ORIGINAL ARTICLE

Fabrication, Physicochemical and Rheological Characterisation of a Drug-therapeutic Oils (Doxycycline Hyclate-*Nigella sativa*-Eugenol) Complex Emulsion Stabilised by Lecithin and Hydroxypropyl Methylcellulose Intended for Delivery Into Periodontal Pocket

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ABSTRACT

Introduction: Earlier attempts to stabilise an emulsion, intended for chronic periodontitis treatment which composed of doxycycline hyclate (DH), *Nigella sativa* oil (NSO), eugenol and several combinations of surfactants failed. To solve the issue, we investigated the ability of lecithin alone and its combination with hydroxypropyl methylcellulose (HPMC) to stabilise the emulsion. **Method:** Compatibility between DH and other ingredients was first investigated using DSC and ATR-IR. The emulsion was characterised, firstly by preparing three phases: doxycycline/preservatives with or without HPMC (varying quantities), NSO/eugenol and lecithin/surfactants as aqueous, oil and emulsifier phases, respectively. The phases were added and emulsified sequentially at 7000 rpm (10 min) with an overhead stirrer and then at 3000 rpm (15min) using a high-shear mixer. DH assay was performed using validated HPLC method. **Results:** All ingredients were found to be compatible with doxycycline based on DSC, ATR-IR and supported by acceptable recovery (98.2±2.2 %) of DH from the emulsion. Stable emulsions were produced with particle size of 198.6±8.2 to 279.3±10.7 nm and zeta potential of -48.2±0.4 to -64.0±3.9 mV. The emulsions showed high viscosity (~200 Pa.s) at zero shear rate and exhibited shear-thinning flow upon increased in shear stress yielding viscosity of ~3 Pa.s at 100 s-1 indicating pseudoplastic behaviour suitable for pre-filled syringe packaging intended for delivery into periodontal pocket. **Conclusion:** Lecithin is an excellent emulsifier that can also impart pseudoplasticity for a complex emulsion constitute of drug and natural oils. This could pave the way for a more complex emulsion formulation fusing contemporary and therapeutic oils

Keywords: Lecithin, Doxycycline, *Nigella sativa*, Eugenol, Chronic periodontitis

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INTRODUCTION

Periodontitis affects the teeth and teeth-supporting structures – the gingiva, the periodontal ligament and the alveolar bone. It involves about 90% of world's population, with the severe form affecting up to 15% of the patients (1,2,3). When it progresses further to a chronic state, the involvement of polymicrobial biofilm bacterial infection (4) and the host response to the microbials that lead to excessive production of destructive enzymes (5) make periodontitis difficult to treat. The mainstay treatment of the chronic periodontitis involves a procedure called scaling and root surface debridement (RSD) (2). Systemic antibiotics can be given as an adjunct to the mainstay treatment up to two weeks (6) based on the severity of the cases. Alternatively, antibiotics can also be administered locally in the periodontal pocket to directly control the microbial flora. For example, an antibiotic fibre strips/gel could be inserted locally inside the periodontal sulcus (7,8). For locally delivered antibiotics, an ideal anti-periodontitis antibiotic must be

targeted to be effective against anaerobic microorganism (9) and possess a prolonged residence time in the pocket to allow complete eradication of the bacteria (10).

