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Original article

Doxycycline hyclate-loaded *in situ* forming gels composed from bleached shellac, Ethocel, and Eudragit RS for periodontal pocket delivery

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ABSTRACT

Polymeric material plays an important role as a matrix former in the modulation of drug release of antimicrobial-loaded *in situ* forming gel (ISG) for efficient periodontitis treatment. This study was conducted to compare three polymers, namely bleached shellac (BS), Ethocel (EC) and Eudragit RS (ERS), as matrix formers of doxycycline hyclate (DH)-loaded solvent exchange-induced ISG. All prepared ISGs, except 25% EC ISG, exhibited the Newtonian flow behaviour. Transformation from solution into matrix-like was achieved rapidly within 5 min. Increasing the amount of these polymers extended the release of DH. DH-loaded EC and ERS ISG systems exhibited high antimicrobial activity, and all ISGs were effective in inhibiting the growth of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, *Porphyromonas gingivalis* and *Candida albicans*. By comparison, the DH-loaded ERS ISG, through the solvent exchange mechanism, was found to be ease in injection with low viscosity and sustained the release with higher concentration, meanwhile, it also exhibited interesting *in vitro* degradability and antimicrobial activities. Therefore, the DH-loaded ERS ISG exhibited a potential use for localized periodontal drug delivery system for the treatment periodontitis.

1. Introduction

Periodontitis is a chronic inflammatory disease of the periodontium caused by the bacteria *Porphyromonas gingivalis* and *Streptococcus* strains that accumulate in periodontal pockets, leading to the loss of teeth (Aminu et al., 2013; 2017; Van Dyke and Sima, 2020). Treatment of periodontitis using systemic antimicrobial drugs is known to cause undesirable side effects; therefore, it would be interesting to explore the use of appropriate drug delivery systems for local antimicrobial drug delivery to the periodontal pocket (Aminu et al., 2019). Concerning with the local drug delivery system, not only the ease in injection, release of the drug in a controlled manner but also the biodegradability, biocompatibility and non-irritating to the tissue should be considered.

An *in situ* forming gel (ISG) is a localized injectable drug delivery system that has the potential to treat periodontitis because of its ability to sustain drug release and make the drug level sufficient for achieving the bacteriostatic or bactericidal effect at the target site (Garala et al., 2013; Phaechamud et al., 2016a,b). ISG reduces the dose of drug and the frequency of administration and thus improves patient compliance (Hatefi and Amsden, 2002; Do et al., 2015). Moreover, it can be administered easily without pain (Xiong et al., 2011). Before administration, the ISG system is in sol state but after administration, upon exposure to the aqueous environment, the exchanged of solvent with this aqueous environment induces the solidification of matrix forming agent from which the drug is released in a controllable fashion (Chantadee et al., 2020a; Parent et al. 2013). The major components of an ISG include a polymer, an active ingredient and a solvent. Typically, the polymer is dissolved in the pharmaceutically acceptable organic solvent

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and upon exposure of the aqueous environment, the polymer precipitation at the injection site attributed to retard the release of drug in a controlled manner. The point is that the aqueous insoluble polymers are used to modulate the drug release but it could be dissolved in the solvent used for formulation. Bleached shellac (BS), Ethocel (EC) and Eudragit RS (ERS) are interesting polymers that are currently being investigated as they are non-toxic, non-allergenic and non-irritant materials (Srichan and Phaechamud, 2017).

Doxycycline hyclate (DH) is an antibiotic drug that has been reported to be efficacious for the treatment of periodontitis (Van Dyke and Sima, 2020; Javali and Vandana, 2012) based on its activity against the major pathogens causing periodontitis, especially *P. gingivalis* (Larsen, 2002). DH has also demonstrated activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, enterococci, anaerobes and *Plasmodium* spp (Sweetman, 2009; Papich, 2016). The molecular weight of DH is 1025.89 g/mol with a pKa of 3.02, and it is soluble in water (1 g/20 mL).

BS is derived by dissolving a polyester resin, harvested from lac, in solvents and then precipitated using sulphuric acid (Buch et al., 2009). It is used for waterproofing in pharmaceutical coating processes (Rowe et al., 2009). EC is a colourless, odourless and tasteless polymer (Zoubari et al., 2019) primarily used in oral drug formulation as a hydrophobic coating agent or as a matrix former for tablets and granules to modify the release of drug and so forth (Rowe et al., 2009; Azoury et al., 1988). ERS is a copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester. It is a white powder with a faint amine-like odour (Porfiryeva et al., 2019). ERS has been widely used as a transdermal patch or as a water-insoluble film for sustained-release products (Mathur et al., 2013). *N*-methyl-2-pyrrolidone (NMP) is a clear solvent that is miscible with water and has low volatility (Jouyban et al., 2010). It has been accepted for use as a strong solubilising agent in the pharmaceutical industry (Jouyban et al., 2010). NMP has been used as an injectable solvent of commercial ISG due to its thermal stability, biodegradability and biocompatibility (Sanghvi et al., 2008). This study was conducted to compare the BS, EC and ERS to be used as aqueous insoluble polymers of DH-loaded ISGs so that to obtain the injectable localized DH-loaded ISG systems with its ability to sustain drug release and make the drug level sufficient for achieving the bacteriostatic or bactericidal effect at periodontal pocket.

2. Materials and methods

2.1. Materials

BS, EC and ERS were obtained from Ake Shellac Co., Ltd., Lumpang (Thailand), the Dow Chemical Company (USA) and EVONIK Röhm GmbH (Germany), respectively. DH (Batch No. 20071121) was purchased from Huashu Pharmaceutical Corporation, China. NMP (lot no. A0251390, Fluka, New Jersey, USA) was used as an organic solvent for polymers and drug. *Staphylococcus aureus* (ATCC 6538P), *Escherichia coli* (ATCC 25922), *Streptococcus mutans* (ATCC 27175), *Porphyromonas gingivalis* (ATCC 33277) and *Candida albicans* (ATCC 17110) were supported by Department of Medical Sciences Ministry of Public Health, Nonthaburi, Thailand.

2.2. Preparation of solutions

The injectable ISG gel bases were prepared by dissolving the different amount of BS, EC and ERS in NMP. Each dispersion was stirred and kept for 24 h until clear solution was formed. Then, the 5% w/w of DH was incorporated into the respective prepared polymer solutions and each system was kept for 24 h.

2.3. Evaluation of physical properties of ISG

2.3.1. Appearance, pH value, viscosity and rheology measurements

The physical appearance of the prepared ISG systems was recorded by visual observation. The pH value was measured by a pH meter using a flat surface probe (Ultra Basic UB-10, Denver Instrument, Bohemia, New York, USA.). The viscosity of the prepared ISG systems was evaluated at 25 °C (room temperature) and at 37 °C (physiological temperature) using a Brookfield DV-III Ultra programmable rheometer (Brookfield Engineering Laboratories Inc, Middleborough, MA, USA) equipped with CP-40 and CP-52 spindles. Viscosity values were recorded at different shear rates at every 15 s of equilibration time. Rheological data were also collected with this instrument. The shear stress of the sample was monitored at several shear rates at both room temperature and physiological temperature (regulated by a water bath; Bushi heating bath B-490, New Hampshire, USA). The flow parameters were calculated using the following exponential formula (Martin, 1993):

$$F^N = \eta G \text{ and } LogG = NLogF - Log\eta$$

Where F is the shear stress, η is the viscosity coefficient, G is the shear rate and N is an exponential constant.

