

FLUID PROPERTIES OF COMPONENTS FOR DEVELOPMENT OF EUDRAGIT RS-BASED IN SITU FORMING GEL AND MICROPARTICLE AND THEIR PHASE INVERSIONS

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Abstract

Solvent exchange-induced in situ-forming gel (isg) and in situ-forming microparticles (ism) have been currently applied as drug delivery approaches for periodontitis treatment. Doxycycline hyclate-loaded Eudragit RS-based isg and ism were prepared in this study. The basic properties such as density, pH, surface/interfacial tension of their solvents including dimethyl sulfoxide (DMSO), N-methyl pyrrolidone (NMP), and 2-pyrrolidone (PYR) in Eudragit RS solution and oils (olive oil and camellia oil) were assessed. This research also tested for apparent viscosity of solvent and drug-free and doxycycline hyclate-loaded ERS-based isg and ism, and their phase transformation rates. Density of PYR was higher than that of DMSO whereas these solvents exhibited the less wettability than oils on glass slide. All fabricated doxycycline hyclate-loaded Eudragit RS-based ism formulations showed good emulsion physical stability with no phase separation for 1 hour. The apparent viscosity of ism was significantly less than that of isg. DMSO-based isg and ism exhibited a significantly less viscosity than that of PYR-based formulations. Rapid phase inversion was evident after isg and ism exposure to agarose gel indicating fast solvent exchange. The loose barrier of camellia oil from its less viscosity facilitated a water diffusion inward Eudragit RS-based ism easier than using viscous olive oil. Solvent exchange-induced doxycycline hyclate-loaded Eudragit RS-based ism using DMSO as the solvent and camellia oil as the continuous phase exhibited various appropriate physicochemical characteristics for applying as drug delivery system for periodontitis treatment.

Keywords: Eudragit Rs; Fluid Characteristic; In Situ-Forming Gel; In Situ-Forming Microparticle

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Introduction

In situ forming systems have been currently applied as drug delivery approaches which solvent exchange-induced *in situ* forming system has been interesting to deliver the antibiotic drugs to periodontal pocket for periodontitis treatment. *In situ*-forming gel (isg) exhibits a sol-to-gel phase transformation which its dissolved polymer changes from a solution state into gel-like or solid state at targeted site with its appropriate residence time (Fogueri and Singh, 2009). By comparison *in situ*-forming microparticles (ism) is fabricated using an anhydrous emulsion template comprising the drug-incorporated internal phase comprising polymer dispersed in the oil. Typically, this emulsion of ism is obtained by pushing two phases containing suitable emulsifiers via two syringes coupled with a connector (Voigt *et al.*, 2012). The droplets of the internal phase of this emulsion solidified and simultaneously altered into the spherical gel-like or solid matrix owing to emulsion template. Nonetheless, the relatively low physical stability of anhydrous ism emulsion should be considered (Voigt *et al.*, 2012). This investigation employed the isg comprising Eudragit RS as the internal phase of ism using *N*-methyl-pyrrolidone (NMP) and 2-pyrrolidone (PYR) as the solvents of Eudragit RS whereas olive oil and camellia oil as the external phase of these anhydrous emulsions.

Eudragit RS has been distributed commercially as powder or granule forms for various applications in the field of pharmaceuticals and related. The presence of the quaternary ammonium group makes the polymer permeable for some amount of water (Wadher *et al.*, 2011; Thakral *et al.*, 2013). Practically, Eudragit RS is employed as a time-controlled liberation of active excipients owing to its insoluble properties, low permeability, and pH-independent drug release (Porwal *et al.*, 2011; Thakral *et al.*, 2013; Singh *et al.*, 2015). It has been fabricated into micro-balloons using evaporation technique of non- aqueous emulsion template to modulate the release of drug such as propranolol HCl. ERS micro-balloons exhibited a biphasic drug - released pattern since it is composed of initial burst drug release followed by retardation of drug liberation (Porwal *et al.*, 2011). The isg containing Eudragit RS as a polymer loading clove oil was fabricated to deliver antimicrobial agent for periodontitis treatment. Clove oil notably minimized the burst drug liberation while increased ERS content could diminish the diffusion rate of biocompatible organic solvent and environmental aqueous resulting to reducing the initial burst drug liberation (Phaechamud *et al.*, 2018a). The adding

peppermint oil into Eudragit RS-based isg decreased initial fast drug release and the sustainable drug liberation were gained with oil concentration dependence and its drug release profile complied well with diffusion control mechanism (Phaechamud *et al.*, 2018b).