Our earlier work has shown that the fusion of a contemporary antibiotic and a natural product worked synergistically in vitro as an antibiofilm agent (11). Anticipating similar effect, here, we selected doxycycline hyclate as the antibiotic of choice to be formulated in combination with Prophetic and natural medicines namely *Nigella sativa* oil (NSO) and eugenol. Doxycycline hyclate is a semi-synthetic derivative of tetracycline that has been used to treat various kinds of aerobic and anaerobic gram-positive and gram-negative bacteria. Currently, a marketed doxycyline hyclate gel, under the brand name Atridox® has been one of the treatment modalities in the periodontitis (7). The Atridox® is supplied in two separate pre-filled syringes consisting of doxycycline hyclate and the polymer that has to be freshly mixed prior to the application into the periodontal pocket, perhaps due to stability issue. (12). *Nigella sativa*, a centuries-old medicinal agent in traditional medicine (13), has been selected because of its oil extract's actions against a wide range of multi-drug-resistant gram-positive and gram-negative bacteria (13), including a remarkable anti-biofilm property (14). Extensive studies had been performed to investigate chemical compositions of this oil using varying methods of extraction. Pinar and Veysel (2017) have investigated NSO in Turkey and studied the chemical constituents in terms of sterol composition, fatty acids composition, triacylglycerols and thymoquinone apart from its physical properties (15). The major sterol was β-Sitosterol while the major unsaturated fatty acids were linoleic and oleic acid with palmitic acid as the major saturated fatty acid. In the same study, it was found that at least 12 triacylglycerols (TGA) made up the component of the oil of which the majority was trilinolein (LLL) (15). Previous studies have reported several combinations of diethyl ether extract of NSO with antibiotics such as gentamicin and streptomycin which exhibited antibacterial synergism against *Staphylococcus aureus, Pseudomonas aueruginosa* and *Escherichia coli* (13). The extracts also showed additive antibacterial action when combined with tobramycin, doxycycline, erythromycin and nalidixic acid. It has also been reported that the combination of thymoquinone which is the main active compound in NSO with anti-fungal drug such as Amphotericin-B were more effective than the drug alone (13).

The third ingredient, eugenol, is a phenolic compound obtained from clove oil. It has been used as an analgesic, anaesthetic, anti-inflammatory, and antibacterial agents (16,17). As a lipophilic substance, eugenol penetrates the lipopolysaccharide membrane of the gram-negative bacteria and changes the structure of the cell causing intracellular leaks (18). It could cause the disintegration of the bacterial biofilm layer (19) and suppress the development of dental caries by downgrading the virulence features of harmful bacteria (20). Recently, eugenol nanoemulsion gel was formulated, demonstrating non-irritating antimicrobial, anaesthetic and anti-inflammatory activities when tested in rats for periodontitis treatment (21).

Our initial attempt to stabilise the complex doxycycline hyclate-NSO-eugenol emulsion using combinations with several sorbitan-based surfactants was not successful. In this study, Tween 80 and Span 20 were used alone (5%) or in combinations (up to 10%) of these sorbitan-based surfactants, emulsified using the T10 Basic Ultra-turrax homogenizer (IKA, Germany). The formulations were unstable as reflected by the presence of phase separation immediately upon stopping homogenizer.

A closer look at the chemical structure of NSO, eugenol and doxycycline hyclate revealed a highly complex emulsion attributed to various constituents. Hence, here we investigated the effect of other stabilisers i.e lecithin and hydroxypropyl methylcellulose (HPMC), in addition to the selected sorbitan-based surfactants, to our emulsion preparation. Lecithin is an amphipathic molecule with two long non-polar chains and with positively and negatively charged polar heads (22). In contrast, HPMC is a long chain cellulose polymer, non-toxic, biocompatible and possess unique property such as able to dissolve in aqueous and non-aqueous solvent (23) apart from having water holding capacity (24). Both were selected mainly due to the presence of bulky molecules that could arrange the NSO molecule according to polar-hydrophobic regions of the emulsifiers in the complex emulsion.

MATERIALS AND METHODS

Materials

Doxycycline hyclate (DH) was purchased from Yancheng Suhai Pharmaceutical Co., Ltd. (Jiangsu Province, China). *Nigella sativa* oil (NSO) was purchased from Chironton Trading Co. (Bangladesh) and eugenol from C. V. Aroma & Co. (Medan, Indonesia). Lecithin was bought from Tianjin Hexiyuan Lecithin Technology Co., Ltd. (Tianjin, China). Hydroxypropyl methylcellulose (HPMC), sodium propyl paraben (SPP) and sodium methyl paraben (SMP) were purchased from Euro Chemo Pharma Sdn Bhd. (Selangor, Malaysia). Polysorbate 80 (T80) was purchased form Merck (Germany) whereas sorbitan monooleate (S80) was from Guangdong Runhua Chemisrty Co. Ltd. (Guangdong, China).