2.3.2. Injectabililty test through syringe

For assessing the injectabililty at room temperature, the prepared ISG was filled into a 1-mL syringe connected with an 18-gauge needle, and then the upper probe of the texture analyser (TA.XT plus, Stable Micro System, UK) was moved downwards (1.0 mm s^{-1}) with a constant force of 0.1 N until the probe contacted with the barrel base. The distance of the barrel was fixed at 20 mm. Force displacement data were recorded and analysed at a distance of 10 mm. The expulsive work was also calculated based on the area under the curve (n = 3) (Kelly et al., 2004).

2.3.3. Assessment of in vitro gel formation and aqueous diffusion into ISG

The phase inversion from solution to gel or matrix-like of the prepared ISG systems or the gel formation was recorded by injecting the formulation into an aqueous medium of PBS (pH 6.8) at room temperature. A volume of 1 mL of ISG was filled into a plastic syringe connected with an 18-gauge needle and injected into a test tube containing 5 mL of PBS (pH 6.8) (simulated gingival crevicular fluid). Subsequently, pictures of the transformation were taken at 0, 1 and 30 min. To investigate the aqueous diffusion that induced the matrix formation, the prepared ISGs were individually filled into a 6-mm-diameter transparent plastic tube, submerged into PBS (pH 6.8), and then the change from the transparent solution to the turbid matrix due to water diffusion into the ISG was measured at room temperature. The turbid distance front was recorded at 0, 4 and 24 h, and the rate of water diffusion was calculated using the following formula: rate = distance of water front diffusion (mm)/time (min) (n = 3).

2.3.4. Drug release study

The dialysis membrane method was used in this experiment, in which a sample of ISG (1 g) was added into a dialysis tube (Spectrapor, MW cutoff: 6000–8000), submerged into 100 mL of PBS (pH 6.8) at 37 °C, and then maintained at a rotational speed of 50 rpm using a shaking incubator (Model SI4; Shel Lab, Cornelius, USA). Aliquots, each of 10 mL, were withdrawn from the release medium at various time intervals, and each aliquot was replaced with 10 mL of fresh medium. The concentration of the drug in the sample solution was detected using a UV–vis spectrophotometer at 349 nm. All experiments were performed in triplicate, and the mean cumulative drug release ± S.D. was calculated (n = 3).

Membrane-less diffusion method was also used in this study, in which 0.4 g of ISG was added into a porcelain cup (10 × 12 mm), submerged into 100 mL of phosphate buffer (pH 6.8) at 37 °C, and then maintained at a rotational speed of 50 rpm using a shaking incubator (Model SI4, Shel Lab, Cornelius, USA). Then, the medium was collected and analysed for DH release using the same procedure applied in the dialysis membrane method (n = 3).

2.3.5. Surface morphology

ISG remnants from the drug release test were dried in a freeze dryer (Triad™ Labconco, Missouri, USA) for 48 h and then sprayed and covered with gold particles. These dried matrix surfaces were examined under a scanning electron microscope (SEM) (Maxim 200 Camscan, Cambridge, England) at an accelerating voltage of 15 kV.

2.3.6. In vitro ISG degradation study

A sample of 1 g of ISG was submerged into 10 mL of PBS (pH 6.8) and then incubated at 37 °C with shaking at 50 rpm. After 7 days, the ISG remnants were dried in a hot air oven with temperature adjusted to 65 °C. The percentage of weight loss was calculated using the following equation: % weight loss = 100 × (initial weight - final weight)/(initial weight) (n = 3).

2.3.7. Antimicrobial activity test

The agar-cup diffusion method was used to determine the antimicrobial activity of the ISG systems containing 5% w/w DH against *Staphylococcus aureus* (ATCC 6538P), *Escherichia coli* (ATCC 25922), *Streptococcus mutans* (ATCC 27175), *Porphyromonas gingivalis* (ATCC 33277) and *Candida albicans* (ATCC 17110). The 100 μL samples were loaded into the cylinder cups (8-mm diameter and 10-mm height) that were placed on the microbial culture plates. The culture plates were then incubated at 37 °C for 48 h followed by the measurement of the diameter of the inhibition zone (n = 3). For evaluating the antimicrobial activity against anaerobic bacteria, the experiment was performed in an anaerobic incubator (Forma Anaerobic System, Thermo Scientific, Ohio, USA).

2.4. Statistical analysis

All sub-experiments were conducted in triplicate, and values were expressed as mean ± standard deviation (S.D.). The relationship between variables was analysed using Pearson correlation coefficients. Statistical significance of the values was analysed using one-way analysis of variance (ANOVA), followed by post hoc test and the least significant difference (LSD), and the significance level was set at $P < 0.05$.

3. Results and discussion

3.1. Appearance and pH value of drug solutions

The DH-loaded BS, EC and ERS ISGs were clear. There was no precipitation or phase separation occurred during preparation and storage. They were yellowish in colour primarily due to the colour of DH. The pH value of these ISGs varied from 3.77 ± 0.05 to 4.42 ± 0.02 as shown in Table 1. Although the pH value of the NMP solution was approximately 8, the pH values of the prepared solutions were apparently lower because of the acidity of DH (Convention USP, 2009). However, the side effects that could possibly be caused by the low pH of these ISGs could be minimised due to the application of only a low amount (20 μL) by injection into the periodontal pocket and also due to the buffering capacity of gingival crevicular fluid and its circulation (Harish et al., 2009; Chaudhary and Verma, 2014).

3.2. Viscosity and rheology

The gellation depends not only on the type and amount of polymer used but also on the time where the complete gellation may occur with time. After administration, ISG system exposed with the fluid in the periodontal pocket where the solvent diffused out and exchanged with the environmental fluid leading to the phase inversion and polymer precipitation as gel like matrix. Therefore, there may be a period for diffusion in and out of the environmental liquid and solvent which in turn and there may be some lag phase for polymer precipitation. During this time, the ISG system may still in solution state. The fast solvent exchange mechanism and rapid in phase inversion may be observed when the viscosity of the system in periodontal pocket is low. The temperature at the periodontal pocket (37 °C) may induce for reduction in the viscosity of the ISG. Thus, it is necessary to investigate the flow property of the solution before and after the administration conditions (25 °C and 37 °C). The present study investigated the viscosity and rheological property of the system (in a solution state) at 25 °C and 37 °C. The experiment showed that the viscosity of all solutions at 37 °C was lower than that at 25 °C (Table 1). The higher temperature vulnerable to cause loses in the structure of the network of the gel solution and it also proved that owing to the loose structure at a higher temperature caused the sharp decrease in the shear stress at 37 °C (Phaechamud et al., 2016a,b). The present study proved the increased amount of the polymer concentration with decreasing amount of NMP lead to increase in fluid viscosity of the ISG. When the interactions between the polymer chains are favoured, the viscosity of the solution is increased. On the other hand, a dominance of polymer–solvent interactions results in min-

Table 1
Physical properties of DH-loaded ISG comprising different polymers (n = 3).