The official solvents for fabricating isg or ism are usually aqueous miscible vehicles including dimethyl sulfoxide (DMSO), NMP and PYR (Voigt *et al.*, 2012). The suitable solvents for *in situ* systems should be to gain appropriate characteristics in term of water miscibility, less viscosity, high solvation power for the polymeric material and its safety (Brodbeck *et al.*, 1999). The minute viscosity of vehicle should be beneficial to further provoke an uncomplicated injection of formulations or good injectability. In the case of formulations fabricated with aqueous-immiscible solvent and exhibited a viscous feature resulting to difficulty for injection require a warm-up step before used (Chen and Singh, 2005). Practically, it presents the advantage of achievement the higher polymer and drug loading which further reduces the injection volume.

Doxycycline hyclate incorporated isg and ism were fabricated using bleached shellac as polymer for eradicating pathogens and periodontal healing (Phaechamud *et al.*, 2016a,b). Types of organic solvent markedly influenced on drug liberation and formulation degradation patterns upon exposure to aqueous phase because of the chemical interaction and solvent exchange. The comprehension of the behavior of solvent including DMSO, NMP, and PYR in Eudragit RS solution and oils from preparation process of isg and ism is necessary. The crucial fluid characteristics such as density, pH, surface/interfacial tension of these solvents and an apparent viscosity of solvent and fabricated ERS-based isg and ism dosage forms have not been undertaken previously. Thus, the desired purpose of this current investigation is to better gain the knowledge for the effect of interesting characteristics of solvent on fluid characteristics of components for development of Eudragit RS-based isg and ism involving their phase transition.

Materials and Methods

Materials

The main polymeric material such as Eudragit RS (lot no. G140338039, Evonik Rohm GmbH, Germany) was employed as acquired. Doxycycline hyclate was used as an antibiotic drug of this research kindly supplied by Bangkok lab and

cosmetics Co., Ltd., Rachaburi, Thailand. Dispersing media or external phases of the emulsion was organic camellia tea oil (Naturel®, Samutprakarn, Thailand) and olive oil (Bertolli® Florence, Italy). The aprotic solvents involving dimethyl sulfoxide (DMSO) (lot no. 453034), 2-pyrrolidone (PYR) (lot no. BCBF5715V), and NMP (lot no. A0251390) from Fluka, New Jersey, USA were utilized to solubilize polymer and drug. Glyceryl monostearate (GMS) (PC Drug, Bangkok, Thailand) was employed as a stabilizer compound.

Fluid Property Tests Polymer Dissolution and Miscibility Test

This study determined the possible solubility of 30%w/w Eudragit RS which is an aqueous insoluble polymer and 10%w/w doxycycline hyclate was utilized as antibiotic agent by dissolving in NMP, DMSO, and PYR. The mixtures were mixed using vortex stirrer for 24 hours. These solvents which could dissolve this polymer and drug was checked their miscibility with olive oil and camellia oil by filling 3 ml. of each solvent in the test tube followed by adding 3 ml. of olive oil and camellia oil and investigating the phase separation or miscibility of them. The basic physical properties of component of isg and ism including DMSO, PYR, PBS, water, olive oil, and camellia oil were determined including density, contact angle, surface tension, and interfacial tension as following.

Physical Properties of Each Component Density

The apparent density value of all components including solvents and oils was measured employing a density meter (Densito 30PX, Mettler Toledo (Thailand) Ltd., Portable Lab TM, Bangkok, Thailand) at 25°C (n=3). In addition, the density values have been to include during surface tension measurement.

Contact Angle

The contact angle of all solvents and oils were determined by a goniometer (FTA 1000, First Ten Angstroms, Cambridge, UK). The sample was loaded into a 1-mL syringe and injected through an 18-gauge blunt needle to generate the drop of fluid on a glass slide. The contact angle after 5 sec was recorded at 25°C (n=3).

Surface Tension and Interfacial Tension

The measurement of surface tension was performed by sessile drop technique using a goniometer as above mentioned by filling the isg system in 1 ml. syringe and expelling it through a 24-gauge blunt needle to obtain the drop suspended in the air (n=3). Interfacial tension was measured by

using this goniometer by adding olive oil and camellia oil in a quartz cuvette then the tip of the needle containing formulation was put into oil and pushed a syringe plunger to inject the formulation. The interfacial tension value between the biggest drop of formulation and the oil was recorded (n=3).

