J. Indian Chem. Soc.,
Vol. 96, December 2019, pp. 1499-1504

Preparation and characterization of PEGylated capric acid liposomes for intravenous delivery system

V. R. Eh Suk*[a], N. A. Basrowi[a], K. Khalid[b] and M. Misran[a]

[a]Colloid Laboratory, Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

[b]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarters, Persiaran MARDI-UPM, 43000 Serdang, Selangor, Malaysia

E-mail: vicitrizal@um.edu.my, konsrangfun@yahoo.com

Manuscript received online 16 August 2019, revised 20 August 2019, accepted 05 December 2019

Fatty acid liposomes showed a promising alternative as a carrier for various active ingredients to distribute intravenously. However, upon storage, fatty acid liposomes are unstable due to oxidation and aggregation. In this study, capric acid has been explored as a functional material for the preparation of liposomes. Fourier-transform infrared spectra, thermal activity, and phase transition of capric acid have been evaluated from the present experiment. Capric acid was spontaneously formed liposomes at pH 5.4 to 7.8. Incorporation of the fatty acid anchored poly ethylene glycol, namely 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamide-N-[methoxy (poly ethylene glycol)-2000] (DOPEPEG2000) reduced the average particle size of liposomes to 110.8 nm and –8.5 mV at the molar ratio of capric acid to DOPEPEG2000 of 1:0.020. PEGylated capric acid liposomes showed a significantly stable upon storage as compared to bare capric acid liposomes. Physical characterization showed that PEGylated capric acid liposomes have the characteristics that may compatible with intravenous delivery.

Keywords: Liposomes, capric acid, PEGylated, stability, palm oil.

Introduction

Malaysia is the world's second-largest palm oil producer, where the palm oil industry alone contributed RM42.8 billion to the national economy in 2016[1]. Palm oil can be extracted from the mesocarp, namely crude palm oil (CPO), or from the inside kernel, namely palm kernel oil (PKO)[2]. Palm oil contains a unique fatty acid (FA), which dominated by palmitic, oleic, linoleic, and stearic fatty acids plus smaller proportions of myristic, lauric, linolenic, and capric acids[3–5].

Fatty acid is widely applied as emulsifiers, texturizing agents, wetting agents, stabilizing agents, cosmetics, and pharmaceuticals[6–8]. Currently, fatty acid either saturated or unsaturated has been explored as a carrier for delivering the active ingredients such as nutrients, drugs, or even antibody to the targeted sites such as fatty acid emulsion[9], nanostructured fatty acid carrier[10], and fatty acid liposomes[11–13].

Fatty acid liposomes are self-assembled spherical particles that compose of one or more lipid bilayers entrapping their aqueous dispersion medium[12,14–17]. Generally, liposomes can be classified by their size and number of bilayers, into unilamellar and multilamellar liposomes. Unilamellar liposomes are spherical vesicles entrapping aqueous solution and bounded by a single bilayer of an amphiphilic lipid or a mixture of such lipids. Unilamellar liposomes can be divided into small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). On the other hand, multivesicular vesicles (MVV) and multilamellar vesicles (MLV) have an onion structure entrapping smaller liposomes inside the body of liposomes separated by layers of water[16]. Different type of liposomes can be obtained by using different techniques of preparation. Preparation of liposomes starts with the formation of amphiphilic lipid molecules[18], and hydration of lipid films followed by sizing of liposomes[19].

In this study, we explored capric acid, a 10 carbon saturated chain with the formula of $CH_3(CH_2)_8COOH$ as a material to prepare the liposomes for intravenous delivery. However, conventional liposomes will encounter many challenges such as oxidation, metabolite by the process of phagocyto-

sis, instability in plasma, short half-life, and rapidly removed from the blood circulation due to the formulation that containing simply phospholipids[16]. Hence, the fatty acid anchored poly ethylene glycol, namely DOPEPEG2000, was incorporated to stabilize the liposomal formulation. Characterization and morphological studied on liposome has been done in order to evaluate the suitability of PEGylated capric acid liposomes as a carrier of active ingredients through intravenous delivery.