Polymer type	Polymer content (% w/w)	pH	Viscosity (cPs)		Exponential constant (N)		Expulsive work (N.mm)
			25 °C	37 °C	25 °C	37 °C	
BS	15%	4.16 ± 0.05	15.86 ± 0.30[a]	10.57 ± 2.29[b]	0.99 ± 0.01	1.03 ± 0.02	13.32 ± 2.12[c]
	20%	4.09 ± 0.01	43.61 ± 0.52	25.11 ± 2.29	1.02 ± 0.01	1.02 ± 0.01	25.89 ± 2.89
	25%	4.13 ± 0.01	186.35 ± 3.96	64.76 ± 2.29	1.00 ± 0.02	1.04 ± 0.01	37.29 ± 4.76
	30%	3.93 ± 0.04	ND	251.11 ± 24.23	–	0.99 ± 0.01	115.23 ± 3.05
EC	5%	3.95 ± 0.05	43.61 ± 0.71	27.75 ± 0.80	1.06 ± 0.01	1.07 ± 0.01	18.58 ± 1.32
	10%	4.05 ± 0.01	306.61 ± 2.29	188.99 ± 2.29	0.99 ± 0.01	1.00 ± 0.02	24.78 ± 1.32
	15%	4.36 ± 0.01	1457.47 ± 11.45[a]	903.98 ± 6.87[b]	0.97 ± 0.01	0.97 ± 0.01	26.73 ± 1.47[c]
	20%	4.42 ± 0.02	ND	3232.66 ± 13.92	0.95 ± 0.01	0.96 ± 0.00	46.33 ± 1.71
ERS	15%	3.86 ± 0.04	33.16 ± 0.57[a]	22.54 ± 0.20[b]	0.99 ± 0.01	1.01 ± 0.01	9.09 ± 0.82[c]
	25%	3.92 ± 0.01	151.99 ± 2.29	99.12 ± 0.30	0.99 ± 0.02	1.00 ± 0.01	14.02 ± 0.74
	30%	3.93 ± 0.01	404.41 ± 7.80	228.64 ± 8.25	1.00 ± 0.01	1.00 ± 0.01	17.04 ± 0.86
	35%	3.77 ± 0.05	1001.78 ± 18.74	564.33 ± 6.06	1.00 ± 0.01	1.00 ± 0.02	24.72 ± 1.35

ND = not determined; the asterisk ([a,b,c]) represents a significant difference ($P < 0.05$)

imal viscosity of the solution (Camargo et al., 2013). When compared, the system containing EC gave the highest viscosity which was followed by the system containing ERS and BS. Since, the increasing amount of polymer contributed to the high resistance in the solution to flow, the viscosity data for the 30% w/w BS and 20%w/w EC ISGs could not be determined. Typically, viscosity is a critical internal factor that potentially affects the flow property and the injectability of an ISG. A highly viscous ISG may cause a difficulty in the injection process leading to pain in patient. The DH-loaded BS and ERS solutions did not change their viscosity when the shear rate was increased, signifying the Newtonian flow behaviour. Hence, the force of expulsion did not affect the viscosity of the solution, and the solution was convenient for usage (Watt et al., 2019). The 5% – 15% w/w EC solutions also exhibited the Newtonian flow behaviour.

3.3. Injectability through syringe

During administration, the ISG should be injected easily and rapidly through a syringe without causing pain (Xiong et al., 2011). The energy required for injecting the ISG was determined by the injectability test, wherein the expulsive work for each DH-loaded solution varied from 9.09 ± 0.82 to 115.23 ± 3.05 N.mm (Table 1). All of exponential constant values were close to 1 indicating of Newtonian flow of them. An increase in work force for expulsion indicates a lower injectability (Chang et al., 2002). The required force increased when the concentration of EC and ERS was increased. Comparison between the polymers at 15% w/w concentration revealed that the energy required for injecting the DH-loaded ERS solution was lower than that for injecting DH-loaded BS and EC, respectively ($P < 0.05$). The linear relationship between the expulsion force and the viscosity of the solution should be obtained (Ryl and Owczarz, 2020). Although ERS ISG systems showed higher viscosity, their expulsive work was apparently lower than that of BS ISG systems. The 3D-network formation within the BS ISG led to a significant enhancement in strength and viscoelasticity of these formulations (Phaechamud et al., 2018). In addition, the adhesiveness property of BS with plastic deformation has been reported previously (Senarat et al., 2020); therefore, these characters of BS promoted the higher expulsive work of BS ISG systems. Therefore, the highest viscosity of the EC solutions imparted their lower injectability. However, the entire expulsive work required for injecting each solution, except for 30% w/w BS solution, was lower than 50 N.mm, indicating their acceptability for the criteria of injection (Philippot et al., 2005).

3.4. In vitro gel formation

The mechanism of solvent exchange promoted the transformation of the ISG from the drug-loaded aqueous insoluble polymeric solution into a gel or matrix-like from which the drug is released gradually in a controlled manner. Therefore, this transformation characteristic is necessary for the ISG, whereas a rapid transformation provides patient compliance (Gokulanathan et al., 2014; Yamamoto et al., 2002). This study demonstrated that all the prepared DH-loaded polymeric solutions, except the 15% w/w BS formula, were able to transform efficiently into matrix (Fig. 1) and the process was complete within 5 min, which was acceptable for application considering patient compliance (Anneken et al., 2001). In detail, the obtained transformed drug-loaded ISGs, such as 20% BS matrix, were soft and the ERS matrix was stiffer than the BS matrix, but the most rigid structure was evident for the EC matrix as observed by the similar polymer content. Overall, the matrix of the ISGs containing a higher polymer concentration was harder than the matrix containing a lower polymer concentration. The appropriate amount of EC was 15% w/w, whereas that of

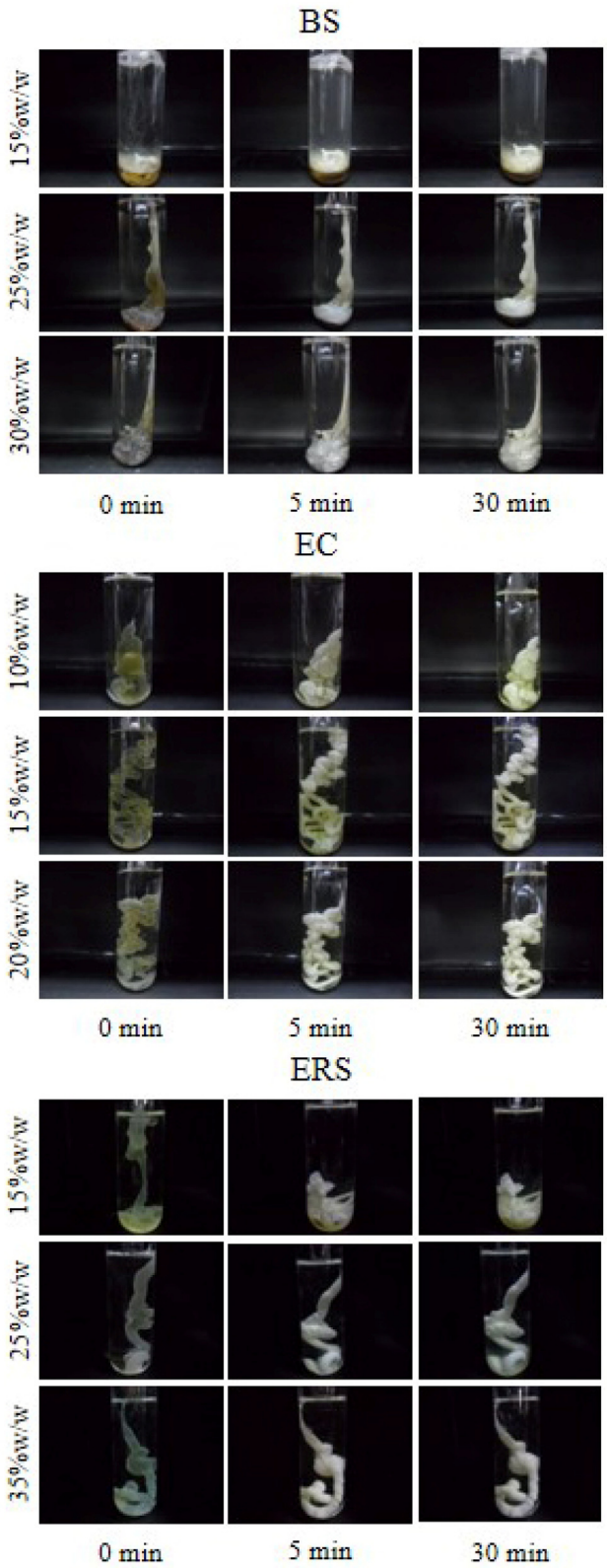

Fig. 1. Transformation of DH-loaded ISG into gel or matrix-like using different polymers in PBS (pH 6.8) and at various time points.