Drug-excipients compatibility studies

Method from Bharate et al. (2010) was adopted (24). DH-excipient binary mixtures were thoroughly mixed by mortar and pestle in 1:1 ratio. The reason for preparing the mixtures at a ratio different to the actual formulation is to increase the chances for detecting any possible interaction. The following were the excipients

tested by combining DH powder and the individual excipient: NSO, eugenol, sorbitan monooleate or Span 80 (S80), polysorbate 80 (T80), lecithin, HPMC, sodium propylparaben (SPP) and sodium methylparaben (SMP). The mixtures were subjected to Differential Scanning Calorimetry (DSC) and Attenuated Total Reflection (ATR) – Infrared (IR) (ATR-IR) spectroscopy.

Differential Scanning Calorimetry (DSC)

A modified method from Aimen et al. (2013) was adopted (26). Briefly, 3 mg of DH and each of the binary mixtures were accurately weighed with analytical balance (Model No. MS204S, Mettler Toledo GmbH, Switzerland) into a pin-holed 40 µL aluminum crucibles, evenly spread and hermetically sealed. Samples were isothermally equilibrated with a reference pan (an empty pin-holed, hermetically sealed aluminum crucible) at 25 °C for 5 min to ensure isothermal starting conditions. Temperature was then then reduced to -30 °C at a cooling rate of 5 °C /min. The temperature was then maintained at -30 °C for 5 min before it was heated up to 280 °C at a heating rate of 10 °C /min (DSC 3 model, Mettler Toledo GmbH, Switzerland). The scanning was conducted under nitrogen purge at 1 mL/min flow rate. The thermograms were analysed using Mettler STARe software (version 0.19) (Mettler Toledo GmbH, Switzerland).

ATR-IR

The spectra were measured in the 4000 to 400 cm^{-1} regions using an ATR-IR spectroscopy Perkin-Elmer Model 1600 coupled with universal ATR which consists of a diamond disc as an internal reflection element interfaced with a software to analyse the bands (Spectrum™ 2 Universal ATR, Perkin-Elmer GmbH, Germany). Each sample (from individual powder and binary mixtures) was placed on the ATR crystal in such a way that the sample was intimately in contact with the ATR surface. The samples were pressed into the diamond crystal of ATR at a standardized pressure using a manometer for each spectrum and then the spectrum was recorded. The spectrum of air was used as a background before each sample analysis. Background and sample spectra were taken in a room with a temperature around 21–23 °C, at a spectral resolution of 4 cm^{-1} performing 8 scans per measurement. The ATR crystal was cleaned using isopropanol-sprayed cellulose paper prior to loading any sample. The generated spectra were then analysed using the software. The wavenumbers corresponding to relevant characteristic peaks were then compared with infrared spectra database or scientific papers to identify and verify qualitatively the types of the functional groups.

Fabrication of the emulsion

Two-steps emulsification modes were employed to fabricate six emulsion formulations (A to F) by varying the amounts of lecithin and HPMC (Table I). Briefly, three phases were firstly prepared, i.e the aqueous, the oil and the emulsifier phases. For the aqueous phase, DH was firstly dissolved in purified water followed by dissolution of the preservatives for the lecithin-incorporated emulsion (LE). The method was repeated with additional dissolution step to dissolve HPMC for lecithin-HPMC-incorporated emulsion (LHE). Dissolution and mixing were carried out using the overhead stirrer for 5 min at 7000 rpm. For the oil phase, eugenol was added to the NSO gradually while mixing with overhead stirrer for 5 min at 7000 rpm. This was followed by preparation of the emulsifier phase by wetting of the lecithin in purified water assisted by all surfactants in this phase.

Table I: Detail of six formulations fabricated using a common set of processing parameters.

Formulation / Ingredients	Lecithin Emulsion (LE)			Lecithin-HPMC Emulsion (LHE)		
	A (%, v/v)	B (%, v/v)	C (%, v/v)	D (%, v/v)	E (%, v/v)	F (%, v/v)
DH	5	5	5	5	5	5
NSO	5	5	5	5	5	5
Eugenol	1	1	1	1	1	1
Lecithin	19	20	21	12	12	12
HPMC	-	-	-	0.7	0.8	0.9
T80*	6	6	6	6	6	6
S80*	5	5	5	5	5	5
SPP#	0.02	0.02	0.02	0.02	0.02	0.02
SMP#	0.08	0.08	0.08	0.08	0.08	0.08
Water	58.9	57.9	56.9	65.2	65.1	65.0

*T80 – Tween 80; S80 – Span 80; both function as surfactants.
#SPP – sodium propylparaben; SMP – sodium methylparaben; both function as preservatives.