Experimental

Materials:

Capric acid was purchased from Sigma-Aldrich (St. Louis, USA). DOPEPEG2000 was purchased from Avanti Polar Lipids, Inc., USA. Chloroform, acetone, and immersion oil were purchased from Merck (Germany). Sodium hydroxide (NaOH) was purchased from Sigma-Aldrich (St. Louis, USA), while hydrochloric acid 37% and phosphate buffered saline tablet were purchased from Spectrum (New Brunswick, NJ).

Fourier-transform infrared (FTIR) spectroscopy:

FTIR spectrum of capric acid and DOPEPEG2000 were analyzed at 30°C using Fourier-Transform Infra-Red Spectroscopy (Perkin-Elmer, USA). The Attenuated Total Reflection crystal was cleaned with a delicate task wiper (Kimberly-Clark Worldwide, Inc., USA) and acetone to eliminate the presence of residues on the optic window[20]. About 5 mg of the sample was carefully transferred on the optic window with a diamond crystal and scanned at the resolution of 4 cm^{-1} from 4000 to 400 cm^{-1} wavelength[21,22]. The peaks obtained were compared with the literature.

Equilibrium curve of capric acid:

Capric acid sodium salt was prepared by slowly mixed capric acid in 0.05 mol dm^{-3} of NaOH solution. The mixture was sonicated using JAC Ultrasonic 1505 (Jeio Tech, Korea) bath sonicator, followed by stirring overnight using the magnetic stirrer to produce a colorless solution[23]. One ml of stock solution was poured into 14 ml glass vials and various amount of 0.05 mol dm^{-3} HCl were then added in order to form the solution with various pH values. The mixtures were stirred overnight using C-MAG HS 7 Magnetic stirrer (IKA, China) and the pH of the samples was measured by using a pre-calibrated PH510 pH meter (Eutech Instruments Pte Ltd., Singapore).

Differential Scanning Calorimetry (DSC):

The thermal behavior of capric acid and DOPEPEG2000 was determined by DSC Q20 Differential Scanning Calorimetry (TA Instruments, USA). About 5 mg of the sample was transferred into the Hermetic aluminum sample pan and sealed using Tzero sample press (TA Instrument, USA). The samples were scanned at a heating rate of 5°C min^{-1}, and temperature ranges from –30 to 150°C, with the presence of a nitrogen atmosphere of 20 mL min^{-1}. Each sample was measured three times, and the average value was used.

Preparation of PEGylated capric acid liposomes:

PEGylated capric acid liposomes were prepared using thin film hydration method as described by previous researchers[12,24]. Various molar ratios of capric acid and DOPEPEG2000 were homogenized with the presence of 5 ml chloroform by using a bath sonicator. One ml of the clear solution was then transferred into the round bottom flask and the chloroform was removed by using R114 rotary evaporator (Büchi®, USA). This process was repeated until all chloroform was discarded, forming layers of thin films at the wall of the round bottom flask. The thin film was then ionized with 0.05 mol dm^{-3} of NaOH and hydrated with warm PBS solution. The pH of the solution was adjusted to pH 7.4 by using 0.05 mol dm^{-3} of HCl or NaOH solutions and stirred overnight.

Morphological observation:

The presence of PEGylated capric acid liposomes was observed at room temperature by using Q500MC Optimizing Polarizing Microscope (Leica, UK). 200 μL of the sample was dropped onto a clean glass (Omano, China) and covered with a glass coverslip. A drop of immersion oil was spiked on the glass coverslip. The micrograph was taken using a 50×objective lens at 500 times magnification using light and dark phases.

Average particle size and zeta potential:

The average particle size and zeta potential of the liposomes were quantified using a Zetasizer NanoZS (Malvern Instruments Ltd., United Kingdom) in entre for Fundamental and Frontier Sciences in Self-Assembly (FSSA), UM[25]. A four clear sided disposable cuvette of 1 cm path length was used to measure the particle size while a U-shaped polycarbonate with the gold plated electrodes cell was used to measure the zeta potential. All measurements were carried out in triplicates at 25°C for a period of 30 days.

