Optimization of Hydrocortisone-Loaded Nanoemulsion Formulation Using D-Optimal Mixture Design

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Received: 25 October 2017;

Accepted: 2 February 2018;

Published online: 28 February 2018;

AJC-18799

Optimization of hydrocortisone-loaded nanoemulsion formulation was achieved by low energy emulsification method. D-optimal mixture design was used with the variables parameters of Tween-20, lipoid S75, palm kernel oil ester (PKOE) and deionized water towards a response (viscosity). Hydrocortisone, ethanol and phenonip were kept constant (1.0, 24 and 0.5 wt. %). The mixing rate and time were kept constant at 400 rpm and 4 h, respectively. The optimum formulation conditions were successfully predicted. From the results obtained, it shows that the higher the concentration of palm kernel oil ester, the less the viscosity of the nanoemulsion. The optimized formulation showed good stability with the viscosity of 2.85 Pa.s. The particle size, polydispersity index and zeta potential of the optimized nanoemulsion gave a result of 172.56 nm, 0.184 and -50.03 mV, respectively. The results are in agreement with the TEM micrograph. The viscosity of the optimized nanoemulsion decreased with increased shear rate, showing a shear thinning behaviour (pseudoplastic) suitable for pharmaceutical application.

Keywords: Hydrocortisone, Nanoemulsions, Hydrophobic drug, Viscosity.

INTRODUCTION

Hydrocortisone is one of the steroidal drugs in the glucocorticoid class of hormones [1]. The problem of hydrocortisone when prescribed orally is the difficulty in adjusting the dosage, due to the short half-life of hydrocortisone [2]. Other problems in the prescription of hydrocortisone orally include nausea, headache and coughing up blood. The transdermal delivery of steroids is a rapidly-expanding field, especially in the treatment of inflammatory diseases [3].

There are several routes of drug delivery system, such as oral, injection, inhalation, transdermal and topical routes. Although these routes are capable of delivering drugs to the targeted cell, they also can cause stomach pain, gastrointestinal disturbances and vomiting. Among these routes, transdermal delivery is suitable to deliver drugs through the skin into the bloodstream. The advantages of transdermal delivery are it is non-invasive, there is longer duration of drug activities and also it reduces side effects such as nausea and vomiting [4].

Nanoemulsion is an emulsion having diameters between 30 to 200 nm [5]. There are two types of nanoemulsion, oil-in-water (O/W) and water-in-oil (W/O) nanoemulsions. Good kinetic stability and small particle size are the reasons nanoemulsions have been selected as a delivery system for pharmaceutical agent [6]. Nanoemulsions can be prepared by two

methods: either using high-energy or low-energy emulsification methods (both covering the particle size ranging less than 500 nm) [7,8]. High energy emulsification methods include high pressure homogeniser, microfluidization and ultrasonication, while the low energy emulsification method include phase inversion composition (PIC), phase inversion temperature (PIT) and dilution of microemulsion [9].

In the formulation of nanoemulsion, surfactants played an important role as a stabilizing agent. Surfactants are surface-active agents that contain hydrophilic heads and hydrophobic tails. They can increase the stability of the system by decreasing the surface tension of a liquid and making it easier to spread. Among the surfactants, non-ionic surfactants are the least toxic and not easily ionized in aqueous solutions. They also improve the solubility of poor water-soluble drugs [10].

The long-term physical stability of nanoemulsions can be accessed through the use of rheology. Rheology is the test of the flow and deformation of matter under applied force. Normally rheology has two types of common behaviour: the Newtonian behaviour and non-Newtonian behaviour. Newtonian behaviour is the shear rate directly proportional to the shear stress. Non-Newtonian behaviour is shown when the shear stress and shear rate are not linear and there is shear-thickening and shear-thinning behaviour. Shear-thickening behaviour is when the viscosity of the matter increases when the stress applied is

increased. On the other hand, shear-thinning behaviour is when the viscosity of the matter decreases when the stress applied is increased. Rheological techniques can be used to investigate the different processes that occur in emulsions, such as flocculation, Ostwald ripening, coalescence and phase inversion [11].

No study has been done on the rheology properties of hydrocortisone-loaded nanoemulsions. Hence, we used the rheometer to study the rheology and also statistical design to get the design viscosity. D-optimal mixture experimental design is used to solve the optimization issue. It is an effective technique of response surface methodology (RSM) for optimizing purposes [12]. Response surface methodology is a mathematical and statistical tool for optimizing the processes or products by establishing the relationship between the independent variables and the observed results [13].