ERS was 35% w/w. The mechanism of matrix formation could be described through the solvent exchange behaviour of the ISG, in which the swollen/dispersed polymer exhibited a phase inversion and formed a solid matrix while its solvent moved out from the system (Phaechamud and Setthajindalert, 2018).

3.5. Aqueous diffusion into ISG

The movement of external environmental aqueous into ISG by solvent exchange influences the matrix formation behaviour, which in turn impacts the drug release behaviour (Phaechamud et al., 2016a,b). Therefore, the rate of aqueous diffusion into the ISG is one of the important role in formation of polymeric matrix. This study demonstrated that the diffusion rate of water into ISGs slowed down and decreased with time (data not shown) due to matrix formation upon exposure to the influx water that acted as a barrier for further diffusion of water into the matrix. The concentration of BS had no significant influence on the water diffusion rate, whereas the rate in the EC system was decreased when the polymer concentration was increased ($P < 0.05$). However, the diffusion rate in the ERS system was not associated with the polymer amount during the first 4 h, but it exhibited a relationship with the polymer content at 24 h ($P < 0.05$). When compared after the 4-h measurement, the formula containing 15% EC exhibited the highest water diffusion rate, which accounted for 0.0088 ± 0.0004 mm/min, whereas this rate of BS and ERS ISGs was not different significant. However, at 24 h, the EC ISG demonstrated the slowest rate of water diffusion, which confirmed their thick barrier from EC matrix for further diffusion of water. Furthermore, the most rigid structure observed in the *in vitro* gel formation study supported the decreased water diffusion rate of EC ISG. On the other hand, the highest water diffusion rate of the ERS system after the 24-h measurement was due to its ability of better swelling and more water permeability (Sánchez-Lafuente et al., 2002).

3.6. Drug release

The transformation from solution into matrix-like was observed in drug release test. Upon administration of the ISG, the exchange of solvent with the aqueous environment lead to precipitation of the polymer to form a matrix from which the drug was released with controlled rate. However, there may be a lag time between the injection and formation of matrix where the burst release of drug may be encountered and thus, leading to systemic toxicity (Hatefi and Amsden, 2002). It has been reported that the polymer phase inversion dynamics has significant effect on the initial burst release (Liu and Venkatraman, 2012). Therefore, the dialysis tube method was applied to detect the initial burst effect of the systems while the membrane-less method allows the direct contact of systems with the medium permitting to enter a large amount of fluid into the systems without delay. Meanwhile, the solvent exchange mechanism and precipitation of the polymer were happened and the release of the drug was observed with limited rate.

The higher polymer content promoted the more prolongation of DH release (Fig. 2A). DH release from EC and ERS ISGs was quite rapid during the initial period but slowed down in the late stage due to the increase in the polymeric matrix formation. The drug release from BS ISG was slower than that from EC ISG, whereas EC ISG prolonged the drug release longer than that of ERS ISG. The comparatively higher drug release of ERS ISG confirmed that the amount of DH retained in the ERS polymer matrix was less than that in the other two polymer matrices; however, the diffusion of hydrophilic DH through the EC matrix could be hindered by the more hydrophobic behaviour of EC. It has been reported that the rate of drug diffusion through the polymer matrix and the drug release could be altered by the drug–polymer molecular interaction (Wischke and Schwendeman, 2008; Chao et al., 2015). Upon injection, the exposure of ISG with aqueous environment caused the transformaiton of gel like matrix which act as the barrier for the drug release. Although the *in vitro* gelaiton experiments showed the evidence of insufficient gelation of 15% w/w BS, the drug release study confirmed the retardation of drug release of BS gel at this concentration. The slow release of DH from BS ISG could be due to the interaction between the positively charged DH molecules and the negatively charged carboxylic acid BS components (Phaechamud et al., 2016a,b). However, the cumulative drug release at 48 h from EC and ERS ISGs was not significantly different. These data also indicated that the rate of drug release from the ISG decreased with an increase in the amount of each polymer ($P < 0.05$). Furthermore, the results obtained from the release study using the dialysis method also revealed the same trend (Fig. 2B). The BS ISG retained the drug by adsorption between the resin and DH, as reported in a previous study (Chao et al., 2015), whereas EC and ERS formed a matrix structure surrounding the drug as depicted in other studies (Quadir et al., 2005; Diaf et al., 2012; Boza et al., 1999). In addition, the EC matrix was more complex than the ERS matrix (Fig. 3) and extended the drug release for longer time. However, the higher ERS-loaded ISG was achieved with the acceptable expulsive work and prolonged drug release

Release data of all formula were fitted to four different mathematical models (first order, Higuchi's, zero order and power law (Korsmeyer-Peppas) model) to characterize the mechanism of drug release. The high value of coefficient of determination (r^2) or model selection criteria (msc) indicated a superiority of the release profile fitting to mathematical equations (Chantadee et al., 2020b; Lwin et al., 2020). The r^2 and msc from curve fitting to first order, Higuchi's, zero order, and power law equations after the release studies using dialysis membrane method are shown in Table 2. The DH release from all EC formula using dialysis membrane method were fitted well with first order model since r^2 and msc from curve fitting were higher than Higuchi's model and zero order curve fitting. Whereas, the DH release profile of all BS using dialysis membrane method were best explained by power law model, but a close relationship was also noted with first order kinetics. The DH release from ERS (15% and 25%w/w) using dialysis membrane method were fitted well with first order model, whereas the DH release from ERS (30% and 35%w/w) were fitted well with power law model. Whereas the DH release from ERS (30% and 35%w/w) showed a close relationship were noted with first order kinetics. It has been reported that the drug release from hydrophobic polymer matrix in water provided the first order release profile (Khairuzzaman et al., 2006).

The release exponent values (n) for power law of all formulations from the release studies using dialysis membrane method are shown in Table 3. The n value obtained from power law equation of the formula (EC (5–10%w/w), BS (15–30%w/w) and ERS (15–35%w/w)) using dialysis membrane method ranged from 0.15 to 0.42. The results indicated that the formula showed drug release by Fickian diffusion mechanism. The drug release rate was decreased as a function of time due to a decrease in the concentration gradient. The drug release from system containing EC (15–20% w/w) were anomalous or non-Fickian diffusion ($0.45 < n < 0.89$) indicated that the drug release was controlled by both mechanism of diffusion and polymeric chain relaxation (Pahwa et al., 2011). The drug release rate (k) parameter after releasing studies using dialysis membrane method was investigated. The increased polymer amounts significantly decreased the drug release rate (k) ($p < 0.05$). Since the viscosity of the higher polymer amount formula is higher than that of lower polymer amount formula. The results showed the slower drug release therefore the polymer amount could sustain DH release from these systems (Table 3).