Results and discussion

Fourier-transform infrared (FTIR) spectroscopy:

Fig. 1 shows the FTIR spectrum of DOPEPEG2000 (a) and capric acid (b) at 30°C. The peaks at 2884 and 2858 cm^{-1} in DOPEPEG2000 and peaks 2954, 2917, and 2850 cm^{-1} in capric acid were attributed to the stretching of C-H bonds[26]. The strong single peak at 1600 to 1800 cm^{-1} in all samples was attributed to the C-O stretching vibration[27]. The peak 1466 cm^{-1} in DOPEPEG2000 and peak 1428 cm^{-1} in capric acid were due to the bending of C-H bond in methylene groups. The stretching of C-O bonds was observed at the peak 1279 and 1103 cm^{-1} of DOPEPEG2000 and peak 1296 cm^{-1} of capric acid[28]. The peak of 962 cm^{-1} in DOPEPEG2000 and peak 930 cm^{-1} in capric acid was attributed to the C-H deformation vibration of the samples[28,29].

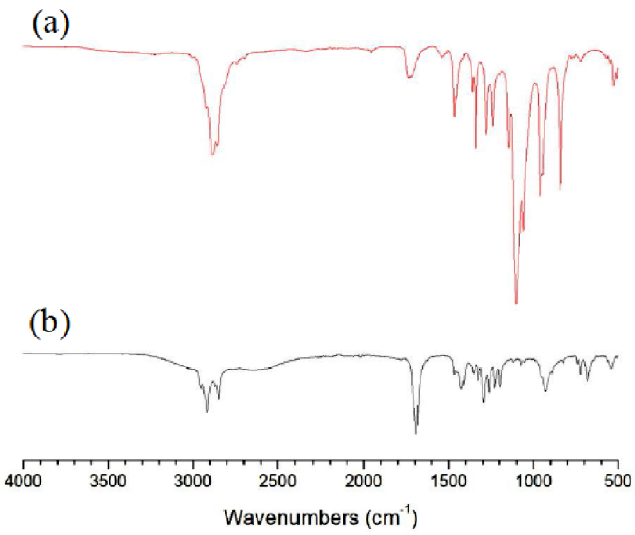

Fig. 1. FTIR spectrum of (a) DOPEPEG2000 and (b) capric acid at 30°C.

Equilibrium curve of capric acid:

Evaluating the equilibrium curve of fatty acid is crucial in determining the most suitable pH for the formation of liposomes.

Fig. 2 shows the phase transition of capric acid as the HCl was introduced into the system. At high pH, the solution appeared colorless, which was due to the ionic repulsion between the adjacent carboxylate head groups[23,30].

Addition of HCl protonated the carboxylate groups of sodium caprate and reduced the pH of the solution. As approximately half of the ionized carboxylate head groups were protonated, the ionized and non-ionized carboxylate groups were spontaneously rearranged due to hydrogen bonding and formed the bilayer membranes of liposomes[17]. At this pH, the solution turned turbid, which the liposomes were formed abundantly, and can be observed through a microscope. The plot showed that capric acid liposomes were formed within pH 5.4 to 7.8, which is suitable for intravenous delivery because the normal pH of the arterial blood is pH 7.35–7.45[31].

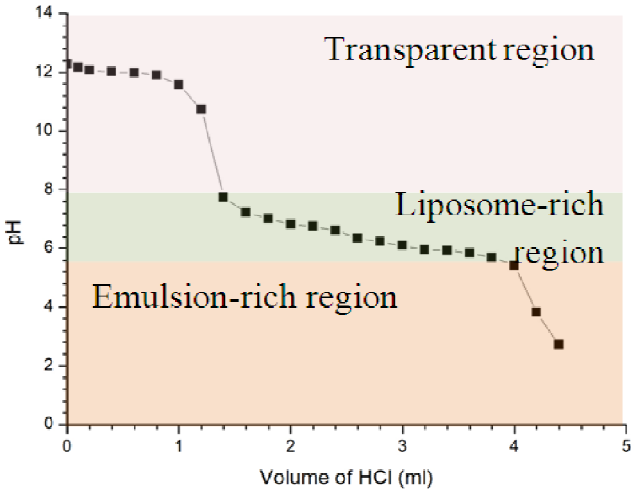

Fig. 2. Equilibrium curve of capric acid as a function of added 0.05 mol dm^{-3} hydrochloric acid at room temperature. The standard deviation was less than 0.5.