Therefore, the aim of this work is to formulate an optimal hydrocortisone-loaded nanoemulsion using D-optimal experimental design in order to evaluate simultaneously the main effect from the variable factors (composition of Tween-20, lipoid S75, palm kernel oil ester (PKOE) and deionized water) towards viscosity. By encapsulating the hydrocortisone into this nanoemulsion, it offers an opportunity for better bioavailability and reduced side effects.

EXPERIMENTAL

Palm kernel oil ester (PKOE) was prepared in our laboratory according to the method used by Keng *et al.* [14] Tween-20 was purchased from Merck (Hohenbrunn, Germany). Lipoid S75 and ethanol were obtained from Sigma-Aldrich (Munich, Germany). Hydrocortisone was purchased from Alfa Aesar (Britain). Phenonip was purchased from Gattefosse (North America, USA). Water was deionized and Milli-Q (Millipore, Billerica, MA) filtered in our laboratory.

Preparation of oil-in-water (O/W) hydrocortisone-loaded nanoemulsion: The preparation of nanoemulsion containing hydrocortisone was according to the method by Da Costa *et al.* [15] with slight modification. In oil phase, the lipoid S75 was dissolved in ethanol. Hydrocortisone was added into the mixture and continuously stirred for 15 min and then the PKOE and phenonip were added. In water phase, Tween-20 (Polysorbate 20) was mixed with water. The water phase was added dropwise to the oil phase and stirred using an overhead stirrer for 4 h.

Experimental design: The experiment mixture design was employed to study the effect of the independent variables: Tween-20 (A), lipoid S75 (B), PKOE (C) and deionized water (D) on the response variable, the viscosity value (Y). The other ingredients were kept constant. Design Expert software (version 7, Stat-Ease Inc., Minneapolis, MN, USA) was used to run 18 experiments to get a design. There was a lower limit (Lj) and an upper limit (Uj) in the D-optimal design. The design constraints of the component proportions are shown in Table-1. The lower and upper limits chosen were closed to the best experiment obtained from the preliminary study. 18 sets of experiment in different proportions of components (% w/w) were obtained. The nanoemulsions were prepared according to the proportions of the components.

TABLE-1
DESIGN CONSTRAINTS OF THE
INDEPENDENT PARAMETER PROPORTIONS

Independent parameters	Lower limit (Lj)	Upper Limit (Uj)
Tween-20 (A)	10.00	20.00
Lipoid S75 (B)	20.00	30.00
PKOE (C)	5.00	15.00
Water (D)	9.50	39.50

Note: A + B + C + D = 74.50 wt. %.

Particle size and polydispersity index (PDI) analysis:

The particle size and PDI of the samples were measured with the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) using dynamic light scattering, scattered at an angle of 173° and a temperature of 25 °C. The mean hydrodynamic diameter (z-average mean) was calculated from the autocorrelation function of the intensity of light scattered from the particles. The software used was DTS Nano version 5.03, supplied by the manufacturer (Malvern Instruments Ltd). All the samples were diluted with water prior to measurement. Polydispersity indexes lower than 0.2 are ideal, as they indicate a narrow range of size distribution.

Zeta potential analysis: The rate of particle movement under the influence of an external oscillating electrical field with a voltage of 150 V (electrophoretic mobility) was measured with the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The measured electrophoretic mobilities were converted to zeta potentials by the instrument's software (Dispersion Technology Software, version 5.03; Malvern Instruments Ltd) using Henry's equation (1):

$$U_e = \frac{2\varepsilon\zeta f(\kappa\alpha)}{3\eta} \quad (1)$$

where U_e is the electrophoretic mobility, ε is the dielectric constant, ζ is the zeta potential, η is the viscosity of the dispersant and $f(\kappa\alpha)$ is the Henry function. The Smoluchowski approximation, $f(\kappa\alpha) = 1.5$, was used for high ionic strength media and the Debye-Hückel approximation, $f(\kappa\alpha) = 1$, was used for low dielectric medium [16].

Rheological measurement: Rheological measurements were carried out using Rotational rheometer (Kinexus, Malvern, UK). The geometry used in the measurements was plate geometry (diameter 6) and the measurement gap was 1 mm. The temperature for the measurement was room temperature. The viscosity value was selected at 5 s shear rate.

