

IDENTIFICATION OF PHASES OF VARIOUS OIL, SURFACTANT/ CO-SURFACTANTS AND WATER SYSTEM BY TERNARY PHASE DIAGRAM

HAROON K. SYED and KOK K. PEH*

School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

Abstract: The objective of this study was to select appropriate surfactants or blends of surfactants and oil to study the ternary phase diagram behavior and identify various phases obtained from the oil and surfactant/surfactant mixture combinations of different HLB. The phases include conventional emulsion, gel/ viscous and transparent/translucent microemulsion. Pseudoternary phase diagrams of water, oil and S/S_{mix} of various HLB values range of 9.65–15 were constructed by using water titration method at room temperature. Visual analysis, conductivity and dye dilution test (methylene blue) were performed after each addition and mixing of water, to identify phases as microemulsion, o/w or w/o emulsion (turbid/milky) and transparent gel/ turbid viscous. High gel or viscous area was obtained with Tween 80 and surfactant mixture of Tween 80 and Span 80 with all oils. The results indicated that non-ionic surfactants and PG of different HLB values exhibited different pseudoternary phase diagram characteristics but no microemulsions originated from mineral and olive oils. The w/o emulsion occupied a large area in the ternary phase triangle when HLB value of the surfactant/ S_{mix} decreased. The o/w emulsion area was large with increasing HLB value of surfactant/ S_{mix} .

Key words: Pseudoternary phase diagram, nonionic surfactants, emulsion

Generally, emulsions, micro or nanoemulsions, are dispersed systems of different ratios of oil, surfactant(s) and aqueous phase. The different phases, their behavior and changes in volume fraction of different phases of the system can be checked by using pseudoternary phase diagram. A system consisting of water, oil, surfactant (or surfactants mixture) with various phases may be depicted on a phase tetrahedron whose apexes, respectively, present the pure components. The phase behavior can easily report on pseudoternary triangles. Apparently, a fixed (weight or volume) ratio must be selected for any two of the components and one of the triangle vertices presents 100% of the binary mixture. Multicomponent phase diagrams are generally based on constant ratios of surfactant to water or co-surfactant-to-surfactant (1).

Oil/lipid based formulations have been developed in the past by using phase diagrams. To identify regions like o/w microemulsion or nanoemulsion, coarse emulsions and gel/viscous by dissolving specific oil/surfactant/surfactant mixture ratio upon dilution of water can be obtained by using phase diagram. The past research studies showed the importance of phase diagram for developing oil based

drug delivery systems, their identification and characterization (2–9).

For making a productive formulation, study of phases obtained from several combinations of oil, surfactant/surfactant and co-surfactant mixture, water and their behavior is required. Numerous oils and surfactants are considered as satisfactory food grade materials or also being used in the pharmaceutical industry (10). The selection and choice of surfactant and co-surfactant is of great importance. Generally non-ionic surfactants are chosen because of their good cutaneous tolerance, lower irritation potential and toxicity (11). Ionic surfactants are used rarely in special cases (12). Non-ionic surfactants (13, 14), short and medium chain alcohols (15, 16), alkanolic acids, alkanediols and alkyl amines (17) can function as co-surfactants that can reduce surface tension and increase the flexibility of the interfacial film.

The aim of this study was to identify and select the surfactant or optimal blends of surfactants and oil using HLB values, and then, investigate the pseudoternary phase diagram behavior of mixtures of these surfactants with different oil and water. The pseudoternary phase diagrams were construct-

* Corresponding author: e-mail: kkpeh@usm.my; kkpehken@gmail.com; mobile: 006-0124623399

ed in order to identify the types of dispersion systems formed by the mixtures at different concentrations of their components. Our interest at this time is in viscous or gel phase. The systems composed of Tween 80, water, four oils such as isopropyl myristate (IPM), eucalyptus oil (EO), olive oil (OO) and mineral oil (MO) and co-surfactants i.e., propylene glycol (PG), Span 80, Span 20 were selected for screening. Molecular structure and HLB value of the surfactants are presented in Table 1. All these surfactants, co-surfactant and oils are generally accepted as safe, nonirritant and nontoxic for use in a number of pharmaceutical, cosmetic, and food products (18).