Further addition of HCl exceeding pH 5.4 produced an emulsion region, where oil droplets started to form, showing that all the carboxylate head groups were fully protonated.

Differential Scanning Calorimetry (DSC):

The information on the melting temperature is important for the preparation of liposomes as liposomes can only be prepared above this temperature[32]. Fig. 3 displays the thermograms of DOPEPEG2000 and capric acid. The sharp peaks indicated the melting temperature of the samples. The bulk structure of DOPEPEG2000 gave out the higher melting point of 52.0°C as compared to the simpler structure of capric acid at 35.7°C. At this temperature, the chains come out of their ordered arrangements and begin to move around freely. The narrow melting temperature range is recognized to have better thermal performance because it is easy to absorb or release latent heat[33].

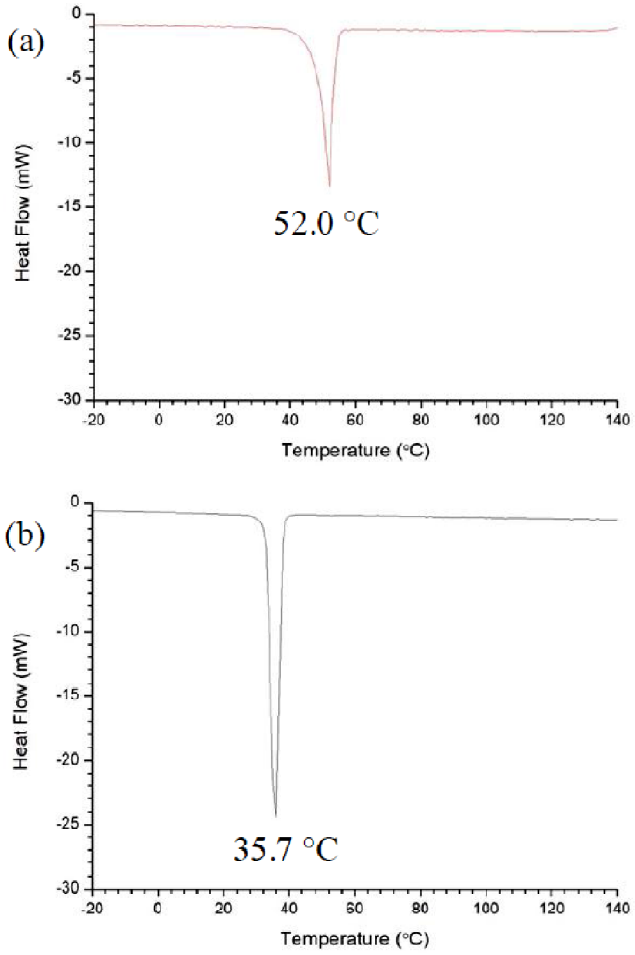

Fig. 3. Differential Scanning Calorimetry (DSC) thermogram of (a) DOPEPEG2000 and (b) capric acid.

Morphological observation:

Micrographs showed the formation of multilamellar vesicles (MLV), where they are having an onion structure entrapping smaller liposomes inside the body of liposomes separated by layers of aqueous[34]. The presence of liposomes can be verified by their unique active properties under polarised light[35].

Fig. 4 shows the presence of blue-yellow spherical PEGylated capric acid liposomes viewed under the light phase OPM due to the birefringent effect. Under the dark phase OPM, liposomes showed their active Maltese cross property. This phenomenon happened as the results of different in refractive index at the ordered assembly of the bilayer membrane of liposomes[36,37].