Next, all the three phases were mixed together to produce emulsion. For the first emulsification step, the mixing was accomplished with the overhead stirrer for 10 min at 7000 rpm to ensure proper dispersion of lecithin into the oil and aqueous phases. For the second emulsification step, the mixing was done using an overhead homogenizer (IKA T 10 Basic ULTRA-TURRAX, IKA Works Asia Sdn. Bhd., Malaysia) for another 15 min at 3000 rpm to yield homogenous emulsions. The emulsions were then stored at 4°C for further investigation.

Forced phase-separation test

Method from Patel et al. (2015) was adopted (27). Freshly prepared 50 mL of emulsion for each formulation was transferred into a 50 mL Falcon tube and centrifuged for 15 minutes at 4000 rpm (25 °C) using HeraeusTM MegafugeTM 8 benchtop centrifuge (Thermo Fisher Scientific, Massachusettes, USA). Separation of the phases was identified and measured using a ruler three times.

Droplet size, polydispersity index (PDI) and zeta potential

Method by Nurul Hafizah et al. (2014) was adopted (28). Malvern Zetasizer Nano series (Malvern Instruments, UK) with Zetasizer Nano software v3.30 were used

to analyse droplet size and PDI. 100 µL of the sample was dispersed in distilled water at the ratio of 1:10000 and placed in a polystyrene cuvette. The reading was obtained at 173° detection angle at 25 °C for every triplicate samples.

Assessment on rheological behaviour

The tests for viscosity and rheological behaviour of the emulsions were performed by Haake Mars III Rheometer with Pp35Ti spindle of 35 mm diameter and 1 mm gap at 25 °C. Samples was placed onto the plate until the shape of the spindle was covered. The shear rate was increased from 0.0001/s up to 100.01/s within 120 second and maintained at the maximum rate for 5 second before it was reduced back to the lowest shear rate. The data analyses were digitally analyzed by Haake Rheo-Win 3.61.0000 software (Thermo-Scientific, Waltham, MA, USA).

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Tukey test was performed on parametric data while the non-parametric data was analysed with Mann U Whitney test. The analysis was performed using Minitab 18 software.

Quantification of DH from emulsion dosage form

The quantification of DH in the emulsion was performed by using HPLC instrument (Waters e2695, Waters Corporation, Milford, US) connected with photodiode array (PDA) detector (Waters 2998, Waters Corporation, Milford, US), applying a validated analytical method. The latter was established by adopting method from USP National Formulary 2018. 60 mg of the emulsion were diluted in a 50 mL volumetric flask to a known concentration of 1.2 mg/ mL. The samples were prepared in triplicates. Samples were run using L21 column with injection volume 20 µL at 1 mL/min flow rate for 40 min run time. Detection wavelength was 270 nm. A buffered monobasic potassium phosphate and sodium hydroxide mixture were used as the mobile phase. The percent recovery of doxycycline hyclate from the emulsion was calculated using the following equation:

Percent recovery = $(r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$

r_u: peak response from the sample solution
r_s: peak response from the standard solution
C_S: Concentration in mg per mL, of doxycycline hyclate in the standard solution
C_U: Concentration in mg per mL, of doxycycline hyclate in the sample solution
P: potency of doxycycline in USP doxycycline hyclate RS (µg/mg)
F: conversion factor, 0.001 mg/µg

RESULTS

Compatibility assessment based on DSC

DSC thermograms (Fig. 1) depicted a common signature endothermic peaks at 168.67 °C indicating melting point of a pure doxycycline hyclate (F) and its exothermic crystallization peak at 225 °C. The appearance of the melting and crystallization peaks were maintained as indicated by the binary mixtures thermograms for all ingredients tested with shifted peaks were manifested by only two mixtures which were DH-SPP and DH-SMP. Several binary mixtures seemed to indicate a reduction in the heat capacity needed to melt and crystallise the doxycycline hyclate, reflected by the reduction in the height of the heat capacity curves of the DH binary mixtures (A, B, C, D, E, G, H, I). For the binary mixtures of DH with powdered paraben materials i.e SPP and SMP, the trend of thermograms are obvious whereby the first peak appeared before DH melting peak was either the melting or the decomposition points of the respective materials (~60 °C for SPP and ~100 °C for SMP) which also shifted to lower temperatures as compared to the data in respective Material Safety Data Sheet (MSDS): SPP (~90 °C) (29) and SMP (125 °C) (30).

