraffin, eucalyptus, lippia and peppermint oils using the turbidimetric method

Test sample	Linear regression equation
Liquid paraffin	$Y_1 = -4.333 + 7.782X$ ($r = 0.916$); $Y_2 = 239.578 - 12.727X$ ($r = -0.972$)
Eucalyptus oil	$Y_1 = -17.943 + 10.062X$ ($r = 0.982$); $Y_2 = 115.425 - 3.688X$ ($r = -0.867$)
Lippia oil	$Y_1 = -55.774 + 11.930X$ ($r = 0.886$); $Y_2 = 199.510 - 9.314X$ ($r = -0.878$)
Peppermint oil	$Y_1 = -29.798 + 8.592X$ ($r = 0.891$); $Y_2 = 250.094 - 13.841X$ ($r = -0.848$)

Y_1, Y_2 = turbidity values. $Y_1 = Y_2$ at the required HLB value X.

Table 3
Effect of HLB values on degree of creaming of liquid paraffin emulsions

HLB	24 h	7 day	14 day	28 day
10.3	8.5 (1.3)	32.7 (0.6)	55.3 (0.6)	68.0 (1.0)
10.7	0	15.5 (1.3)	31.3 (1.0)	61.5 (1.3)
11.3	0	6.3 (1.0)	19.5 (1.3)	59.7 (1.5)
11.6	0	0	0	31.0 (1.2)
11.8	0	0	0	30.3 (0.6)
12.0	0	0	0	30.1 (1.0)
12.2	0	0	0	30.5 (0.6)
12.4	0	0	13.0 (1.0)	45.0 (1.4)
12.9	5.0 (1.0)	30.8 (2.2)	51.5 (1.3)	69.2 (1.3)

Figures in parenthesis are S.D.

the emulsifier blend (Gullapalli and Sheth, 1999), and would give the highest turbidity over a period of storage due to low creaming rate. The above assumptions and the methods involved were validated by determining the required HLB of liquid paraffin whose value fell within the range 10–12 reported in literature (Lund, 1994; Prinderre et al., 1998). The credibility for using the turbidimetric method in evaluating the required HLB of the oils was affirmed by the demonstration of existence of correlation between the droplet size and the turbidity of the emulsions. The negative correlations indicated that the degree of turbidity of the emulsion, a manifestation of its stability, increases with decrease in droplet size. The droplet size analysis and the turbidimetric methods are, therefore, considered useful in the determination of the required HLB values of essential oils whose very low temperature stability precludes the use of the PIT method.

While an initial small droplet size may indicate stability of an emulsion, the main criterion for its stability would be the retention of such over a period of storage. Thus, the percentage increase in the mean droplet diameter (when centrifugal force was applied) was found a useful indicator for comparing the relative effectiveness of the different emulsifier blends in resisting the applied stress. A minimum value would indicate optimal stability, which thus determines the HLB of the system. We found that the method produced an insignificant shift of $+0.2$, $+0.4$ and $+0.2$ in the required HLB values of eucalyptus, lippia and peppermint oils, respectively, when compared with the values obtained from the droplet size analysis of the uncentrifuged emulsions. The use of percentage increase in droplet size in determining the required HLB of the essential oils would be as good as using the droplet size analysis of the emulsions.

The degree of dispersion (size distribution) of an emulsion gives an indication of the emulsifying power of the emulgent used (Levius and Drommond, 1953). The geometric standard deviation was used by Smith and Jordan (1964) in describing the skewness or dispersion of a size distribution curve. A meaningful application of the method in the determination of HLB of an emulsion would require comparative evaluation of the degree of dispersion of both the centrifuged and uncentrifuged emulsions. A dispersion ratio of 1 would indicate practically no change in the droplet size distribution of the emulsion when subjected to centrifugal force. Since centrifugation induces coalescence of droplets, a shift in the droplet size distribution, and hence, a dispersion ratio greater that 1 should be expected. The HLB of an emulsifier blend providing a minimum dispersion ratio should be acceptable as the required HLB of the oil. The required HLB of lippia oil obtained by this method was practically the same as that obtained from the droplet size analysis. However, there was a shift of $+0.2$ in the required HLB values of both eucalyptus and peppermint oils. The method can, therefore, be considered applicable for the determination of the required HLB values of the essential oils.

In conclusion, the required HLB values of the essential oils based on droplet size analysis, percentage increase in mean droplet diameter, degree of dispersion of the droplet size and turbidimetric method are summarized in Table 4. The calculated confidence intervals for the effective prediction of the required HLB at 5% level of significance are also included. Despite the use of different methods, the obtained results were not significantly different ($P > 0.05$). The methods can, therefore, be used to support one another.