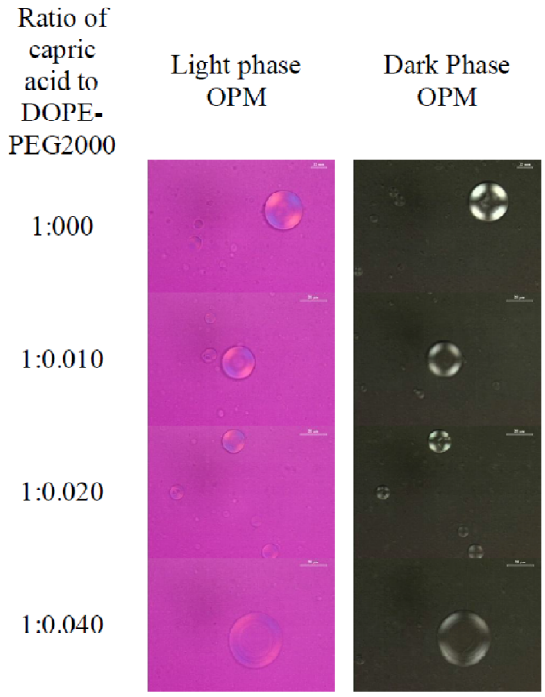

Fig. 4. Optical Polarising Micrograph (OPM) of 1st day old PEGylated capric acid liposomes at room temperature. The scale is 20 µm.

Average particle size and zeta potential:

Determination of particle size is important in designing the carrier for intravenous delivery as the size of the particle has to be compatible with the size of blood capillary, which is 4000 nm[38].

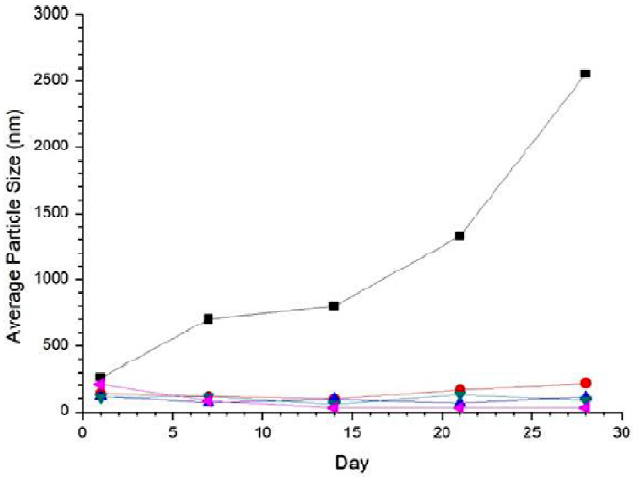

Fig. 5. Average particle size of PEGylated capric acid liposomes at 25°C. The standard deviation was less than 10. The molar ratios of capric acid to DOPEPEG2000 were 1:0 ( ), 1:0.005 ( ), 1:0.010 ( ), 1:0.020 ( ) and 1:0.040 ( ).

Fig. 5. shows the average particle size of liposomes as incubated for 28 days. On the 1st day, the average particle size of capric acid liposomes was largest, which was at 262.0 nm and smallest in PEGylated capric acid liposomes with the ratio of capric acid to DOPEPEG2000 of 1: 0.02 at 110.8 nm. Upon incubated in 7°C for 28 days, capric acid liposomes showed a significant increase in the average particle size, proving that DOPEPEG2000 was able to stabilize the capric acid liposomes. This may be due to the aggregation of capric acid liposomes. The changes in average particle size of PEGylated capric acid liposomes with the ratio 1:0.005, 1:0.010, 1:0.020, and 1:0.040 over 28 days incubation period was 50.1, –3.5, –20.7, and –84.4%, respectively.

Table 1. Zeta potential of PEGylated capric acid liposomes at 25°C. The standard deviation was less than 1

Ratio of capric acid to DOPEPEG2000	Average zeta potential (mV)	
	Day 1	Day 28
1:0.000	–15.7	–47.1
1:0.005	–7.2	–38.4
1:0.010	–2.9	–32.6
1:0.020	–8.5	–18.3
1:0.040	–2.8	–12.8

The zeta potential of fatty acid liposomes was usually found to be negative due to the presence of free fatty acids[39]. Table 1 shows the zeta potential of day 1 liposomes was most negative in capric acid liposomes, which was at –15.7 mV. Incorporation of DOPEPEG2000 altered the surface of liposomes, which increase the zeta potential up to –2.8 mV in PEGylated capric acid liposomes with the molar ratio of capric acid to DOPEPEG2000 of 1:0.040. This zeta potential allowed encapsulation of highly negative zeta potential so that it can be compatible with the zeta potential of the blood, which is around –15 mV[40]. On day 28, the average zeta potential became more negative, which may be due to the presence of free fatty acid due to the breakage of the bilayer membrane. The zeta potential was most negative in capric acid liposomes and the zeta potential gradually became less negative with the addition of DOPEPEG2000 in the formulation.