EXPERIMENTAL

Materials

Eucalyptus oil and olive oil were purchased from Jiaxing, Sunlong Industrial and Trading Co. Ltd., China. Mineral oil was purchased from Moksha Life Style Products, New Delhi, India. Isopropyl myristate was purchased from Derifats Chemicals Sdn. Bhd., Malaysia. Propylene glycol, Tween 80, Span 20 and Span 80 were purchased from Sigma-Aldrich, USA. Other reagents or chemicals used were of analytical grade. The materials were used as received.

METHODS

Construction of ternary phase diagram

The oils employed were olive oil (OO), mineral oil (MO), isopropyl myristate (IPM) and eucalyptus oil (EO). The physico-chemical properties of the oils are presented in Table 2. The molecular volumes (v) of the oils were calculated as:

$$v = 1.66 (Mr/d) \quad (19)$$

where Mr represents the relative molecular mass (in g/mol) of the oil and d is the density (in g/cm³). The values of Mr and d were taken from the manufacturer's specifications.

The pseudoternary phase diagrams consisting of oil, water and surfactant/surfactant and co-surfactant mixture of different HLB values were constructed using water titration method. The ratio of surfactant to co-surfactant was fixed at 1 : 1 on the weight basis. Each oil was mixed with surfactant or surfactant and co-surfactant mixture at ratios of 0.5 : 9.5, 1.0 : 9.0, 1.5 : 8.5, 2.0 : 8.0, 2.5 : 8.5, 3.0 : 7.0, 3.5 : 6.5, 4.0 : 6.0, 4.5 : 5.5, 5.0 : 5.0, 6.0 : 4.0, 7.0 : 3.0, 8.0 : 2.0 and 9.0 : 1.0 (w/w). Four types of surfactant (Tween 80, Tween 80 and Span 20, Tween 80 and Span 80, Tween 80 and propylene glycol) were used. Distilled water was added in increments of 100 μ L by micropipette at room temperature ($26 \pm 2^\circ\text{C}$) to the oil and surfactant or surfactant and co-

Table 1. Selected surfactants for screening.

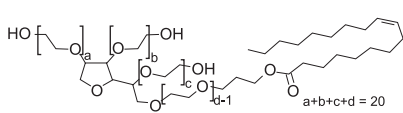
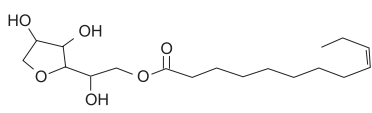
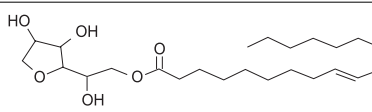
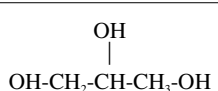
Name	HLB	Molecular structure
Tween 80 (polyoxyethylene sorbitan monooleate)	15	 <p>Polysorbate 80</p>
Span 20 sorbitan laurate (sorbitan monododecanoate)	8.6	 <p>Sorbitan monolaurate</p>
Span 80 sorbitan monooleate (sorbitan (Z)-mono-9-octadecenoate)	4.3	 <p>Sorbitan monooleate</p>
Propylene glycol	4.45	 <p>OH OH-CH₂-CH-CH₂-OH</p>

Table 2. Chemical structures, relative molecular masses (M_r), density (d) (at 20°C) and calculated molecular volumes (v) of oils investigated.

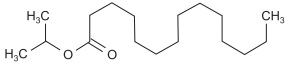

Oil	Chemical structure	M_r (g/mol)	d (g/cm ³)	v (cm ³ /mol)
Isopropyl myristate		270.45	0.854	525.7
Eucalyptus oil		154.25	0.913	280.45
Mineral oil	As specified in USP	–	0.875	–
Olive oil	As specified by manufacturer	–	0.909	–

Table 3. Percentage of IPM, Tween 80 and PG or water using 1.0 : 9.0 oil and surfactant or surfactant and co-surfactant ratio.

IPM (μL)	Tween 80 & PG (μL)	Water* (μL)	Total volume (μL)	IPM (%)	Tween 80/PG (%)	Water (%)
100	900	100	1100	9.09	81.81	9.09
100	900	200	1200	8.33	75.00	16.66
100	900	300	1300	7.69	69.23	23.07
100	900	400	1400	7.14	64.28	28.57
100	900	500	1500	6.66	60.00	33.33
100	900	600	1600	6.25	56.25	37.5
100	900	700	1700	5.88	52.94	41.17
100	900	800	1800	5.55	50.00	44.44
100	900	900	1900	5.26	47.36	47.36
100	900	1000	2000	5.00	45.00	50.00
100	900	1100	2100	4.76	42.85	52.38
100	900	1200	2200	4.54	40.90	54.54
100	900	1500	2500	4.0	36.00	60.00
100	900	2000	3000	3.33	30.00	66.66
100	900	5000	6000	1.66	15.00	83.33
100	900	10000	11000	0.90	8.18	90.90