Preparation and Evaluation of isg and ism Systems Containing Eudragit RS Preparation of isg

Free-drug isg formulations were fabricated by solubilizing 30% w/w Eudragit RS in two solvents including DMSO and PYR as shown in Table 1. The binary mixture was prepared by dissolving using a roller tube mixer (Scilogex MX-T6-S analog tube roller, Scilogex, Connecticut, USA) for 48 hours until obtained as a homogeneous and transparent solution. To prepare doxycycline hyclate-added systems, 10% w/w doxycycline hyclate was added to a prepared polymer solution, and subsequently the mixture was mixed for 24 hours until turned into a transparent solution.

Preparation of ism

The isg formulation was further employed as the internal phase of the ism formulation. The 7.5% w/w of GMS was dispersed into olive oil and camellia oil as shown in Table 1 and mixed with heat at 60°C using a hotplate and magnetic stirrer (C-MAG HS7, IKA, Germany) until a clear solution was obtained and were used as the external oil phase. Two obtained phases were promptly mixed into an emulsion with a ratio of 1:1 using a 3 ml. disposable syringe connected with a butterfly lure lock by pressing forward and backward through the syringe for 1 minute. For doxycycline hyclate-loaded formulations they were fabricated by incorporating 10% w/w doxycycline hyclate in the internal polymeric phase. Subsequently, the doxycycline hyclate mixture and external oil phase were mixed into ISM emulsion by a ratio of 1:1 using the two syringes connector as above-mentioned procedure to prepare 5% w/w doxycycline hyclate-loaded ism.

Evaluation of isg and ism Appearance, Phase Separation of Emulsion and Matrix Formation

The physical emergencies including colour and transparency of prepared isg and ism were noticed. The separation of oil from the internal phase of the fabricated ism emulsion within a disposable plastic syringe was detected at room temperature. The 0.8 ml ism dosage form was filled into 1 ml. plastic syringe and this syringe was vertically placed in a stainless rack with checking its volume of oil separation.

Table 1. Formulation of free-drug and doxycycline hyclate-loaded ERS isg and ism formulations containing different solvents and oils

Formulation	Doxycycline hyclate	Eudragit RS	%w/w				GMS
			DMSO	PYR	Olive oil	Camellia oil	
F1		30	70				
F2	10	30	60				
F3		30		70			
F4	10	30		60			
F5		15	35		42.5		7.5
F6		15	35			42.5	7.5
F7	5	15	30		42.5		7.5
F8	5	15	30			42.5	7.5
F9		15		35	42.5		7.5
F10		15		35		42.5	7.5
F11	5	15		30	42.5		7.5
F12	5	15		30		42.5	7.5

pH Measurement

The determination of a pH value of solvents, oils, free-drug loaded, and drug-loaded of isg and ism dosage forms were undertaken using a pH meter (Ultrabasic UB-10, Denver Instrument, Bohemia, New York) (n=3).

Viscosity

The measurement for viscosity and rheology of all related systems as mentioned in 2.3.3.2 were conducted using a Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories, Inc., USA) with spindles (CP40 and CP52) at ambient temperature (n=3). The estimate parameters for measurement were recorded at 15-sec equilibration time of different shear rates (n=3).

Phase Transformation of isg and ism

The gradual transformation by time from solution of isg or emulsion of ism into gel-like or solid matrix could be observed under stereoscope after them contact the aqueous phase such as agarose gel. Before testing, the preparation of agarose gel could be attained by dissolving 0.6% w/w agarose powder in PBS. Then the prepared solution was filled into 9-cm diameter plastic petri dishes and cooled down for setting into gel state. Subsequently, a cylindrical agarose well was fabricated by drilling manually using a 6-mm-diameter cylindrical stainless-steel cup. The 150 µl of the prepared formulation was filled to the cylindrical agarose well and observed the transformation under a stereomicroscope (Motic SMZ-171 Series, Kowloon, Hong Kong). The diffusion length of the opaque ring of a gel-like or solid matrix was performed at five positions. The data is exhibited as average value $\pm$ standard deviation (S.D.), subsequently, their diffusion rate values were calculated (n=3).