The r^2 and msc from curve fitting to first order, Higuchi's, zero order, and power law equations after the releasing studies using membrane-less method are shown in Table 4. The DH released from EC (5–15%w/w), BS (15–25%w/w) and ERS (15–30%w/w) formula after release studies using membrane-less method were fitted well with first order model. Whereas the DH released from EC (20%w/w), BS (30%w/w) and ERS (35%w/w) formula were best

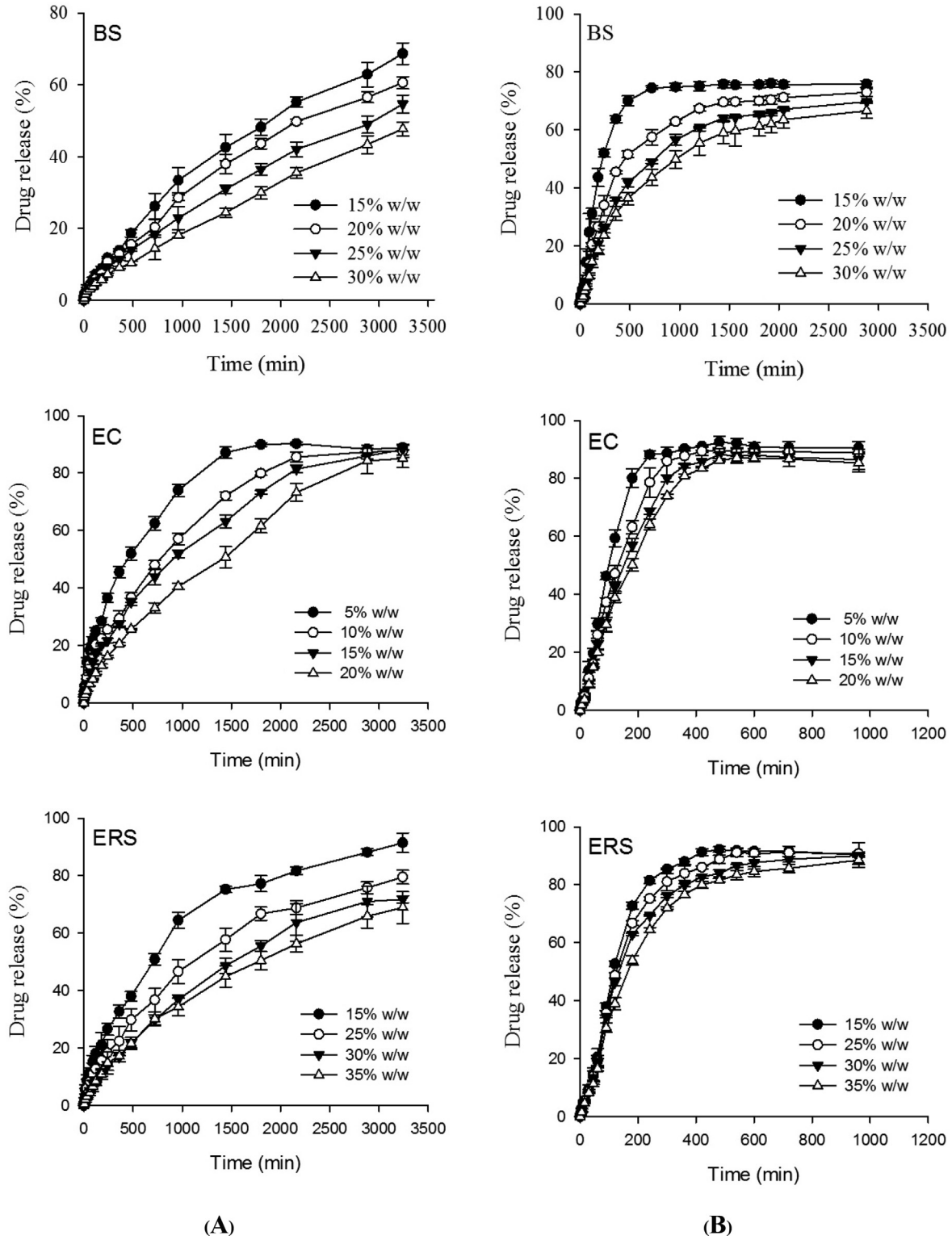

Fig. 2. DH release from DH-loaded ISG assessed using membrane-less method (A) and dialysis tube method (B) in PBS (pH 6.8) (n = 3).

explained by power law model, but a close relationship was also noted with first order kinetics. The release exponent values (n) for power law of all formula from the release studies using membrane-less method are shown in Table 5. The n value obtained from power law equation of all formula (EC (5–20%w/w), BS (15–30%w/w) and ERS (25–35%w/w)) after release studies using membrane-less method ranged were anomalous (non-Fickian) diffusion controlled release ($0.45 < n < 0.89$) except ERS (15%w/w) that showed drug release by Fickian diffusion. Considering the drug release rate (k) parameter after release studies using membrane-less method, it was indicated that the increased polymer amounts tended to decrease the drug release rate (k) ($p > 0.05$) that similar to the previous release studies using dialysis membrane method. The factors affecting release kinetic are liquid

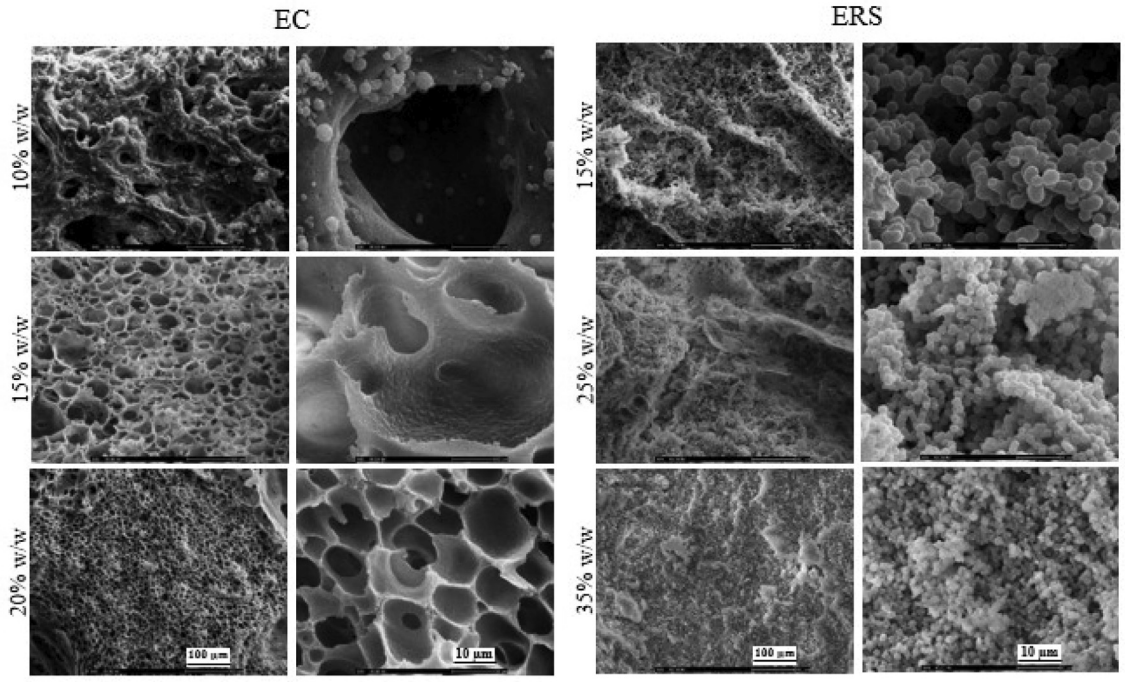

Fig. 3. SEM micrograph of the dried ISG systems; 5%w/w DH-loaded EC ISG (left) and 5%w/w DH-loaded ERS ISG (right) with different concentrations of matrix former at different magnifications (200 × and 2000 ×).