*The amount of water is varied to provide a water concentration in the range of 9% to 90% of total volume.

surfactant mixture until 90.90% w/w. The samples were vigorously mixed with a vortex mixer for 2 min and kept at room temperature ($26 \pm 2^\circ\text{C}$) for 24 h to reach equilibrium before the next addition of water. The percentages of IPM, Tween 80 and PG and water for oil and surfactant or surfactant and co-surfactant at ratio of 1.0 : 9.0 are presented in Table 3.

Visual inspection

Visual inspection was made after each addition of water to the oil and surfactant or surfactant and co-surfactant mixture. The samples were identified as microemulsions when they appear as transparent/translucent and easily flowable liquid. No attempt was made to distinguish between oil-in-water, water-in-oil or bicontinuous type microemul-

sions. The samples were identified as emulsions when they appeared as milky or turbid liquids. The samples were identified as gel when they did not show a change in the meniscus after tilting to an angle of 90°. All these categories were plotted on a triangular graph as ternary or pseudoternary phase diagram using Chemix School 3.51 software, Arne Standnes USA.

Physical appearance of emulsions

The visual qualities of the emulsion mixtures were noted as turbid or milky liquids.

Electrical conductivity test

A pair of electrodes (Sanwa YX-360TRD Multitester, Japan) connected to an electric bulb was dipped into an emulsion. If the emulsion is o/w type, the electric bulb glows.

Staining test/dye-solubility test

Water soluble dye, methylene blue solution of 10 μL was added to the emulsion. If the continuous phase is water (o/w emulsion), the dye will dissolve uniformly throughout the system. If the continuous phase is oil (w/o emulsion), the dye will remain as cluster on the surface of the system (20).

RESULTS AND DISCUSSION

A wide variety of structures and phases can be formed by mixing oil, water and surfactants in different ratios. Molecular and structural examinations, concentration of surfactants and other ingredients

can expose the existence of microemulsions, typical emulsions, crystalline and lamellar structures depending on the ratio of the components. Recognition of different phases and structures can be achieved by simple visual inspection of their physical appearance (e.g., microemulsions (ME) are transparent/translucent, emulsions are nontransparent and phases separate after a while; and gels (21, 22). The appearance of ME, emulsion and gel is presented in Figure 1.

It is important to find the chemical type of surfactant which best matches that of the oil, because the chain length compatibility of a surfactant and oil is critical for the formation of emulsion systems. Surfactant type plays a major role in determining the rheological properties and droplets size of the systems (9). Choice of surfactant is crucial to obtain the desired formulation. Each oil and surfactant has a specific HLB value. The HLB of the selected surfactant or surfactant and co-surfactant that matches the HLB of the selected oil provides the lowest interface tension between the oil and water phases. The HLB of the selected surfactant(s) reflects the stability of the system and can be obtained when the HLBs of the surfactant and oil are similar (8). The stability of emulsions is improved if a combination of surfactants is used because solubilization reaches the maximum and the smallest particles are formed when the hydrophilic-lipophilic balance of a surfactant is optimal in a given oil-water system. Likewise, a type of oil, whether it is triglyceride form or long chain hydrocarbon, can change the physical properties of the systems.

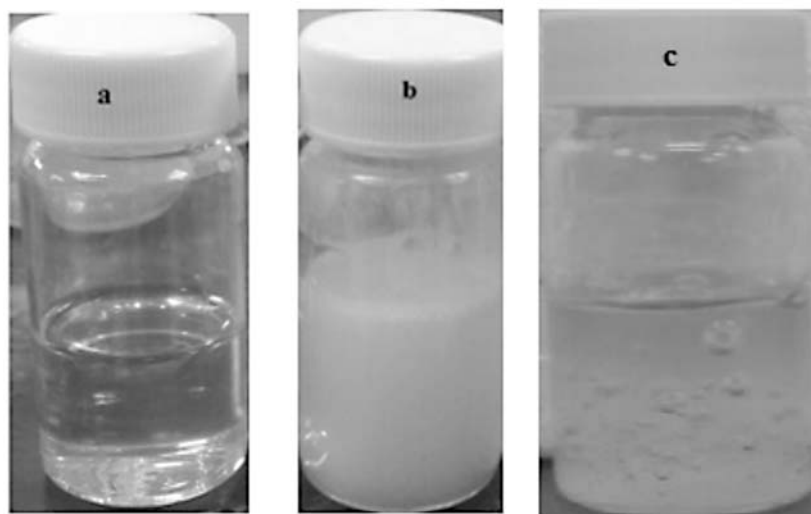


Figure 1. a – Transparent ME, b – turbid emulsion, c – gel (left to right)