Reduction in average particle size and zeta potential was happening may be due to the oxidation of the membrane which leads to the rupture of the liposomal bilayer membrane.

Conclusions

The multilamellar PEGylated capric acid liposomes were successfully prepared by using the repeated thin lipid hydration technique. Capric acid liposomes were found to be the most abundantly present between the pH 5.4 to 7.8, which allowed the preparation of liposomes for intravenous delivery at pH 7.4. The presence of liposomes was confirmed by OPM through the presence of spherical multilamellar particles that underwent birefringent effect and Maltese cross effect under polarised light. Incorporation of DOPEPEG2000 reduced the average particle size of liposomes and significantly stabilized the liposomes. These stable PEGylated liposomes demonstrated a high potential to be a carrier for active ingredients for intravenous delivery. Further experiments such as biocompatibility tests, encapsulation of various active ingredients, and upscale test are needed for further development.

Acknowledgements

This research was supported by the Geran Penyelidikan Fakulti under Grant No. GPF065B-2018, the Fundamental Research Grant Scheme (FRGS) under Grant No. FP075-2018A, and the Prototype Research Grant Scheme (PRGS) under Grant No. PR002-2018A.

Supporting information

The raw data is available upon request.

References

1. A. Norhidayu, M. Nur-Syazwani, R. Radzil, I. Amin and N. Balu, *Int. J. Econs. & Mgmt.*, 2017, **11(3)**, 591.
2. O. I. Mba, M.-J. Dumont and M. Ngadi, *Food Biosci.*, 2015, **10**, 26.
3. M. Koushki, M. Nahidi and F. Cheraghali, *J. Paramed Sci.*, 2015, **6(3)**, 117.
4. M. I. Al-Widyan and A. O. Al-Shyoukh, *Bioresour. Technol.*, 2002, **85(3)**, 253.
5. K. Acurio, A. Chico-Proano, J. Martínez-Gómez, C. F. Ávila, Á. Ávila and M. Orozco, *Constr. Build. Mater.*, 2018, **192**, 633.
6. K. Kawai, S. Takato, M. Ueda, N. Ohnishi, C. Viriyarattanasak and K. Kajiwara, *Int. J. Food Prop.*, 2017, **20(7)**, 1500.
7. A. Bialek, M. Bialek, M. Jelinska and A. Tokarz, *Int. J. Cosmetic Sci.*, 2016, **38(4)**, 382.
8. D. Perinelli, S. Lucarini, L. Fagioli, R. Campana, D. Vllasaliu, A. Duranti and L. Casettari, *Eur. J. Pharm. Biopharm.*, 2018, **124**, 55.
9. J. Haqq, L. M. Howells, G. Garcea and A. R. Dennison, *Mol. Nutr. Food Res.*, 2016, **60(6)**, 1437.