Statistical analysis

The statistical analysis presented as a statistical significance of the measurement data was conducted using a one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) *post hoc* test or Duncan's test. The significant level was set at $p < 0.05$.

Results and Discussion

Polymer Dissolution, Miscibility and Density of Oils and Solvents

Eudragit RS at concentration of 30% w/w could dissolve in NMP, DMSO and PYR for applying as a structure-forming agent of isg and ism dosage forms in this research. The lower concentration of this polymer could not induce the sufficiently dense gel formation whereas the higher concentration made into too viscous mass for injection. NMP had a partial miscible with oil; thus, provoked a fast phase separation. DMSO and PYR were immiscible with olive oil and camellia oil since the volume of them did not change after mixing them together. It notably evidenced that an initial prepared emulsion was not physically stable and was not able successfully to be attained using the internal phases dissolved in NMP. This evidence of unsuccessful achievement was owing to the partial miscibility between NMP and oil phase promoting unstable emulsion after mixing two phases together. Therefore, DMSO and PYR were selected as the vehicles for producing anhydrous emulsion of ism with suitable properties for local administration because of their low viscosity and their immiscibility with oils as mentioned these required characteristics previously (Khang *et al.*, 2021; Tuntarawongsa *et al.*, 2021). In this research, the density values of the solvents and oils are presented

in Table 2 Typically, the density is an important parameter affecting contact angle, surface tension and interfacial tension. Moreover, the density value has to include for calculation of the surface tension. The density of PBS was 1.0035 g/cm^3 and nearly that of the water (1.0001 g/cm^3) whereas their values were apparently higher than that of the oils. PYR presented the highest density (1.1061 g/cm^3) followed by DMSO (1.0935 g/cm^3) which were higher than that of the PBS solution.

Contact Angle of Oils and Solvents

The angle created between the target surface and the line tangent to the edge of the fluid drop is indicated as a contact angle (Marmur *et al.*, 2017). The contact angle of the oils and solvents are presented in Table 2 and Figure 1. The water was measured and employed as the standard liquid and it exhibited the highest contact angle with 52.37° . PBS, the aqueous-based comprising various ionic species, showed the rather low contact angle compared to the water because of the interaction between negative surface charge of silanol group (SiO^-) and buffer ion species; thereafter promoting adhesion with low contact angle (Behrens and Grier, 2001). DMSO and PYR, the polar aprotic solvents, presented the contact angle of 26.69° and 24.79° , respectively, that markedly lower than that of water about two times. Therefore, their dipole-dipole force was less than H-bonding of water. These results led to their spreadability on glass slide was easier than water because of their weaker intermolecular force. In addition, their more hydrophobicity promoted the lower contact angle; nonetheless, were higher than that of oils. These results also corresponded to the viscosity values of these two solvents as presented in Table 4 since the more viscous characteristic of fluid typically enables to retard the spreadability ability of its droplet on a target surface.

Surface Tension of Oils and Solvents

Normally, the energy superintends for the phenomenon of surface tension could be approximately equivalent to the work or energy needed to remove the surface layer of molecules in a unit area (Hauner *et al.*, 2017). Table 2 and Figure 2 shows the values of surface tension of test samples in this research. The highest surface tension was evident for water because of its intermolecular force from H-bonding (O'Connell and Prausnitz, 1969), that higher than that of PBS. The greater value of surface tension of DMSO than PYR resulted from the dominant H-bond and dipole-dipole intermolecular forces of DMSO (Pope and Oliver, 1966). The 5-membered lactam ring of PYR should exhibit less intermolecular force (Russo and Casazza, 2012). The similar rather low value of

Table 2. Density, contact angle, and surface tension values of oils and solvents (mean $\pm$ S.D.) (n=3)

Substance	Density (g/cm^3)	Contact angle ($^\circ$)	Surface tension (mN/m)
Water	1.0001 ± 0.0005	52.37 ± 2.58	73.71 ± 0.71
PBS	1.0035 ± 0.0005	32.24 ± 0.64	72.82 ± 0.21
DMSO	1.0935 ± 0.0005	26.69 ± 2.52	39.81 ± 0.58
PYR	1.1061 ± 0.0000	24.79 ± 1.12	35.61 ± 0.13
Olive oil	0.9090 ± 0.0005	22.38 ± 2.34	31.68 ± 0.26
Camellia oil	0.9103 ± 0.0007	23.52 ± 1.48	30.77 ± 0.23