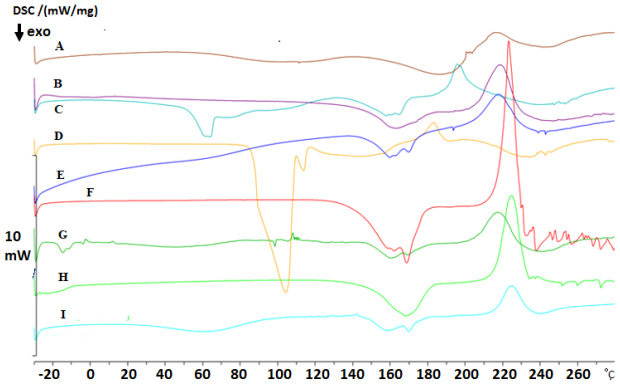

Figure 1: DSC curves of DH alone (F) and mixtures of DH with the following: (A) eugenol, (B) S80 (Span 80) (C) SPP (sodium propyl paraben), (D), SMP (sodium methyl paraben), (E) lecithin, (G) T80 (Tween 80), (H) NSO and (I) HPMC. Melting and crystallization points of the pure DH (F) are shown to occur at 168.67 °C and 225 °C, respectively. Except for binary mixtures consisted of DH-eugenol (A) and DH-SMP (D), otherthermograms of the binary mixtures are showing prominent endothermic melting peaks of DH at around 160-180 °C indicating compatibility of the DH with the other materials. The exothermic crystallization peak of DH in all binary mixtures are also retained although some shifted to the lower temperature as depicted in the region 200 °C to 240 °C

Compatibility assessment based on ATR-IR

Here, we investigated compatibility of our Active Pharmaceutical Ingredient (API) of interest namely doxycycline hyclate (DH), with the selected excipients by analysing the appearance and disappearance of the characteristic peaks of DH and whether these were maintained in the binary mixtures or not as compared to the pure powder of DH.

Fig. 2 is showing the absorption bands of a pure DH at (a) 3327.50 cm^{-1} and 3276.70 cm^{-1} (O-H stretch); (b) 1663.70 cm-1 (C=O stretch); (c) 1612.80 cm^{-1} and

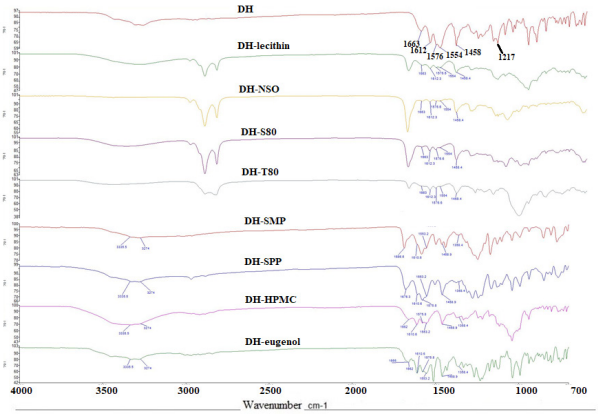

Figure 2: FTIR-ATR spectra of the pure doxycycline hyclate (DH) and binary mixtures consisted of DH and the following materials: lecithin, NSO (Nigella sativa oil), S80 (Span 80), T80 (Tween 80), SMP (sodium methyl paraben), SPP (sodium propyl paraben), HPMC (hydroxypropyl methylcellulose) and eugenol. The spectra depict characteristic peaks of DH at 3200-3400 cm^{-1} (O-H stretch and N-H stretch), 1663.70 cm^{-1} (C=O stretch), 1554 cm-1 (N-H bending), 1458 (C-H2 bending) and 1217 cm^{-1} (C-N stretching) which were retained in the binary mixtures indicating compatibility of DH with all materials tested here.