10. S. Khan, M. Ganguli, A. Aditya, S. Khan, S. Baboota and J. Ali, *J. Drug Deliv. Sci. Tec*., 2019, **52**, 138.
11. A. Arouri, K. E. Lauritsen, H. L. Nielsen and O. G. Mouritsen, *Chem. Phys. Lipids*, 2016, **200**, 139.
12. V. R. Eh Suk and M. Misran, *Colloid Surf. A*, 2017, **513**, 267.
13. M. L. Rogerson, B. H. Robinson, S. Bucak and P. Walde, *Colloid Surf. B*, 2006, **48(1)**, 24.
14. A. D. Bangham and R. W. Horne, *J. Mol. Biol*., 1964, **8(5)**, 660-IN10.
15. D. D. Lasic, *Trends Biotechnol*., 1998, **16(7)**, 307.
16. M. S. Mufamadi, V. Pillay, Y. E. Choonara, L. C. Du Toit, G. Modi, D. Naidoo and V. M. Ndesendo, *Drug Deliv*., 2011, **2011**, 939851.
17. J. R. Kanicky and D. O. Shah, *J. Colloid Interface Sci*., 2002, **256(1)**, 201.
18. J. Dua, A. Rana and A. Bhandari, *Int. Phar. Sci. Re. Res.*, 2012, **3**, 14.
19. G. Gregoriadis, "Liposome Technology: Liposome Preparation and Related Techniques", CRC Press, Florida, 2016, pp. 1-17.
20. M. Lucarini, A. Durazzo, J. Sánchez del Pulgar, P. Gabrielli and G. Lombardi-Boccia, *Food Chem.*, 2018, **267**, 223.
21. L. Binder, J. Jatschka, D. Baurecht, M. Wirth and C. Valenta, *Eur. J. Pharm. Biopharm*., 2017, **120**, 34.
22. X. Jiang, S. Li, G. Xiang, Q. Li, L. Fan, L. He and K. Gu, *Food Chem*., 2016, **212**, 585.
23. V. R. Eh Suk and M. Misran, *J. Surfactants Deterg*., 2016, **20**, 321.
24. Y. Y. Teo, M. Misran, K. H. Low and S. M. Zain, *Bull. Korean Chem. Soc*., 2011, **32(1)**, 59.
25. L. Q. Ying and M. Misran, *Mal. J. Fund. Appl. Sci*., 2017, **13(3)**.
26. A. Sarı, A. Bicer, A. Al-Ahmed, F. A. Al-Sulaiman, M. H. Zahir and S. A. Mohamed, *Sol. Energy Mater. Sol. Cells*, 2018, **179**, 353.
27. M. Chylińska, M. Szymańska-Chargot and A. Zdunek, *Carbohydr. Polym.*, 2016, **154**, 48.
28. A. Farooq, H. Shafaghat, J. Jae, S.-C. Jung and Y.-K. *Park, J. Environ. Manage*., 2019, **231**, 694.
29. V. K. Singh, P. M. Pandey, T. Agarwal, D. Kumar, I. Banerjee, A. Anis and K. Pal, *J. Mec. Behav. Biomed. Mater*., 2016, **55**, 250.
30. I. A. Chen and J. W. Szostak, *Biophys. J*., 2004, **87(2)**, 988.
31. V. Gibson, "Pulse oximetry and arterial blood gas analysis, in Respiratory Care", Routledge, 2016, pp. 59-72.
32. B. Testa, "Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies", Zürich, Verlag Helvetic Chimica Acta, 2001, pp. 401-411.
33. X. Jin, X. Xu, X. Zhang and Y. Yin, *Thermochim. Acta*, 2014, **595**, 17.
34. A. Akbarzadeh, R. Rezaei-Sadabady, S. Davaran, S. W. Joo, N. Zarghami, Y. Hanifehpour, M. Samiei, M. Kouhi and K. Nejati-Koshki, *Nanoscale Res. Lett*., 2013, **8(1)**, 102.
35. M. Placzek and M. Kosela, *Acta Pharm.*, 2016, **66(1)**, 1.
36. S. W. Chia and M. Misran, *J. Surfactants Deterg*., 2013, **17(1)**, 1.
37. H. W. Tan and M. Misran, *Int. J. Pharm*., 2013, **441(1-2)**, 414.
38. T. Zhang, Y. Li and A. Mueller, *Chem. Phys. Lipids*, 2011, **164(8)**, 722.
39. T. Ujilestari, N. D. Dono, B. Ariyadi, R. Martien and Z. Zuprizal, *Mal. J. Fund. Appl. Sci.*, 2018, **14(3)**, 360.
40. L. Ciani, S. Ristori, C. Bonechi, C. Rossi and G. Martini, *Biophys. Chem*., 2007, **131(1-3)**, 80.