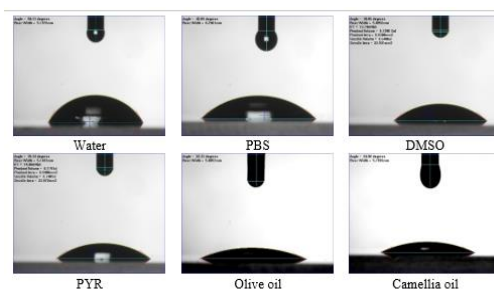


Figure 1. Photographs of contact angle of different samples

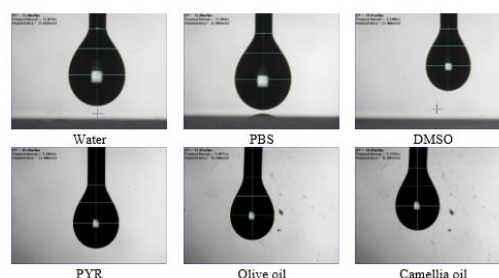


Figure 2. Photographs from surface tension measurement of different samples

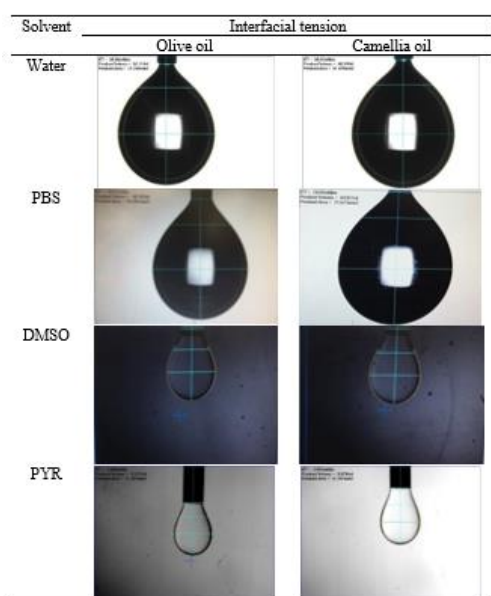
surface tension was obtained for these used two oils because of their similar hydrophobic force. The understanding for these basic surface tension value of components could be applied subsequently for their interfacial behaviours. In addition, the liquid with higher density should be gravimetrically dropped into the liquid with less density for easier measurement of interfacial tension.

Interfacial Tension of Oils and Solvents

This measurement aims to check the molecular attraction force at the interface of two interested fluids (Kronberg *et al.*, 2014). The measurement of interfacial tension between the internal phase and the external phase is crucial for insightful knowing the emulsion stability. Normally, the diminishment of the interfacial tension between the two phases minimizes the free energy and enhances the inherent thermodynamic stability of the prepared emulsion (Harkins and Humphery, 1915). For ism, the

Table 3. Interfacial tension of solvents against olive oil and camellia oil (n=6)

Solvent	Interfacial tension $\pm$ S.D. (mN/m)	
	Olive oil	Camellia oil
Water	20.72 ± 0.05	28.36 ± 0.27
PBS	18.06 ± 0.11	24.23 ± 0.16
DMSO	4.20 ± 0.07	4.64 ± 0.02
PYR	3.44 ± 0.06	3.58 ± 0.01

**Figure 3. Droplet photograph from interfacial tension measurement of solvents against olive oil and camellia oil**

polymeric solution was dispersed in olive oil or camellia oil for fabricating into the emulsion. Nevertheless, the viscous polymeric solution is difficult to injection as droplet formation into the external phase during interfacial tension measurement. To signify the physical stability of emulsion, the interfacial tensions between the selected solvent and the oil was tested and reported in Table 3 and Figure 3. The highest interfacial tension was found for water in camellia oil with the largest drop shape owing to the high polarity of water. The present of electrolytes in PBS promoted a lower value of interfacial tension against the oil that corresponded to the previous data. Notably, the interfacial tension of NMP and PYR was lower for 7 times against camellia oil and 5 times against olive oil compared to the water due to their lower polarity. Thus, both of these solvents presented the markedly minute drop shape in these oils. The interfacial tension of selected solvents was significantly diminished ($p < 0.05$) in which the density profile at the interface was ostensibly forced by the minimization of the free energy into the interface

(Miller and Neogi, 2008; Pichot, 2010). The densities of mass in the interface should be intermediate values between the density of olive oil and that of selected solvent. Typically, the density varies abruptly at interface phase. The interactions between compounds having the same density are more probable at broad interface than at sharp interface. Therefore, the sharper interface, the greater is the density difference (Cahn and Hilliard, 1958). The system with a minute density gradient efficiently diminished the free energy after adding solvent into oil phase. Therefore, DMSO and PYR presented as the potential vehicles of the internal phase for preparing the anhydrous emulsion using olive oil and camellia oil as the continuous phases. Additionally, the incorporation of GMS in oils should enhance the ability to minimize the interfacial surface tension for more stable emulsion preparation.