Table 4
Experimentally determined and calculated required HLB values of eucalyptus, lippia and peppermint oils

Types of oil	Methods of determination/HLB value					
	Droplet size analysis		% increase in droplet size	Degree of dispersion	Turbidimetric method	Calculated confidence interval (df 5, $P < 0.05$)
	Uncentrifuged emulsion	Centrifuged emulsion				
Eucalyptus	9.7	9.7	9.9	9.9	9.9 (9.7)[a]	9.8 ± 0.1 (0.04)[b]
Lippia	12.0	12.0	12.4	12.0	12.1 (12.0)[a]	12.1 ± 0.2 (0.07)[b]
Peppermint	12.2	12.2	12.4	12.4	12.2 (12.5)[a]	12.3 ± 0.1 (0.05)[b]

[a] Derived from linear regression equations.

[b] S.E.M.

5. Uncited references

Deans and Ritchie, 1987

References

Ansel, H.C., Popovich, N.G., Allen, L.V. Jr. (Eds.), 1995. Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th ed. Lea and Febiger Book, Philadelphia, pp. 375–378.

Aulton, M.E. (Ed.), 1995. Pharmaceutics: The Science of Dosage Form Design. Churchill Livingstone, New York, pp. 290–292.

Balsam, M.S., 1972. Frangrance. In: Balsam, M.S., Sagarin, E. (Eds.), Cosmetics—Science and Technology, vol. 2, 2nd ed. John Wiley & Sons, Inc, New York, pp. 599–634.

Bechner, P. (Ed.), 1965. Emulsion Theory and Practice, 2nd ed. Chapman & Hall, London, pp. 152–153.

Body, J., Parkinson, C., Sherman, P., 1972. Factors affecting emulsion stability and the HLB concept. J. Colloid Interface Sci. 41, 359–370.

British Pharmacopoeia, 1980. Her Majesty's Stationary Office, London, pp. A108–A112.

Deans, S.G., Ritchie, G., 1987. Antibacterial properties of plant essential oil. Int. J. Food Microbiol. 5, 165–180.

Elworthy, P.H., Florence, A.T., 1969. Stabilization of oil-in-water emulsions by non-ionic detergents: electrical and entropic contributions to stability. J. Pharm. Pharmacol. 21 (Suppl.), 79S–90S.

Griffin, W.C., 1949. Classification of surface active agents by HLB. J. Soc. Cosmet. Chem. 1, 311–326.

Gullapalli, R.P., Sheth, B.B., 1999. Influence of an optimized non-ionic emulsifier blend on properties of oil-in-water emulsions. Eur. J. Pharm. Biopharm. 48, 233–238.

Hammer, K.A., Carson, C.F., Riley, T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86, 985–990.

Levius, H.P., Drommond, F.G., 1953. Elevated temperature as an artificial breakdown stress in the evaluation of emulsion stability. J. Pharm. Pharmacol. 5, 743–756.

Lin, T.J., Kurihara, H., Ohta, H., 1977. Prediction of optimum o/w emulsification via solubilization measurements. J. Soc. Cosmet. Chem. 28, 457–479.

Lund, W. (Ed.), 1994. The Pharmaceutical Codex—Principles and Practice of Pharmaceutics, vol. 516. Pharmaceutical Press, London, pp. 86–87.

Oladimeji, F.A., Orafidiya, O.O., Ogunniyi, T.A.B., Adewunmi, T.A., 2000. Pediculocidal and scabicidal properties of *Lippia multiflora* essential oil. J. Ethnopharmacol., 305–311.

Orafidiya, L.O., Oyedele, A.O., Shittu, A.O., Elujoba, A.A., 2001. The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil. Int. J. Pharm. 224, 177–183.

Orafidiya, O.O., Elujoba, A.A., Iwalewa, E.O., Okeke, I.N., 2000. Evaluation of antidiarrhoeal properties of *Ocimum gratissimum* volatile oil and its activity against entroaggregative *Escherichia coli*. Pharm. Pharmacol. Lett. 10, 9–12.

Oyedele, A.O., Orafidiya, L.O., Lamikanra, A., Olaifa, J.I., 2000. Volatility and mosquito repellency of *Hemizygia Welwtochii* Rolfe oil and its formulation. Insect Sci. Appl. 20, 123–128.

Pharmaceutical Codex, 1979. The Pharmaceutical Press, London, pp. 656–657.

Prinderre, P., Piccerelle, Ph., Cauture, E., Kalantzis, G., Reynier, J.P., Joachim, J., 1998. Formulation and evaluation of o/w emulsion using experimental design. Int. J. Pharm. 163, 73–79.

Sherman, P., 1968. General properties of emulsions and their constituents. In: Sherman, P. (Ed.), Emulsion Science. Academic Press, London, pp. 157–162.

Shinoda, K., 1968. Using the phase inversion temperature and hydrophile–lipophile balance value for choosing an emulsifier. Nippon Kagaku Zasshi 89, 435–442.

Smith, J.E., Jordan, M.L., 1964. Mathematical and graphical interpretation of the log-normal law for particle size distribution. J. Colloid Sci. 19, 549–559.