Table 2
Comparison of degree of goodness-of-fit from curve fitting of the release profiles of DH in phosphate buffer pH 6.8 using dialysis membrane method to different release models.

Formula (%w/w)	First order		Higuchi's		Zero order		Power law	
	r^2	msc	r^2	msc	r^2	msc	r^2	msc
EC								
5%	0.9926	4.41	0.9621	2.70	0.9545	2.52	0.9774	2.93
10%	0.9963	5.17	0.9845	3.60	0.9398	2.37	0.9905	3.91
15%	0.9968	5.40	0.9796	3.49	0.9653	2.91	0.9826	3.45
20%	0.9980	5.86	0.9900	4.21	0.9780	3.37	0.9900	4.01
BS								
15%	0.9552	2.62	0.8798	1.67	0.9245	2.01	0.9953	4.51
20%	0.9261	2.27	0.8563	1.69	0.8705	1.60	0.9596	2.61
25%	0.9179	2.25	0.9277	2.23	0.9235	2.17	0.9727	3.23
30%	0.9244	2.32	0.9745	3.27	0.9289	2.28	0.9861	3.90
ERS								
15%	0.9766	3.49	0.9477	2.38	0.9265	2.11	0.9712	2.80
25%	0.9787	3.58	0.9429	2.36	0.9365	2.26	0.9734	2.96
30%	0.9710	3.27	0.9544	2.59	0.8798	1.67	0.9755	3.11
35%	0.9760	3.46	0.9738	3.20	0.9311	2.27	0.9864	3.70

Table 3
Estimate parameter from curve fitting of DH release in phosphate buffer pH 6.8 using dialysis membrane method to power law expression.

Formula (%w/w)	k ± S.D.	tl ± S.D. (min)	n ± S.D.	Release mechanism
EC				
5%	0.1015 ± 0.0083	40.47 ± 1.85	0.40 ± 0.02	Fickian
10%	0.0784 ± 0.0101	40.35 ± 0.71	0.42 ± 0.02	Fickian
15%	0.0499 ± 0.0115	36.37 ± 2.27	0.49 ± 0.04	Anomalous
20%	0.0448 ± 0.0059	35.86 ± 1.18	0.49 ± 0.02	Anomalous
BS				
15%	0.2821 ± 0.0345	135.58 ± 33.40	0.15 ± 0.02	Fickian
20%	0.1131 ± 0.0148	110.92 ± 3.34	0.25 ± 0.02	Fickian
25%	0.0700 ± 0.0045	108.79 ± 0.61	0.30 ± 0.01	Fickian
30%	0.0507 ± 0.0074	103.05 ± 5.53	0.34 ± 0.01	Fickian
ERS				
15%	0.0774 ± 0.0037	41.86 ± 1.15	0.43 ± 0.01	Fickian
25%	0.0568 ± 0.0080	57.37 ± 1.11	0.32 ± 0.00	Fickian
30%	0.0209 ± 0.0032	59.14 ± 0.37	0.24 ± 0.00	Fickian
35%	0.0101 ± 0.0017	54.51 ± 1.22	0.38 ± 0.01	Fickian

k = release rate; tl = lag time and n = diffusional exponent

Table 4
Comparison of degree of goodness-of-fit from curve fitting of the release profiles of DH in phosphate buffer pH 6.8 using membrane-less method to different release models.

Formula (%w/w)	First order		Higuchi's		Zero order		Power law	
	r^2	msc	r^2	msc	r^2	msc	r^2	msc
EC								
5%	0.9937	4.77	0.9874	3.98	0.9315	2.35	0.9927	4.33
10%	0.9956	5.16	0.9857	3.97	0.9673	3.11	0.9876	3.89
15%	0.9966	5.41	0.9933	4.71	0.9480	2.65	0.9943	4.74
20%	0.9925	4.59	0.9828	3.73	0.9740	3.34	0.9951	4.87
BS								
15%	0.9971	5.49	0.9932	4.59	0.9698	3.14	0.9970	5.20
20%	0.9956	5.05	0.9851	3.81	0.9711	3.18	0.9943	4.63
25%	0.9989	6.45	0.9809	3.56	0.9899	4.19	0.9985	5.89
30%	0.9984	6.03	0.9591	2.80	0.9970	5.42	0.9990	6.29
ERS								
15%	0.9931	4.69	0.9718	3.28	0.9398	2.45	0.9814	3.55
25%	0.9938	4.77	0.9894	4.24	0.9291	2.34	0.9895	4.09
30%	0.9965	5.32	0.9865	3.97	0.9548	2.76	0.9915	4.27
35%	0.9965	5.33	0.9954	5.06	0.9655	3.03	0.9983	5.86

Table 5
Estimate parameter from curve fitting of DH release in phosphate buffer pH 6.8 using membrane-less method to power law expression.

Formula (%w/w)	k ± sd	tl ± sd (min)	n ± sd	Release mechanism
EC				
5%	0.0253 ± 0.0017	8.06 ± 7.41	0.49 ± 0.01	Anomalous
10%	0.0152 ± 0.0034	−52.41 ± 17.84	0.62 ± 0.03	Anomalous
15%	0.0093 ± 0.0014	−11.49 ± 13.20	0.58 ± 0.02	Anomalous
20%	0.0053 ± 0.0013	18.02 ± 21.74	0.64 ± 0.03	Anomalous
BS				
15%	0.0062 ± 0.0028	100.76 ± 9.84	0.59 ± 0.06	Anomalous
20%	0.0043 ± 0.0010	87.14 ± 16.44	0.62 ± 0.03	Anomalous
25%	0.0019 ± 0.0007	57.32 ± 9.29	0.70 ± 0.04	Anomalous
30%	0.0007 ± 0.0002	29.38 ± 6.37	0.82 ± 0.07	Anomalous
ERS				
15%	0.0321 ± 0.0039	47.01 ± 5.30	0.42 ± 0.01	Fickian
25%	0.0181 ± 0.0063	62.65 ± 3.14	0.48 ± 0.05	Anomalous
30%	0.0096 ± 0.0037	87.61 ± 1.77	0.55 ± 0.06	Anomalous
35%	0.0084 ± 0.0021	100.98 ± 11.66	0.55 ± 0.03	Anomalous

k = release rate; tl = lag time and n = diffusional exponent

diffusion rate and polymeric chain relaxation rate. When the liquid diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian, whereas when the relaxation process is very slow compared with the diffusion, the case II transport occurs. When liquid diffusion rate and polymer relaxation rate are of the same order of magnitude, anomalous or non-Fickian diffusion is observed (Perioli et al., 2004). Solute transport from non-degradable polymeric systems is mainly considered as diffusion driven. Non-degradable polymers can be fabricated into "reservoir-" and "matrix-" type devices, which can be a rate-controlling membrane (Ful et al., 2010). For matrix-type devices, drug release is more likely to be Fickian diffusion driven, which is associated with concentration gradient, diffusion distance, and the degree of swelling (Chantadee et al., 2020c; Siepmann & Siepmann, 2008).