Physical Properties of isg and ism Systems using ERS as Matrix Forming Agent

Appearance

All isg dosage forms exhibited as transparent solutions. The doxycycline hyclate-loaded isg formulations shown as yellowish transparent solutions because of the color of this active compound. After mixing with the dispersing oil phase, all of the ism dosage forms presented a good physical stability of obtained emulsion since there was no phase separation for 1 hour. The emulsifier such as GMS has been applied for diminishing a droplet coalescence of an anhydrous emulsion because GMS promoted a viscous environment of dispersed phase and its liquid crystalline layer embedded between two phases (Phaechamud *et al.*, 2019; Rein *et al.*, 2020; Chuenbarn *et al.*, 2022). Moreover, GMS enabled to perform as gelling agent of oil phase from GMS network to diminish a droplet coalescence of emulsion (Hodge and Rousseau, 2005; Daniel and Marangoni, 2012). Some previous investigations claimed the good stabilization of emulsion by GMS (Chuenbarn *et al.*, 2022; Rein *et al.*, 2020). The development of ism dosage form aims to fabricate as a freshly prepared injection dosage form; thus, the physical emulsion stability with no phase separation for 1 hour should be sufficient prior to conducting *via* injection by the physician or dentist (Jain *et al.*, 2012)

pH

To apply Eudragit RS-based isg and ism for periodontitis treatment, pH value of gingival crevicular fluid at periodontal pocket should be considered in which its pH is close to 6.8 (Blackstock, 1989; Eggert *et al.*, 1991; Galgut,

2001). Table 4 shows the pH of intact solvents and the free drug-loaded Eudragit RS formula. The pH value of DMSO and PYR was 11.40 and 11.20, respectively. The free-drug loaded Eudragit RS formulas showed the pH values between 7.50-9.82. The isg and ism formulations containing DMSO as a solvent (F1, F2, F5, F6, F7, and F8) presented more alkaline than PYR (F3, F4, F9, F10, F11, and F12) because DMSO has a high pH value. Moreover, the pH of doxycycline hyclate-loaded formulations was markedly declined ($p < 0.05$) owing to the acid of this active compound for enhancing the stability of this drug and diminished its degradation (Jantravid *et al.*, 2010; Redelsperger *et al.*, 2011). The recovery of doxycycline hyclate at 93.8% and 84% could be achieved from an acidified reverse-osmosis water with pH value as 2.6 after filling in for 2 weeks (Redelsperger *et al.*, 2011). In addition, some acidic pharmaceutical dosage forms were acceptable to inject into periodontal pockets (Blackstock, 1989; Eggert *et al.*, 1991). Eudragit RS also performed as an interesting material in acidic pH because of its solubility with pH-independent (Phaechamud *et al.*, 2017; Srichan *et al.*, 2017).

Viscosity

The selected solvents exhibited rather a minute apparent viscosity of less than 15 cps, while both oils presented apparently higher viscosity as presented in Table 4. The density value of PYR was higher than that of DMSO (Table 4) corresponded with the density value as exhibited in Table 2. From the preliminary solubility test of polymer, Eudragit RS at concentration of 30% w/w could be as a structural forming agent in this research. By comparison, ism formulations with isg formulations, the free-drug loaded ism formulations including F5, F6, F9, and F10 presented significantly less viscosity than the isg formulations such as F1 and F3 ($p < 0.05$) due to the lubrication effect of the oil as a continuous phase of ism emulsion (Karmakar *et al.*, 2017; Phaechamud *et al.*, 2018c). Nonetheless, the viscosity of isg and ism comprising different oils as the external phases showed the same trend of viscosity characteristic as the free-drug loaded formulations. By comparison, DMSO-based systems in both isg and ism formulations presented a significantly less viscosity than that of PYR-based systems ($p < 0.05$). This result indicated that DMSO posed as a good solvent for Eudragit RS in which Eudragit RS-solvent interactions prevailed over polymer-polymer interactions, whereas PYR presented a high viscosity, especially in the isg, signifying that polymer-polymer interaction was dominated (Phaechamud *et al.*, 2019; Rein *et al.*, 2020). Some research mentioned that isg using large amount of