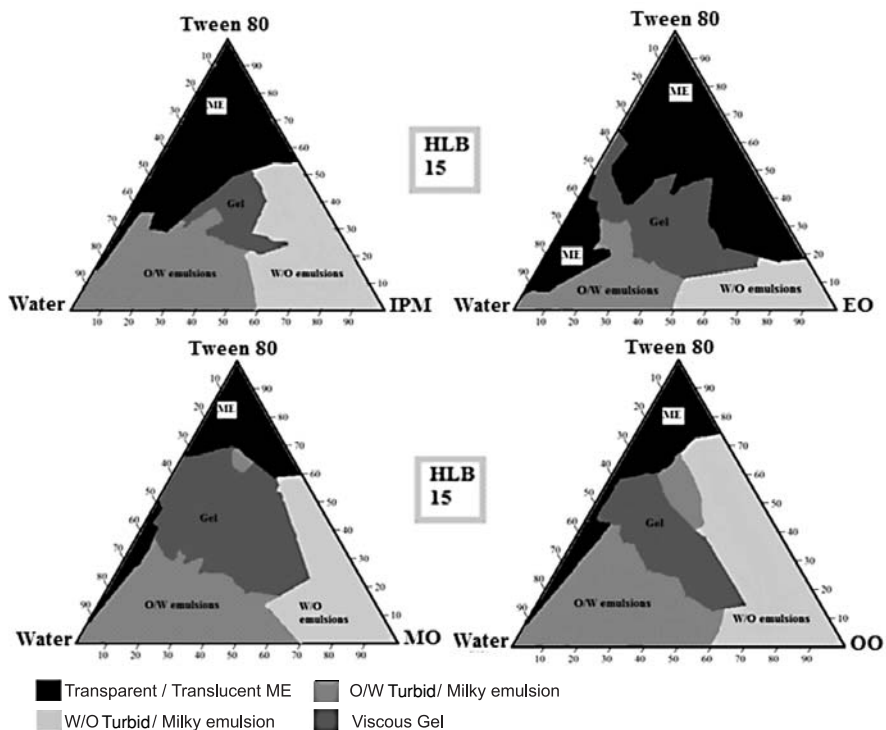


Figure 2. Ternary phase diagrams of isopropyl myristate (IPM), eucalyptus oil (EO), mineral oil (MO) and olive oil (OO) with Tween 80 and water