Statistical analysis: Analysis of variance (ANOVA) was used to determine the statistical significance between the independent variables. Reduced model ($p < 0.05$) and multiple regressions were employed in analyzing the experimental data. The interaction between the independent variables and responses were shown in the three-dimensional surface response.

Stability studies: The samples were centrifuged for 15 min and 4000 rpm and then observed for phase separation. Samples without any phase separation were then stored at room temperature and observed visually for phase separation every day for a month.

Morphology: The morphology of the particles in the nanoemulsion formulation was visualized with the transmi-

ssion electron microscope. The samples were dropped to a 200 mesh formvar-coated copper grids and were negatively stained with 50 mL of 2 % (w/v) phosphotungstic acid (PTA) for 5 min, at room temperature. Excess liquid was removed with a piece of Whatman filter paper and dried at room temperature. The samples were observed with Hitachi H-7100 Transmission Electron Microscope (Japan). The acquired digital images were processed with Adobe Photoshop® software.

RESULTS AND DISCUSSION

Preliminary study: The preliminary study was carried out to find the suitable range to be added in the mixture design. The formulation of hydrocortisone-loaded nanoemulsion adapted from previous work of Costa *et al.* [15] was chosen. Nanoemulsion was prepared by low energy emulsification method. The particle size (160.10 nm), polydispersity index (0.292), zeta potential (-70.80 mV) and viscosity (0.209 Pa.s at shear rate, $\dot{\gamma} = 5$ s) were obtained. The viscosity was chosen at 5 sec shear rate due to the non-Newtonian fluid behaviour of the sample and in this range the viscosity started to drop tremendously [17].

Besides that the preliminary study also included the two different ways to produce hydrocortisone-loaded nanoemulsion: the high-emulsification energy method and low-emulsification energy method. The high-emulsification method in this study used high shear stirrers. The result showed that when the mixing rate of the high shear increased, the particle size of the emulsion decreased. The mixing time of the high shear was 15 min, which was also the constant variable. Hence, the high shear for 3000 rpm mixing rate had the smallest size and optimum polydispersity index. Table-2 shows that the particle size of formulation using low energy method was not much different when compared to the high energy method. Therefore, low energy emulsification method was used in the D-optimal design to produce 18 sets formulation of nanoemulsions.

Model fitting: The formulation of hydrocortisone-loaded nanoemulsion was optimized through the D-optimal mixture design approach. The significance of the coefficient of the quadratic polynomial models was evaluated by using ANOVA. For any terms in the models, a large F-value and a small P-value indicated a significant effect on the respective response variables. The 18 set experiments were carried out to optimize the four components in the formulation and were shown in Table-3.

TABLE-3
THE MATRIX OF D-OPTIMAL DESIGN:
INDEPENDENT PARAMETERS AND A RESPONSE

Set	Independent parameters				Response
	A: Tween-20	B: Lipoid S75	C: PKOE	D: Water	Viscosity (Pa.s)
1	19.99	21.14	15.00	18.37	0.001
2	14.15	23.55	12.77	24.03	0.087
3	16.45	30.00	7.31	20.74	0.048
4	10.01	20.00	5.01	39.47	1.076
5	10.00	20.01	14.47	30.02	2.850
6	10.00	20.01	14.47	30.02	2.850
7	10.00	25.90	8.69	29.90	0.225
8	19.18	20.00	8.15	27.17	0.045
9	10.01	20.00	5.01	39.47	0.011
10	14.22	20.38	6.80	33.10	0.055
11	19.99	21.14	15.00	18.37	0.220
12	20.00	25.83	5.09	23.57	0.064
13	15.89	26.68	15.00	16.93	0.054
14	20.00	25.83	5.09	23.57	0.037
15	10.03	30.00	14.99	19.47	0.257
16	10.03	30.00	14.99	19.47	0.258
17	10.17	28.94	10.89	24.50	0.067
18	12.61	30.00	5.13	26.76	0.037

Table-4 shows that the quadratic model had higher coefficients for the response of viscosity. The D-optimal mixture design model illustrated that the second-order polynomial used for viscosity determination coefficient was $R^2 = 0.93$ which was close to 1, illustrating that the quadratic model could explain 93.48 % of the response value changes. However, the overall

TABLE-2
COMPARISON BETWEEN LOW ENERGY AND HIGH ENERGY METHOD

Emulsification method	Mixing rate (rpm)	Mixing time (min)	Droplet size (nm)	Poly dispersity index	Zeta potential (mV)
Low energy	400	240	160.10 $\pm$ 2.55	0.292 $\pm$ 0.230	-70.80 $\pm$ 0.46
High energy	3000	15	166.27 $\pm$ 0.90	0.339 $\pm$ 0.005	-50.43 $\pm$ 0.55