Table 4. Physical properties of the solvent, free-drug loaded formulation, and doxycycline hyclate-loaded formulation (n=3)

Formulation	pH $\pm$ S.D.	Viscosity $\pm$ S.D. (cPs.)
Free drug-loaded formulation		
DMSO	13.11 $\pm$ 0.09 ^{ab}	2.26 $\pm$ 0.15 ^c
PYR	11.40 $\pm$ 0.22 ^{ab}	15.72 $\pm$ 0.03 ^c
Olive oil	NA	112.80 $\pm$ 0.17
Camellia oil	NA	86.42 $\pm$ 0.15
F1	8.82 $\pm$ 0.01 ^a	862.57 $\pm$ 4.56 ^c
F3	7.91 $\pm$ 0.00 ^a	6936.00 $\pm$ 75.62 ^c
F5	9.21 $\pm$ 0.01 ^b	513.30 $\pm$ 9.18 ^c
F6	9.82 $\pm$ 0.00 ^b	460.40 $\pm$ 6.96 ^c
F9	8.46 $\pm$ 0.01 ^b	677.33 $\pm$ 16.54 ^c
F10	7.50 $\pm$ 0.01 ^b	621.10 $\pm$ 5.29 ^c
DH-loaded formulation		
F2	3.35 $\pm$ 0.00 ^a	2435.00 $\pm$ 23.79 ^c
F4	3.21 $\pm$ 0.00 ^a	18812.00 $\pm$ 1274.63 ^c
F7	3.30 $\pm$ 0.00 ^b	1714.30 $\pm$ 206.47 ^c
F8	3.24 $\pm$ 0.02 ^b	1261.00 $\pm$ 42.14 ^c
F11	3.14 $\pm$ 0.01 ^b	10782.00 $\pm$ 601.02 ^c
F12	3.18 $\pm$ 0.00 ^b	9679.30 $\pm$ 479.75 ^c

NA = Not determined; the superscripts a-c indicate a significant difference ($p < 0.05$)

PYR exhibited a notably high apparent viscosity because PYR was high viscous and less solubility power (Chantadee *et al.*, 2019). Thus, the aggregation of the polymer markedly enhanced the viscosity of the system (Camargo *et al.*, 2013). The viscosity value of doxycycline hyclate-loaded bleached shellac-based ism prepared employing 2-pyrrolidone as a solvent was markedly higher than formulation prepared with DMSO (Phaechamud *et al.*, 2016b). Regarding influence of oil type, the system using camellia oil as the external phase exhibited a significantly less viscosity than the system using olive oil ($p < 0.05$), due to the lower viscous characteristic of camellia oil. Loading of doxycycline hyclate significantly enhanced the viscosity ($p < 0.05$) since this active compound molecule replaced a content of the solvent in the formulations (McPherson *et al.*, 2015).

Phase Transformation of isg and ism

Practically, isg formulations changed from solution to gel state and ism formulations changed from emulsion to microparticle mass from mechanistic solvent exchange inducing polymer matrix formation (Phaechamud *et al.*, 2019; Rein *et al.*, 2020). Doxycycline hyclate-loaded ism was chosen as the model formulation to determine the diffusion rate and length of water diffusion into systems. Figure 4 presented the visual images from the stereomicroscope of Eudragit RS-based isg and ism transformation. There was a presence of surrounding cloudy mass in agarose well after adding ism indicating the polymeric matrix

formation and this cloudy mass was greater by time. To clearly observe the diffusion length of ism, the water-soluble red dye such as amaranth was dissolved in agarose gel and the results are shown in Figure 4 for F7, F8, F11, and F12. The length of diffusion is shown in Figure 5 that is gradually increased because of the harden polymer matrix and the hydrophobic oil. The formulations employing DMSO as a solvent had longer diffusion than that using PYR as a solvent owing to higher polarity and apparently less viscosity of DMSO (Phaechamud *et al.*, 2019). Moreover, ism prepared from camellia oil had a more diffusion path length than other because the low viscosity of this oil performed as the loose barrier and the water easily diffused into the formula.