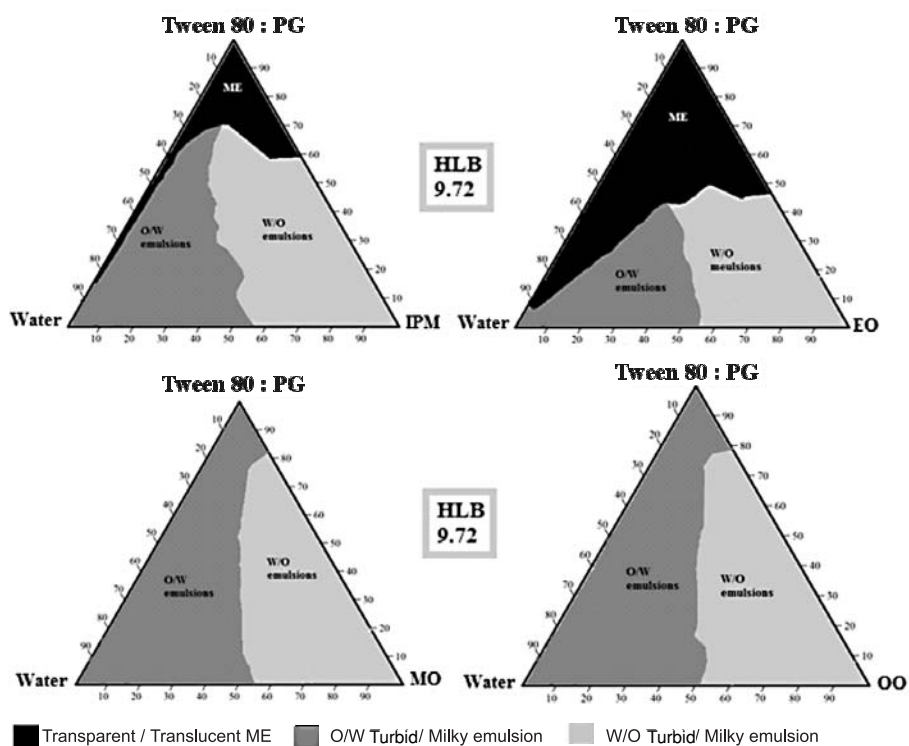


Figure 3. Ternary phase diagrams of isopropyl myristate (IPM), eucalyptus oil (EO) and olive oil (OO) with Tween 80 : Propylene glycol (PG) (Smix 1 : 1), and water

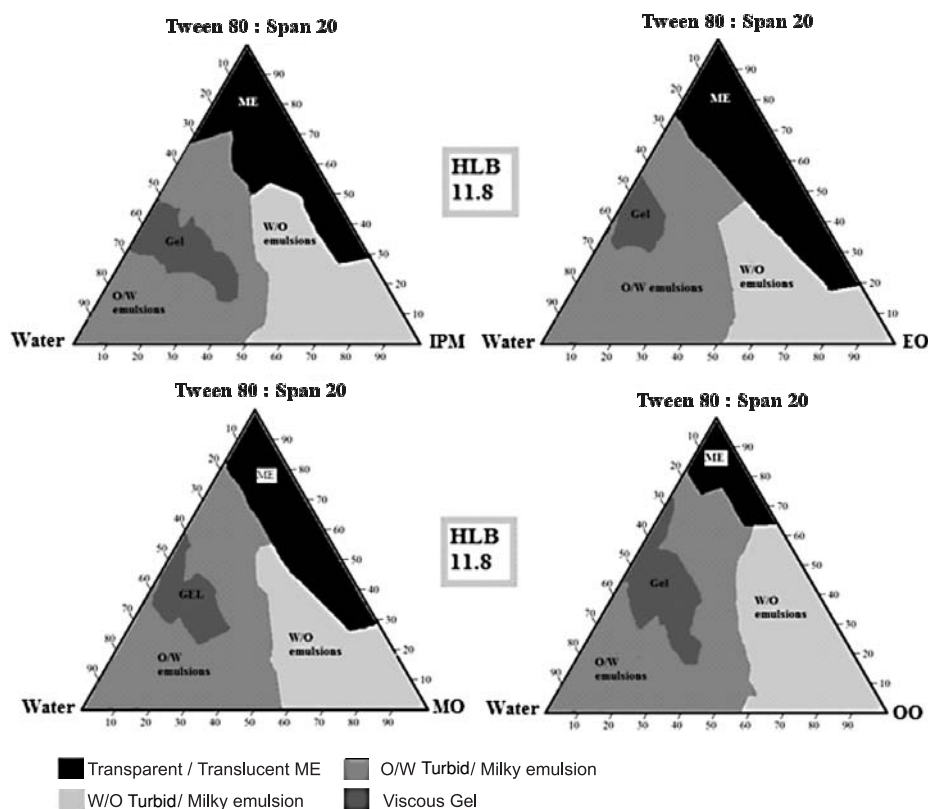


Figure 4. Ternary phase diagrams of Isopropyl myristate (IPM), Eucalyptus oil (EO), Mineral oil (MO) and Olive oil (OO) with Tween 80: Span 20 (Smix 1:1) and water

Non-ionic surfactants in general, Tween and Span in particular are safe agents for all biological tissues in general and for skin specifically (23, 24). These non-ionic emulsifiers are compatible with various ingredients used in the preparation of emulsions and are not affected by pH. A combination of lipophilic and hydrophilic non-ionic surfactants is able to build highly structured emulsions.

Tween 80 is a derivative of Span 80, but is hydrophilic in nature because the hydroxyl groups on the sorbitan ring are replaced and substituted with bulky polyoxyethylene groups. This substitution makes Tween 80 more soluble in water, so it tends to form oil-in-water emulsions. Span 80 is a viscous, lipophilic, emulsifying liquid agent, which tends to form water-in-oil emulsions.