3.7. ISG topography

Typically, the study of morphology of samples by SEM proves the porosity of pore structure, surface structure, and drug crystalline. The release of drug from the matrix was influenced by the drug diffusion and dissolution of the polymeric network (Poursamar et al., 2011) which in turn attributed to the changes in the morphology of the ISG system. Thus, the porosity and pore connectivity revealed by SEM photomicrograph prove the behavior of drug diffusion and dissolution of the polymeric network. The surface morphology of dried DH-loaded EC ISG showed a porous scaffold, whereas the structure of the DH-loaded ERS ISG demonstrated agglomerated spherical particles (Fig. 3). The pore sizes of the DH-loaded EC ISG were minimised with an increase in the polymer concentration, and the particle size of spherical particles in the DH-loaded ERS ISG was decreased with an increase in the ERS amount (Lwin et al., 2020) The ISG system with low polymer concentration may give the lose structure of the polymeric matrix from which the diffusion of drug and dissolution of polymer were taken easily resulting in formation of larger pore size. However, the higher amount of polymer enhanced the denser matrix structure which contributed to the higher tortuosity for NMP and drug molecules to diffuse out indicating the decreased in pores size. On the other hand, the decreased in the size of void inside the structure proved the controlled in drug release which finding are aligned with the drug release study where the increased in polymer concentration promoted the prolongation in drug release (Fig. 2). Otherwise, the DH-loaded BS ISG could not form a dry structure for analysing using SEM after freeze drying, because the amount of NMP in the system after the release study was still high. However, this study suggested that the mechanism of drug release from the EC and ERS ISG systems involved the diffusion of both the drug and NMP. The pores were formed throughout the matrix, indicating that they were formed by drug and solvent diffusion and matrix erosion. In addition, the pore connectivity suggested that the release of DH from the ISG by solvent movement and the

scaffolds were promoted concurrently by the polymeric matrix formation during the phase inversion process (Phaechamud et al., 2016a,b; Marco et al., 2014).

3.8. In vitro degradation

The percentage of weight loss of the DH-loaded EC, BS and ERS ISGs was significantly decreased with an increase in the amount of the polymer (Table 6). The increased in polymer concentration attributed to the denser in matrix formation (Fig. 1) indicating the harder diffusion out of solvent and drug which is the evidence of the lowering in *in vitro* degradation.

Comparison between the polymers at the same concentration (15% w/w) showed that the type of the polymer had an apparent impact on the degradation of the ISG, wherein the order of *in vitro* degradability was as follows: EC > ERS > BS ISGs, respectively ($P < 0.05$). This *in vitro* degradation occurred due to the migration of DH and NMP during medium exchange, and it has also been believed that BS was degraded by de-esterification (Coelho et al., 2012), whereas EC was destabilised by oxidative degradation (McBurney, 1949).

EC formed as a major backbone of timolol maleate-loaded implantable nanoparticle-laden ring-in-hydrogel contact lenses well tolerated displaying insignificant cytotoxicity and ocular irritation (Maulvi et al., 2016). The nanocapsules of beclomethasone prepared for pulmonary delivery using EC as a protective polymer showed the insignificant cytotoxic effect (Chassot et al., 2015). Shellac was compared to another fluoride varnish (Duraphat) and a fluoride containing desensitizing agent (Isodan) with the cytotoxicity test performed on human gingival fibroblasts and through dentin slice on human pulp fibroblasts (Hoang-Dao et al., 2008). Shellac showed an adequate cellular compatibility and a significant effect on human dentin hydraulic conductance. Shellac showed an adequate cellular compatibility with human gingival fibroblasts and a significant influence on human on human dentin hydraulic conductance indicating its safety and effectiveness as potential desensitizing agent (Hoang-Dao et al., 2008). Moreover, shellac is nontoxic, physiologically harmless and biodegradable resin (Irimia-Vladu et al., 2013). Thus, this designed depot system has the potential use for delivering the antimicrobial drug with biodegradable characteristic. Nanoparticles fabricated using ERS as drug carrier licensed for clinical application by the major health authorties of Europe, Japan and USA (Hoffart et al., 2006). Low molecular weight heparin-loaded ERS nanoparticles was proposed as a good delivery system with low cell toxicity (Eidi et al., 2010). Moreover, this positively charged polymer has been used for developing nano- and micro-fibers as scaffolds in tissue engineering (Vaquettea et al., 2008) indicating its safety for use as polymeric material in periodontitis treatment.

Table 6
Percentage of weight loss of DH-loaded ISGs (n = 3).

Polymer	% polymer (w/w)	% weight loss
BS	15	89.14 ± 0.07*
	20	82.59 ± 1.04
	25	72.00 ± 3.55
	30	64.62 ± 5.79
EC	5	99.72 ± 0.31
	10	89.12 ± 0.31
	15	84.95 ± 0.20*
	20	79.47 ± 0.54
ERS	15	86.52 ± 0.49*
	25	75.00 ± 0.25
	30	69.46 ± 0.28
	35	61.17 ± 2.92

The asterisk (*) represents a significant difference ($P < 0.05$)

3.9. Antimicrobial activity

The prepared DH-loaded ISGs comprising 15%w/w polymer demonstrated antimicrobial activities against *S. aureus*, *S mutans*, *E. coli*, *P. gingivalis* and *C. albicans* (Fig. 4). DH has been shown to inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit (Dadashi et al., 2019), while NMP enhances cell permeability (Saw et al., 2007). The type of polymer influenced the antimicrobial activities of DH ISG. Comparison with 15% w/w polymer concentration showed that the DH-loaded ERS ISG demonstrated the highest activity against *S. aureus* (not different from the positive control) and *E.coli.* Otherwise, the DH-loaded EC ISG exhibited the highest activity against *S. mutans* (but not different from the positive control) and *P. gingivalis*, which is the primary pathogen causing periodontitis (Aminu et al, 2013; 2019). In addition, both the ERS and EC ISG systems exhibited the same highest activity (also higher than the positive control) against *C. albicans*, which is the common fungal pathogen infecting the oral cavity (Grimoud et al., 2003). An apparently high antifungal activity of NMP against *C. albicans* has also been reported earlier (Phaechamud et al., 2013); thus, these three ISGs also exhibited this fungal inhibition. However, the DH-loaded BS ISG also

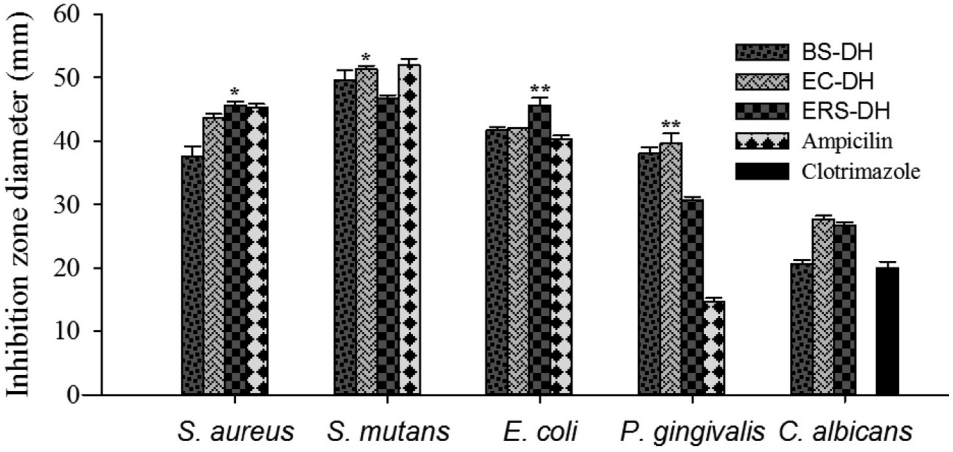

Fig. 4. Antimicrobial activity of 15% w/w DH-loaded ISG (n = 3). Ampicillin (10 μg) and clotrimazole (10 μg) were used as positive control for assessing the antibacterial and antifungal activities, respectively. The asterisk * indicates the significantly larger inhibition zone diameter than that obtained using other ISGs, but not ampicillin, while ** shows the significantly larger inhibition zone diameter than that obtained using other ISGs and also ampicillin ($P < 0.05$).