1610.50 cm^{-1} (C=O stretch); (d) 1557.40 cm^{-1} and 1552.70 cm^{-1} (N-H bending); (e) 1460.30 cm^{-1} and 1459.80 cm^{-1} (C-H2 bending); (f) 1330.90 cm^{-1} (C-H bending); (g) 1217.60 cm^{-1}, 1215.30 cm^{-1} and 1175.70 cm^{-1} (C-N stretching) and (h) 1175.70 cm^{-1}, 1083.30 and 1039.70 (C-O stretching). The characteristic peaks of DH that we identified in the binary mixtures at 1663 cm^{-1}, 1612 cm^{-1}, 1576 cm^{-1}, 1554 cm^{-1}, 1458 cm^{-1} and 1217 cm^{-1} were indicating compatibility of DH with all selected ingredients. All of the characteristic peaks are important functional groups in the chemical structure of DH (31).

Challenge test by centrifugation

None of the six emulsions exhibited signs of phase separation when inspected visually. There was no oil layer formation on the surface, in between layers nor at the bottom. The emulsions stayed as one homogenous phase following centrifugation at high speed (4000 rpm).

Droplet size, PDI and zeta potential

Range of droplet size attained in the six emulsions was 190 to 290 nm (Table II). Higher amount of lecithin in LE significantly ($p<0.05$) produced smaller droplet size and reduction in corresponding PDI. Zeta potential for LE ranged between -50 to -68 mV but did not show any trend in the reading despite lecithin increment.

For LHE whereby the lecithin concentration was reduced almost by half and HPMC was added as co-stabiliser at a very low concentration (less than 1%), increase in HPMC concentration resulted in significant ($p<0.05$) reduction of particle size when compared between LHE groups. Increase in HPMC seemed to give a better uniformity of the droplet size as depicted by

Table II: Droplet size, PDI and zeta potential of the six emulsions formulation with constant amount of doxycycline hyclate, NSO, eugenol and other excipients. Data represented by mean value ± SD for n=3.

Formulation	Droplet Size (nm)	PDI	Zeta Potential (mV)
A (19% L*)	247.8±2.6	0.553±0.048	-57.3±1.5
B (20% L)	221.6±9.3	0.485±0.028	-64.0±3.9
C (21% L)	198.6±8.2	0.448±0.026	-53.1±3.5
D (12% L, 0.7% HPMC#)	259.9±21.4	0.710±0.080	-51.6±0.8
E (12% L, 0.8% HPMC)	279.3±10.7	0.642±0.081	-48.2±0.4
F (12% L, 0.9% HPMC)	213.8±3.3	0.601±0.039	-50.5±0.5

* L – lecithin; # HPMC – hydroxypropyl methylcellulose

significant improvement ($p<0.05$) of the PDI. Similar to LE formulations, the zeta potential was not affected by the slight increase in HPMC in this LHE groups but the values were lower than LE groups (-48 to -52 mV for LHE as compared to -53 – 64 mV for LE).

Rheological behaviour of the emulsions

All six emulsions exhibited plastic flow curves as represented by Fig. 3A (LE groups) and 3B (LHE groups). At zero shear rate, the viscosity of the emulsions was very high i.e about 200 -300 Pa.s. However, the viscosity was reduced as the shear rate and shear stress increased. The viscosity approaching plateau i.e around 2-3 Pa.s when the applied shear rate was 100 s^{-1}. There was no significant ($p>0.05$) change in viscosity when lecithin concentration was increased in LE groups. Similarly, the increase in HPMC concentration also did not incur significant effect on viscosity and flow behaviour of the emulsions in LHE groups.

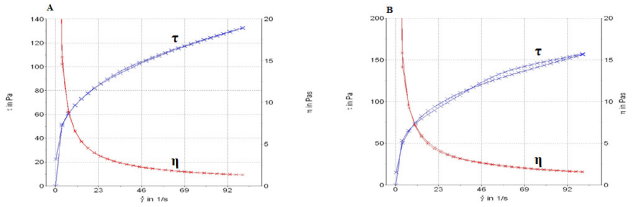

Figure 3: Representative Rrheological behavior depicted by viscosity (η) (y-axis at the right) and shear stress (τ) (y-axis at the left) curves at a different shear rate ( ) (x-axis) of LE (A) and LHE (B) groups showed a typical non-newtonian fluid that exhibited shear-thinning flow in response to increase shear stress.