Polyols such as propylene glycol (PG) and short-chain alcohols are known to act as co-surfactants. Hence, it is presumed that a considerable part

of PG is incorporated into the surfactant layer and will increase the interfacial fluidity, and the other part of PG will decrease the polarity of the water because PG is mainly soluble in water. PG is one of the least hydrophilic simple polyols, that is soluble in water but practically non-soluble in the oil phase (25). In comparison with other alcohols, PG is relatively tolerable by the skin.

Different combinations of oil, surfactants and water when mixed together produce either typical emulsion or microemulsion in the form of o/w or w/o. Bicontinuous system can also be formed in case of microemulsion system where each phase of water and oil is found as a continuous phase. Pseudoternary phase diagram construction is the best way to study all types of formulations that can originate from mixing of surfactants, water and oil. This study is performed to predict the optimized compositions of surfactants, oil and water in the development of ME, emulsion or gel.

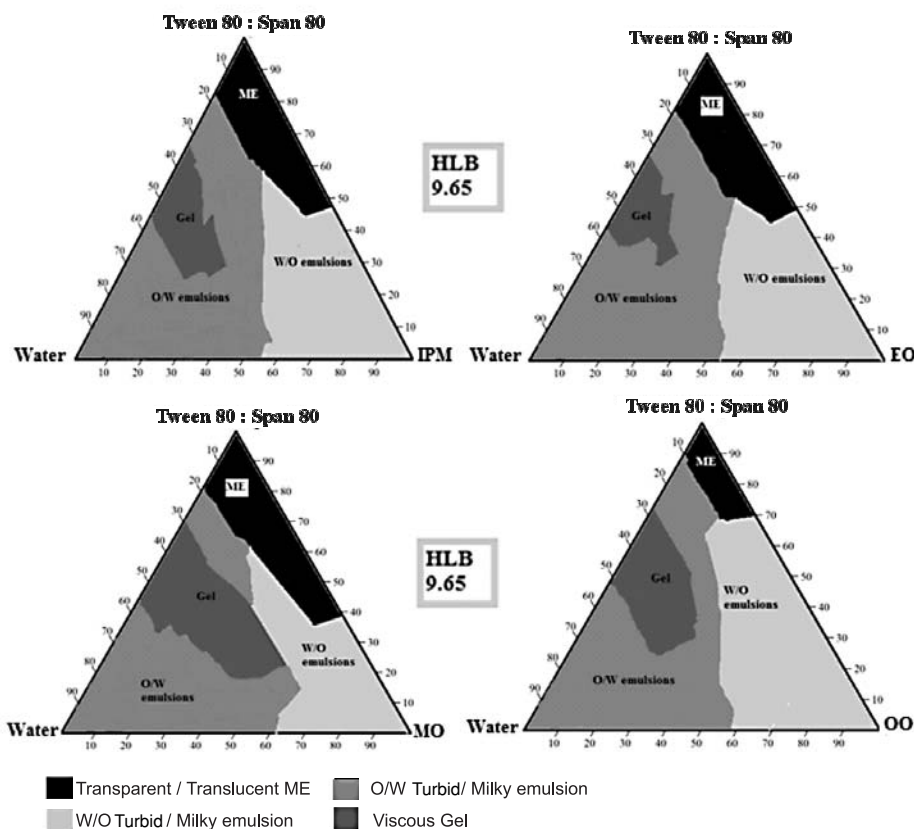


Figure 5. Ternary phase diagrams of isopropyl myristate (IPM), eucalyptus oil (EO), mineral oil (MO) and olive oil (OO) with Tween 80 : Span 80 (Smix 1 : 1) and water

Figures 2 to 5 are the phase diagrams of mixture of IPM, eucalyptus oil, mineral oil, olive oil, water and surfactant or their mixtures with HLB values of 15.00, 9.72, 11.80 and 9.65, respectively. A large area of transparent/clear solution was formed in the oil rich regions. The ME areas were found to be attached to the borders of the diagrams where water and oil ratio was low and the surfactant ratio was high. All figures showed the presence of transparent ME area except Figures 3c and 3d, representing the diagrams of mineral oil and olive oil with surfactant mixtures of Tween 80 : PG and water with HLB value of 178,758 9.72. Attempts to obtain microemulsions using mineral oil and olive oil were not successful. Mineral oil is a mixture of high molecular weight hydrocarbons. Olive oil contains predominantly long-chain triglycerides of oleic acid. Thus, the molecular weight of mineral oil and olive oil is most probably too high to assist in the formation of microemulsion (26). No liquid crystalline structure was observed.