The diffusion rate of isg and ism systems are shown in Figure 6. There was the highest rate of initial diffusion for F4 and gradually slower from barrier effect of polymeric gel. F2 showed the diffusion with a slower rate than F4 in the initial state. Nevertheless, isg using DMSO as a solvent presented the higher diffusion rate after initial state than that using PYR because of the higher water affinity and less viscosity of DMSO (Rein *et al.*, 2020). However, there was no significant difference in the early state meanwhile they were slower with time due to microparticle formation and interfered the diffusion of water. The more apparent in different diffusion rate was found after early 5 min. For example, F8 showed the highest diffusion rate conforming with its long diffusion length but F12 exhibited the slowest diffusion rate due to its high viscosity. The more unsaturated fatty acid of camellia oil than olive oil could interfere the water diffusion into developed systems (Wei *et al.*, 2016).

Conclusions

DMSO and PYR were selected as the solvents to prepare isg and as the internal phase of anhydrous emulsion of ism owing to their immiscibility with oils. DMSO and PYR performed as the potential solvents of the internal phase for preparing the anhydrous emulsion using olive oil and camellia oil as the continuous phases due to their low interfacial tension property. After mixing with the external oil phase, all of the doxycycline hyclate-loaded Eudragit RS-based ism formulations presented a good emulsion physical stability with no sign of phase separation for 1 hour. This physical emulsion stability was sufficient for freshly prepared dosage forms before administration via injection. The free-drug loaded ism formulations presented significantly less viscosity than the isg due to the lubrication effect of the oil in continuous phase.

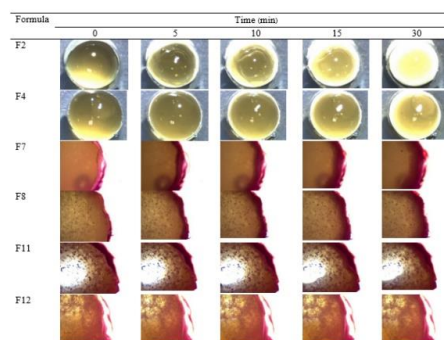


Figure 4. Macroscopic transformation of Eudragit RS from isg and ism formulations under the stereomicroscope (magnification of 10x) in 0, 5, 10, 20, and 30 min

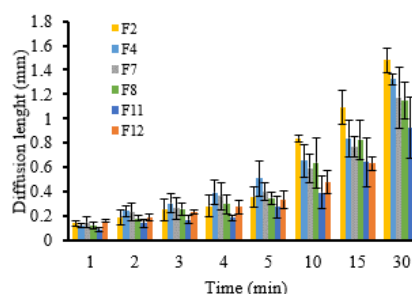


Figure 5. Diffusion length of Eudragit RS-based isg and ism formulations at different times (n=3)

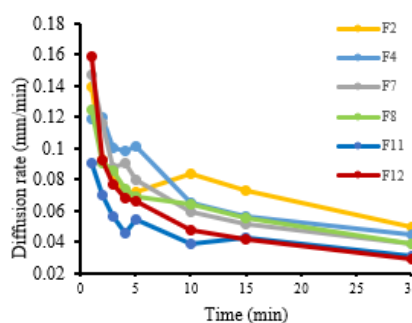


Figure 6. Diffusion rate of Eudragit RS-based isg and ism formulations at different times

Viscosity of isg and ism comprising different oils as the external phases showed the same trend of viscosity characteristic as the free-drug loaded formulations. DMSO-based formulations in both isg and ism formulations exhibited a significantly less viscosity than that of PYR-based formulations. The rapid change of the liquid into the solid gel of isg or microparticle mass of ism was achieved after exposure aqueous phase. In situ forming systems

that using DMSO as a solvent more rapidly phase transformed with solvent exchange than that using PYR. Moreover, Eudragit RS-based ism containing camellia oil exhibited a larger diffusion length than that containing olive oil because of the low viscosity of the camellia oil to facilitate solvent-water exchange, thus the water easily diffuses into the formulation. These finding evidences will be applicable for describing the further research work of in silico study using dynamic modelling for phase transformation mechanism into gel or microparticle and their mechanistic phase inversion of Eudragit RS-based drug-loaded isg and ism for periodontitis treatment.

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