TABLE-4
ANALYSIS OF VARIANCE (ANOVA) FOR D-OPTIMAL MIXTURE DESIGN OF QUADRATIC

Source	Sum of square	DF	Mean square	F value	Prob > F	Significance
Model	12.98	7	1.85	20.48	< 0.0001	Significant
Linear Mixture	6.74	3	2.25	24.82	< 0.0001	
AC	0.77	1	0.77	8.48	0.0155	
BC	2.11	1	2.11	23.33	0.0007	
BD	0.45	1	0.45	5.02	0.0490	
CD	0.13	1	0.13	1.49	0.2507	
Residual	0.91	10	0.09			
Lack of Fit	0.31	5	0.06	0.53	0.7478	
Pure Error	0.59	5	0.12			
R ²	0.9348	17				
R ² (Predicted)	0.8072					Not significant
R ² (Adjusted)	0.8892					
Lack of Fit (p-value)	0.5449					

results proved that the model was suitable to represent the relationship between the variables and the response. The significance of coefficients was represented by the p-value. It was significant when $p < 0.05$ but it was insignificant when $p > 0.05$. Besides that, the purpose of lack of fit was to measure the adequacy of the fit. The F-value of 0.53 and the p-value of 0.7478 as ($p > 0.05$) meant that the lack of fit was insignificant due to pure errors such as experimental errors and personal errors being kept at a minimum.

The model F-value of 20.48 indicated that the model was significant. It meant that there was only a 0.01 % chance that a “model F-value” this large could occur due to noise. A value of “probability > F” being less than 0.05 implied that the model terms were significant [18]. F-value and p-value played a role in determining the significance of each coefficient. The variables were Tween-20 (A), lipid S75 (B), PKOE (C) and deionized water (D). In this case linear mixture components, AC, BC and BD were significant model terms. The model term CD was insignificant because it was greater than 0.100.

Thus, the final quadratic polynomial equation:

$$\text{Viscosity} = -1.38 A + 7.25 B + 14.42 C + 0.50 D - 27.45 AC - 41.50 BC - 12.57 BD - 9.68 CD$$

where A, B, C, D were Tween-20, lipid S75, PKOE and deionized water, respectively.

Consequently, the final reduced model was obtained. Three dimensional response surface plots were constructed to see the interaction effect of the variables on the responses. It was suggested that for a good fit of a model, R^2 should be at least 0.80 and above.

D-Optimal analysis: Fig. 1(a) shows that a higher amount of component C, the oil, gave a higher value of viscosity. This was due to an increase in phase volume of internal phase in emulsion [19]. When the oil content increased, it augmented the number of particles in the matrix. Hence, the presence of a

large number of particles improved the resistance to the flow and increased the apparent viscosity. Besides that, as oil content increased, the particles were closer, leading to packing of the oil droplets and stronger inter-particle interactions. The attractive forces between droplets drove the formation of flocs, which normally could evolve into a space-filling particulate network. According to this explanation, the apparent viscosity of emulsions would be influenced by their oil content [20].

Fig. 1(b) shows the interaction between Tween-20, palm kernel oil ester and deionized water. It was found that the viscosity of the nanoemulsions decreased with increase in water content. The decrease in the viscosity could be due to the production of bigger particle size due to coalescence and effect of over-processing of the emulsification with higher shear force [21].

Optimization of hydrocortisone-loaded nanoemulsion:

From the 18 sets of experiment, set 5 and set 6 were the optimized formulation for hydrocortisone-loaded nanoemulsion as viscosity was the response. According to Rezaee *et al.* [22] the desired viscosity of pharmaceutical nanoemulsion was 5.49 Pa.s. Set 5 and 6 which had the same formulation gave the maximum value of viscosity among the 18 experiments. The viscosity of set 5 and set 6 was 2.85 Pa.s which was the nearest to the desired viscosity. The formulation of set 5 and set 6 were obtained as in Table-5. The viscosity of nanoemulsion was a function of the composition's concentrations. Increasing the water content led to a decrease in viscosity while decreasing the amount of surfactant increased interfacial tension between water and oil, resulting in increase in viscosity.