Incorporation of co-surfactant like PG increased the maximum amount of incorporated water in the oil-surfactant system with the microemulsion zone being increased compared to the surfactant system (Fig. 3b). The presence of PG abolished the region of the gel from the phase diagrams (Fig. 3) compared to the surfactant system (Fig. 2). Breaking of the gel was reported after introduction of short chain alcohol with up to 4 carbon atoms (21).

W/O emulsion occupied the lower right region of the triangle phase diagram (oil rich region). On the other hand, o/w emulsion occupied the middle and left regions (water rich regions) of the triangle phase diagram. The formation of w/o emulsion or o/w emulsion depends on the composition of the emulsifier and its solubility in the oil and water (27). The phase diagrams obtained also showed that areas of w/o dispersions decreased as the HLB value of the surfactant system increased. This is in accor-

dance with the theory of HLB of surfactant, which stated that the types of surfactant used has more tendencies to produce the emulsion type in which it is more soluble in the external phase (9). Tween 80 : Span 80 at an HLB of 9.65 had the tendency to form a large area of w/o emulsion as shown in Figure 4. Similarly, a larger o/w area was formed by the mixture of Tween 80 : Span 20 at a higher HLB value of 11.8 (Fig. 4). Tween 80 formed large oil-in-water emulsion areas compared with water-in-oil emulsion on the oil-water axis (Fig. 1) due to higher HLB value of 15 of Tween 80, which promoted the formation of o/w emulsion (28). O/W and w/o emulsions have been used for a long time in the pharmaceutical industry and cosmetic industry (29, 30).

It can be observed in Figures 2, 4 and 5 that the gel/viscous area was formed when the water content in the system was in the range of 25 to 60%. It was found that water content below 25% was insufficient to hydrate the polyoxyethylene groups, which are critical for the swelling of surfactant chains to demonstrate the gel structure. When the water content was above 60%, the distance between the polyoxyethylene groups increased and destabilized the gel structure resulting in breaking of the swelled gel (31).

Phase diagrams containing Tween 80 (Fig. 2) and those containing surfactant and co-surfactant of Tween 80 (HLB 15) and Span 80 (HLB 4.3) (Fig. 5) exhibited higher gel area compared to Tween 80 and Span 20 (HLB 8.6) (Fig. 4). The results showed that water and surfactant and co-surfactant mixture of Tween 80 and Span 80 having HLB of 9.65 formed larger gel area than that of mixture of Tween 80 and Span 20 having HLB value of 11.8. The results indicated that a mixture of two non-ionic surfactants with big difference in HLB values between them might be able to produce stable preparations. This may be due to the fact that surfactants with very low HLB value dissolved in oil phase and the surfactant with high HLB value dissolved in the water that enable them to function together well enough to exert stronger effect than surfactant and co-surfactant mixture having closer HLB values (8). The results indicated that Tween 80 alone and surfactant mixture of Tween 80 and Span 80 would be better than surfactant mixture of Tween 80 and Span 20 for forming emulsions with gel properties. Since the Tween 80 and Span 20 showed small viscous area, this surfactant combination was excluded from further study.

CONCLUSION

This phase diagram study provides valuable information on the role that structure of the surfac-

tant, co-surfactant, and oil plays in determining the properties of the system at any composition. It was observed that a proper mixture of surfactants is achieved when surfactants at lower and higher HLB are blended. When the difference between the hydrophilic and lipophilic surfactants will be greater, the better would be the coverage by mixtures at the interface. Tween 80 alone and surfactant mixture of Tween 80 and Span 80 produced high viscous or gel area. The interesting results obtained with surfactant mixture of Tween 80 and PG, that supported the formation of ME and turbid emulsion phases but no gel phase was appeared with this combination in all oils. Except Tween 80 and PG combination, the phase diagrams containing other non-ionic surfactant mixture and Tween 80 alone, showed the appearance of different phases with oils. This study will be very useful in formulating a delivery system in pharmaceutical industry as well as in cosmetics and personal care products.

Acknowledgments

The authors thank Universiti Sains Malaysia for the graduate assistant scheme and RU grant (1001/PFARMASI/815071) and PRGS grant (1001/PFARMASI/844074) support.