Quantification of doxycycline hyclate

Average percentage recovery (Table III) of DH from representative emulsion was found to be within acceptance criteria for doxycycline hyclate as stated in the pharmacopoeia (specification is 90-120%) (32).

DISCUSSION

Ideally, any new drug formulation shall start with

Table III: Quantification of DH in representative emulsion sampled at the top, middle and bottom of the mixing vessel.

Sample	Peak Area*	Recovery Amount (mg)	% Recovery	Average Recovery	RSD (%)#
Top	15408132	11.7	99.6		
Middle	15093212	11.2	95.6	98.2±2.2	1.2
Bottom	15402503	11.6	99.3		

*These values were substituted in the linearity curve's equation of the spiked samples, established during method validation to obtain Recovery Amount; y=13795x+130821, R^2= 0.999. #RSD – Relative Standard Deviation; acceptance criteria of RSD are < 2% according to Q2 (R1) subsection of International Council of Harmonisation of Technical Requirements for Pharmaceutical for Human Use (ICH) guideline.

compatibility testing between the Active Pharmaceutical Ingredient (API) and other ingredients that are proposed to be used in the formulation regardless of dosage forms. These compatibility data between drug-excipient or drug-drug are required for the purpose of product registration and commercialization. It is important to establish these data in order to expedite product development especially in the context of novel drug combination such as fusion between contemporary and prophetic/natural/herbal medicine illustrated by the present study.

Doxycyline hyclate (DH) was the selected API in this study and hence its thermogram must be first established using DSC (Fig. 1F) for comparison with the binary mixtures. The signature thermal event that we obtained for the pure DH was consistent with the thermogram discovered elsewhere (33). The binary mixtures of the DH, with all excipients such as NSO, lecithin, SPP, S80, T80 and HPMC indicated evidence of compatibility. However, the thermograms of pure DH were shifted when combined with preservatives SPP and SMP at the ratio 1:1. It is noteworthy that these two preservatives are usually employed at extremely low concentration namely less than 1% and hence if incompatibilities were to exist, the risk of product instability would be less likely. Meanwhile there were no prominent peaks appeared before the melting point of DH for the binary mixtures between DH and viscous sorbitan surfactants i.e S80 and T80 as well as DH with NSO. This is because the melting points for all the liquid materials (oily or non-oily) were well below the range tested, i.e below than -30 °C. Their melting point data are not available in any MSDS. Incidentally, lecithin in pure, viscous liquid was documented to have melting point below -25 °C (34).

Incompatibility of the physical mixtures might be indicated with the absence or a significant shift in the peaks as well as the appearance of new exo/endothermic peaks. In spite of that, it is expected to have a slight change in the peak shape, height and width due to the possible differences in the mixture geometry (35).

ATR-IR is a quick and robust method to identify specific functional group of any organic compounds. The spectrum generated from the analysis of a compound or a material is generally unique for the compound and resulted from the absorption of photons of infrared (IR) radiation by the compound causing specific bond vibrations. In a compatibility study where the compound identity was already known, the ATR-IR is used to investigate whether a combination of any two materials would be compatible or not by analyzing the appearance or disappearance of relevant spectral peaks of the individual compound and comparing it with the spectrum of the binary mixtures. The peaks at specific wavenumbers correspond to specific functional groups; the latter can be compared to IR-database.

Based on the results, we found no definitive evidence of chemical interaction between DH and excipients materials was seen in the ATR-IR spectra (Fig. 2) as indicated by the appearance of DH's signature peaks in all binary mixtures, leading to the conclusion that no interaction took place between DH and the excipients. Based on the compatibility results, all proposed ingredients can be safely used for further formulation development.

Six different formulations were fabricated with variations in the concentration of emulsifiers (i.e. lecithin and/or HPMC) incorporated (Table I). Ingredients types, quantities and processing parameters were kept constant based on prior knowledge and investigations involving preparation of DH-NSO-eugenol emulsion. In the early development of the LHE, the addition of HPMC alone turned the formulation into a gel instead of an emulsion. Thus, the amount of HPMC was reduced to 0.7 to 0.9% v/v and lecithin was added to maintain the emulsion's form, achieving similar viscosity to LE groups.