Rheology: The viscosity and shear stress of optimized formulation was measured at a continuous shear rate. Fig. 2 shows that the optimized formulation was a non-Newtonian fluid since the viscosity changed as the shear rate varied. Furthermore, the viscosity of nanoemulsion decreased with an increase in shear rate, thus showing shear thinning behaviour (pseudoplastic). Shear thinning behaviour is a desirable property in

Fig. 1. Three dimensional surface plot showing the interaction effect between three variables (a) between Tween-20 (A), Lipid S75 (B) and PKOE (C); and (b) between Tween-20 (A), PKOE (C) and deionized water (D), with respect to the viscosity

TABLE-5
COMPOSITION OF OPTIMIZED FORMULATION

Composition (%)							Particle size (nm)	PDI	Zeta potential (mV)
Tween-20	Lipoid S75	PKOE	Water	HC	Ethanol	Phenonip			
10	20	14.47	30.02	1	24	0.5	172.56 ± 1.270	0.184 ± 0.001	-50.03 ± 1.563

Fig. 2. Viscosity as a function of shear rate for the optimized nanoemulsion containing hydrocortisone

topical preparations because it facilitates even application on the skin.

Particle size, polydispersity index, zeta potential and stability evaluation: The mean particle size, polydispersity index and zeta potential of the optimized formulation were 172.56 nm, 0.184 and -50.03 mV, respectively (Table-5). The polydispersity of the optimized formulation was 0.184, which had a narrow size distribution [23]. The morphology of the nanoemulsion was observed by TEM, the sphere of the droplet size correlated well with the results obtained from droplet size analysis using zetasizer (about 180 nm) (Fig. 3). The zeta potential for the optimized formulation showed good stability due to the high negative surface charge. High negative surface charge led to repulsive forces between particles and also improved the stability of the emulsion [24]. The particles in the emulsion repelled each other when they had a high positive or negative zeta potential value. Consequently, it reduced the chance of occurrence of flocculation and coalescence [25]. To confirm these, the stability studies were evaluated. The results showed that the optimized formulation was very stable; no separation was observed within 30 days. This could be due to the Tween-20 provides the steric stabilizing effect which formed a thick steric barrier around the particles to prevent flocculation and coalescence [24].

Fig. 3. TEM image of hydrocortisone-loaded nanoemulsion

Conclusion

The study has shown that D-optimal mixture experimental design is a useful tool for carrying out optimization to the formulation of hydrocortisone-loaded nanoemulsion. The independent variables were Tween-20, lipid S75, PKOE and deionized water. The optimized formulation of hydrocortisone-loaded nanoemulsion was 10 % Tween-20, 20 % lipid S75, 14.47 % PKOE and 30.02 % deionized water, where the other ingredients were kept constant. From the analysis of variance, the model had a low F-value (20.48) and a low p-value (< 0.0001), with a non-significant lack of fit (0.7478) and $R^2 = 0.9348$. The optimized formulation also had good results in terms of particle size, polydispersity index and zeta potential, at 172.56 nm, 0.184 and -50.03 mV, respectively and had a good stability and the droplet size had agreement with the TEM image. The optimized formulation showed a shear-thinning behaviour. Oil content was found to be the more dominant factor in controlling the viscosity of nanoemulsions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and financial from the Malaysian Ministry of Education (MOE) and Universiti Putra Malaysia (UPM) through the Fundamental Research Grant Scheme (FRGS) (vote number 5524939) and Putra Grant IPM (vote number 9456200).