REFERENCES

1. Fanun M.: in Colloid and surface research trends, 1st edn., pp. 107–146, P.K. Fong, New York 2007.
2. Nazzal S., Smalyukh I.I., Lavrentovich O.D., Khan M.A.: *Int. J. Pharm.* 235, 247 (2002).
3. Rao S.V.R., Shao J.: *Int. J. Pharm.* 362, 2 (2008).
4. Zhang P., Liu Y., Feng N., Xu J.: *Int. J. Pharm.* 355, 269 (2008).
5. Elnaggar Y.S.R., El-Massik M.A., Abdallah O.Y.: *Int. J. Pharm.* 380, 133 (2009).
6. Shafiq S., Shakeel F., Talegaonkar S., Ahmad F.J., Khar R.K., Ali M.: *Eur. J. Pharm. Biopharm.* 66, 227 (2007).
7. Kang B.K., Lee J.S., Chon S.K., Jeong S., Yuk S.H., Khang G., Lee H.B., Cho S.H.: *Int. J. Pharm.* 274, 65 (2004).
8. Elrashid S.M., Mohamed H.F.S., Muthanna F.A., Ghassan Z.A., Munavvar A.S., Azmin M.N.: *Drug Des. Devel. Ther.* 5, 311 (2011).
9. Muthanna F.A., Ghassan Z.A., Mohamed H.F.S., Mallikarjun C., Mun F.Y., Elrashid S.M., Ibrahim M.S et al.: *Int. J. Drug. Dev.* 3, 95 (2011).

10. Duro R., Souto C., Gomez-Amoza J.L., Martinez-Pacheco R., Concheiro A.: *Drug Devel. Ind. Pharm.* 25, 817 (1999).
11. Nirmala G., Padmini R., Rashmi M.: *Ind. J. Pharm. Edu. Res.* 45, 100 (2011).
12. Jiao J., Burgess D.: *AAPS. J.* 5, 62 (2003).
13. Sagitani S.H., Friberg.: *J. Disper. Sci. Technol.* 1, 151 (1980).
14. Osborne D.W., Middleton C.A., Rogers R.L.: *J. Disper. Sci. Technol.* 9, 415 (1988).
15. Attwood D., Mallon C., Taylor C.J.: *Int. J. Pharm.* 84, 5 (1992).
16. Aboofazeli R., Patel N., Thomas M., Lawrence M.J.: *Int. J. Pharm.* 125, 107 (1995).
17. Aboofazeli R., Lawrence C.B., Wicks S.R., Lawrence M.J.: *Int. J. Pharm.* 111, 63 (1994).
18. Viyoch J., Klinthong N., Siripaisal W.: *Naresuan Univ. J.* 11, 29 (2003).
19. Richardson, C.J., Mbanefo, A., Aboofazeli, R., Lawrence, M.J., Barlow, D.J.: *J. Colloid Interf. Sci.* 187, 296 (1997).
20. Aulton M.E.: in *Pharmaceutics The Science of Dosage Form Design*, 2nd edn., pp. 282–299, Churchill Livingstone Press, Spain 2002.
21. Alany R.G., Tucker I.G., Davies N.M., Rades T.: *Drug Devel. Ind. Pharm.* 27, 31 (2001).
22. Mads K.: *Adv. Drug Deliv. Rev.* 54, 77 (2002).
23. Nattiya S., Sarunyoo S., Prapaporn B.: 35th Congress on Science and Technology, pp. 1–6, The Science Society of Thailand with BBU (2007).
24. Baroli B., López-Quintela M.A., Delgado-Charro M.B., Fadda A.M., Blanco-Méndez J.: *J. Control. Release* 69, 209 (2000).
25. Whitehead K., Karr N., Mitragotri S.: *Inflammopharmacology* 25, 1782 (2007).
26. Nissim G., Anan Y., Martin E.L., Veronique C., Heribert J.W.: *J. Agric. Food Chem.* 49, 2552 (2001).
27. Djekic L., Primorac M.: *Int. J. Pharm.* 352, 231 (2008).
28. Golemanov K., Tcholakova S., Denkov N., Gurkov T.: *Langmuir* 22, 3560 (2006).
29. Yaghmur A., Campo L., Aserin A., Garti N., Glatter O.: *Phys. Chem. Chem. Phys.* 6, 1524 (2004).
30. Marti-Mestres G., Nielloud F.: *J. Dispers. Sci. Technol.* 23, 419 (2002).
31. Magdassi S.: *Colloids Surf. A. Physicochem. Eng. Asp.* 123, 671 (1997).

Received: 22. 06. 2013