The emulsion formulations were prepared to achieve desirable characteristic taking into account the intended usage of the emulsion which is as a locally-applied antibiotic into periodontal pockets. As the periodontal pocket depth can be as deep as 5 to 12 mm for chronic periodontitis due to receding periodontal gum (7), therefore the stability of the emulsion is not the sole important consideration. Equally important is the rheological properties of the formulation. Pseudoplastic flow characteristic exhibited by the LE and LHE would ensure its ability to be pre-filled in the syringe during high-speed manufacturing, and subsequent application via a blunt needed to be attached to the syringe for the administration into the periodontal pocket. The results obtained here are in agreement with other finding that demonstrated desirable shear-thinning behaviour of a propolis gel formulation intended for periodontal pocket (36). The study also shown good syringeability tested using a texture analyser (36).

Lecithin has phosphatidylcholine, composed of phosphocholine and glycerol residue and non-polar region containing two hydrocarbon of fatty acid chains (37), as its major component. These amphiphilic

molecular structure provides lecithin with excellent emulsification property (38,39) and stability due to its tendency to produce monodisperse emulsions and its resistance to droplet size increment 40). The amount of lecithin incorporated into our emulsion correlated well with the droplet size, zeta potential, and pseudoplastic properties of the emulsion, and these observation were in agreement with other studies that employed lecithin (39, 40). The previous studies suggested that the effects were due to the ability of lecithin to significantly maintain the organisation of the oil droplets thus preventing the occurrence of phase separation (39) and delaying or preventing any formation of instability (38).

Hydroxypropyl methylcellulose (HPMC), a water-soluble polysaccharide derived from a family of cellulose ethers. The hydrophilic structure decreases the flowability and enhances the long term stability of droplets in an emulsion (41). HPMC also influences tensile strength (42) whereby increased HPMC concentration will increase the tensile strength, thus, preventing the occurrence of phase separation. It was initially hypothesized that the combination of HPMC with lecithin may impart not only thixotropicity but also pseudoplasticity to the emulsions' rheological properties, hence its incorporation here was worth a study.

Based on the characteristics of the LHE (Table II) and their rheological data (Fig. 3), there were no significant improvement imparted by the addition of HPMC. Lecithin-incorporated emulsions seemed to produce the emulsions of desirable properties such as sufficiently small particle size (in the range of 200 nm) with nearly monodisperse droplet size (PDI < 0.5) and very stable emulsions depicted by the high value of zeta potential (~ -50 to -60 mV) as well as no phase separation by visual inspection. It was previously reported that lecithin, which is a zwitterionic surfactant, would incur either positively or negatively charged zeta potential to the resultant emulsion (43, 44) depending on the pH of the emulsion. Moreover, lecithin concentration that made up one-fourth of the formulation demonstrated to be compatible with the API, doxycycline hyclate, as indicated by the acceptable percentage of recovery i.e ~98% of DH quantified from the emulsion.

Additionally, it was discovered that the dispersion of the emulsifier was easier and faster if lecithin alone was used as the presence of HPMC seemed to lengthen the overall emulsification process. These could be due to different interfacial properties of both lecithin and HPMC that resulted in different wettability and dispersibility in aqueous media even though the quantities of the lecithin were reduced to almost half in the LHE (D to F) than the LE (A to C) (Table I) despite constant amount of surfactants. It was reported that the surface tensions of HPMC and lecithin were around 55 dyne/cm (23) and 50 dyne/cm, respectively (45). This indicate that slight difference in the surface tension amongst emulsifiers would results not only in different dispersing time but also dispersing method. The latter may involve the use of heat to aid in dispersion as compared to heat-free process. This kind of consideration would also be important during scale-up stage from the lab to the industry scale because any involvement of heat will increase the cost of finished product, once successfully registered and approved for market.

CONCLUSION

Within the limitation of this study, the doxycycline hyclate were found to be compatible with the therapeutic oils and other selected ingredients based on ATR-IR and DSC data. The doxycycline emulsion was successfully stabilised by lecithin and lecithin-HPMC combination based on physicochemical and rheological characterisation. The emulsion exhibited desirable pseudoplastic flow which could be of use to ease clinical administration of the doxycycline emulsion into periodontal pockets. Further study such as in vitro release profile, sensitivity against periodontal microflora and stability study shall be demonstrated before the emulsion deserve to proceed to the preclinical or clinical trial.

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