REFERENCES

1. A.C. Williams and B.W. Barry, *Adv. Drug Deliv. Rev.*, **64**, 128 (2012); <https://doi.org/10.1016/j.addr.2012.09.032>.
2. J. Werumeus Buning, P. Brummelman, J. Koerts, R.P.F. Dullaart, G. van den Berg, M.M. van der Klauw, O. Tucha, B.H.R. Wolffenbuttel and A.P. van Beek, *Psychoneuroendocrinology*, **55**, 36 (2015); <https://doi.org/10.1016/j.psyneuen.2015.02.001>.
3. T. Iannitti, M.F. McDermott, C. Laurino, A. Malagoli and B. Palmieri, *Drug Deliv. Transl. Res.*, **7**, 156 (2017); <https://doi.org/10.1007/s13346-016-0340-9>.
4. M.R. Prausnitz and R. Langer, *Nat. Biotechnol.*, **26**, 1261 (2008); <https://doi.org/10.1038/nbt.1504>.
5. L. Salvia-Trujillo, R. Soliva-Fortuny, M.A. Rojas-Graü, D.J. McClements and O. Martín-Belloso, *Ann. Rev. Food Sci. Technol.*, **8**, 439 (2017); <https://doi.org/10.1146/annurev-food-030216-025908>.
6. J. Komaiko and D.J. McClements, *J. Food Eng.*, **146**, 122 (2015); <https://doi.org/10.1016/j.jfoodeng.2014.09.003>.
7. J. Shen, Y. Wang, Q. Ping, Y. Xiao and X. Huang, *J. Control. Release*, **137**, 217 (2009); <https://doi.org/10.1016/j.jconrel.2009.04.021>.
8. F. Ostertag, J. Weiss and D.J. McClements, *J. Colloid Interface Sci.*, **388**, 95 (2012); <https://doi.org/10.1016/j.jcis.2012.07.089>.
9. N. Salim, N. Ahmad, S.H. Musa, R. Hashim, T.F. Tadros and M. Basri, *RSC Advances*, **6**, 6234 (2016); <https://doi.org/10.1039/C5RA14946K>.
10. F. Podczeczek, A. Maghetti and J.M. Newton, *Eur. J. Pharm. Sci.*, **37**, 334 (2009); <https://doi.org/10.1016/j.ejps.2009.03.003>.
11. T. Moravkova and P. Filip, *Adv. Mater. Sci. Eng.*, **2013**, 1 (2013); <https://doi.org/10.1155/2013/168503>.
12. H. Yin, Z. Chen, Z. Gu and Y. Han, *LWT-Food Sci. Technol.*, **42**, 327 (2009); <https://doi.org/10.1016/j.lwt.2008.04.002>.
13. E. Betiku and A.E. Taiwo, *Renew. Energy*, **74**, 87 (2015); <https://doi.org/10.1016/j.renene.2014.07.054>.
14. P.S. Keng, M. Basri, M.R. Zakaria, M.A. Rahman, A.B. Ariff, R.A. Rahman and A.B. Salleh, *Ind. Crops Prod.*, **29**, 37 (2009); <https://doi.org/10.1016/j.indcrop.2008.04.002>.

15. S. Da Costa, M. Basri, N. Shamsudin and H. Basri, *Asian J. Chem.*, **26**, 2883 (2014);
<https://doi.org/10.14233/ajchem.2014.15929>.
16. Z.E. Allouni, M.R. Cimpan, P.J. Høl, T. Skodvin and N.R. Gjerdet, *Colloids Surf. B*, **68**, 83 (2009);
<https://doi.org/10.1016/j.colsurfb.2008.09.014>.
17. G. Schramm, *A Practical Approach to Rheology and Rheometry*, Karlsruhe: Haake (1994).
18. S.H. Musa, M. Basri, H.R. Fard Masoumi, N. Shamsudin and N. Salim, *Int. J. Nanomedicine*, **12**, 2427 (2017);
<https://doi.org/10.2147/IJN.S125302>.
19. T. Zhang, Z. Xu, Y. Wu and Q. Guo, *Ind. Eng. Chem. Res.*, **55**, 4499 (2016);
<https://doi.org/10.1021/acs.iecr.6b00039>.
20. M. Soleimanpour, A. Koocheki and R. Kadkhodae, *Food Res. Int.*, **50**, 457 (2013);
<https://doi.org/10.1016/j.foodres.2012.04.001>.
21. P.H. Li and B.H. Chiang, *Ultrason. Sonochem.*, **19**, 192 (2012);
<https://doi.org/10.1016/j.ultsonch.2011.05.017>.
22. M. Rezaee, M. Basri, R.N.Z.R.A. Rahman, A.B. Salleh, N. Chaibakhsh and R. Abedi Karjiban, *Int. J. Nanomedicine*, **9**, 539 (2014);
<https://doi.org/10.2147/IJN.S49616>.
23. S. Zainol, M. Basri, H.B. Basri, A.F. Shamsuddin, S.S. Abdul-Gani, R.A. Karjiban and E. Abdul-Malek, *Int. J. Mol. Sci.*, **13**, 13049 (2012);
<https://doi.org/10.3390/ijms131013049>.
24. S. Honary and F. Zahir, *Trop. J. Pharm. Res.*, **12**, 265 (2013);
<https://doi.org/10.4314/tjpr.v12i2.20>.
25. N. Salim, M. Basri, M.B. Rahman, D.K. Abdullah and H. Basri, *Int. J. Nanomedicine*, **7**, 4739 (2012);
<https://doi.org/10.2147/IJN.S34700>.