Table 7
Effect of polymer concentration on the antibacterial activity against *S. mutans* and *P. gingivalis* (n = 3).

Polymer	% polymer (w/w)	*S. aureus*	*S. mutans*	*E. coli*	*P. gingivalis*	*C. albicans*
BS	15	48.22 ± 0.44	51.48 ± 0.82	42.91 ± 0.52	38.82 ± 0.92	22.72 ± 0.81
	20	45.30 ± 0.36	43.30 ± 0.52	36.23 ± 0.51	25.72 ± 1.10	12.98 ± 0.48
	25	44.82 ± 0.52	40.21 ± 0.22	35.28 ± 0.31	26.22 ± 0.82	11.62 ± 0.39
	30	43.71 ± 0.61	41.82 ± 0.36	32.71 ± 0.44	25.31 ± 0.13	10.34 ± 0.22
EC	5	44.82 ± 0.42	52.33 ± 0.52	41.38 ± 0.23	41.00 ± 1.20	28.62 ± 0.42
	10	44.39 ± 0.23	51.33 ± 0.48	41.33 ± 0.32	41.00 ± 1.03	28.17 ± 0.33
	15	42.29 ± 0.22	51.33 ± 0.35	42.06 ± 0.04	39.67 ± 1.44	27.36 ± 0.48
	20	43.27 ± 0.17	50.33 ± 0.54	41.42 ± 0.37	38.33 ± 2.00	27.08 ± 0.14
ERS	15	44.87 ± 1.03	46.67 ± 0.18[a]	45.88 ± 0.52	30.67 ± 0.58[b]	27.91 ± 0.82
	25	42.28 ± 0.76	43.67 ± 0.41	43.22 ± 0.26	28.67 ± 0.36	24.82 ± 0.36
	30	42.01 ± 0.62	42.67 ± 0.27	42.87 ± 0.32	27.33 ± 0.39	22.02 ± 0.62
	35	41.99 ± 0.48	41.67 ± 0.34[a]	41.92 ± 0.27	25.33 ± 0.22[b]	20.01 ± 0.31
Ampicillin disc 10 μg		50.22 ± 0.88	52.00 ± 1.00	43.24 ± 0.62	14.67 ± 0.07	10.08 ± 0.11
NMP		32.65 ± 0.23	28.00 ± 2.65	25.43 ± 0.43	17.00 ± 1.00	45.82 ± 1.06

([a,b]) represents a significant difference ($P < 0.05$)

exhibited activity against all the five tested microorganisms, although it was not at the highest level. Furthermore, the higher concentration of the BS, EC and ERS ISG systems appeared to slightly decrease the antimicrobial activity against *S. mutans* and *P. gingivalis* (Table 7). This result was due to the retardation of DH release through the polymer matrices and thereafter into the agar. Because of the apparent prolongation of DH diffusion through the denser ERS matrix, the diameter of the inhibition zone obtained using DH-loaded 35% ERS ISG against these two microbes was significantly smaller compared to that obtained using DH-loaded 15% ERS ISG ($P < 0.05$). The increased EC amount in the systems did not affect the antimicrobial activity against all microbes ($p > 0.05$).

4. Conclusion

The ISG system, when exposure to the surrounding liquid, the solvent exchange mechanism between influx and efflux of solvent and the water leads to the polymer phase separation and polymer matrix formation from which the drug is released with controlled rate. Thus, to achieve the controllable manner of drug release from ISG system, the formation of matrix is critical factor whereas polymers have been interested as matrix formers. In addition, the ease of injection is also an critical point in order to get the patient compliance. The present study investigated the three different polymers namely BS, EC and ERS. When compared, the ISG system showed the Newtonian flow behaviour except 25% EC ISG. The increased in concentration of polymers attributed to increase in viscosity whereas the highest viscosity of EC solution imparted the lowest injectability while ERS ISG gave the highest injectability. But, owing to the applied force lower than 50 N.mm, all the prepared ISG indicates their acceptability for injection, except for 30% w/w BS solution. Through the solvent exchange mechanism, the increased in concentration of polymer contributed to higer matirx formaiton whcih sustained the drug release. The system contatining BS showed the lowest amount in drug release when compared with other ISGs. The SEM photography also reveals the drug diffusion and dissolution of polymeric network druing the drug release. By comparison, all the prepared ISGs showed equally or more effective against tested microbes than the positive control. Thus, the low viscosity with the high injectability of the DH-loaded ERS ISG confirmed that it can be suitably used as an injectable dosage form accompanied by its interesting antimicrobial activities for treating periodontitis.

Statement of human and animal rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aminu, N., Baboota, S., Pramod, K., Singh, M., Dang, S., Ansari, S.H., Sahni J-K., Ali, J., 2013. Development and evaluation of triclosan loaded poly-ε-caprolactone nanoparticulate system for the treatment of periodontal infections. J Nanopart. Res. 15, Article 2075.

Aminu, N., Chan, S.-Y., Yam, M.F., Toh, S.-M., 2019. A dual-action chitosan-based nanogel system of triclosan and flurbiprofen for localised treatment of periodontitis. Int. J. Pharm. 570,. https://doi.org/10.1016/j.ijpharm.2019.118659 118659.

Aminu, N., Chan, S.-Y., Toh, S.-M., 2017. Roles of nanotechnological approaches in periodontal disease therapy. J. Appl. Pharm. Sci. 7, 234–242. https://doi.org/10.7324/JAPS.2017.70735.

Anneken, C., Treinen, J., Willershausen, B., 2001. Patients compliance in periodontal therapy: a retrospective investigation on the basis of a clinical group. Eur. J. Med. Res. 6, 75–82.

Azoury, R., Elkayam, R., Friedman, M., 1988. Nuclear magnetic resonance study of an ethyl cellulose sustained-release delivery system II: release rate behavior of tetracycline. J. Pharm. Sci. 77, 428–431.

Boza, A., Caraballo, I., Alvarez-Fuentes, J., Rabasco, A.M., 1999. Evaluation of Eudragit RS-PO and Ethocel 100 matrices for the controlled release of lobenzarit disodium. Drug. Dev. Ind. Pharm 25, 229–233.

Buch, K., Penning, M., Wachtersbach, E., Maskos, M., Langguth, P., 2009. Investigation of various shellac grades: additional analysis for identity. Drug Dev. Indus. Pharm. 35 (6), 694–703.

Camargo, J.A., Sapin, A., Nouvel, C., Daloz, D., Leonard, M., Bonneaux, F., Six, J.L., Maincent, P., 2013. Injectable PLA-based *in situ* forming implants for controlled release of ivermectin a BCS Class II drug: solvent selection based on physicochemical characterization. Drug Dev. Ind. Pharm 39, 146–155.

Chang, J.Y., Oh, Y.K., Kong, H.S., Kim, E.J., Jang, D.D., 2002. Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gel on vaginitis. J. Control Release. 82, 39–50.

Chantadee, T., Sawangsri, P., Santimaleeworagun, W., Phaechamud, T., 2020a. Vancomycin hydrochloride-loaded stearic acid/lauric acid in situ forming matrix for antimicrobial inhibition in patients with joint infection after total knee arthroplasty. Mater Sci Eng. C. 115,. https://doi.org/10.1016/j.msec.2020.110761 110761.

Chantadee, T., Santimaleeworagun, W., Phorom, Y., Chuenbarn, T., Phaechamud, T., 2020b. Vancomycin HCl-loaded lauric acid in situ-forming gel with phase

inversion for periodontal pocket delivery. J. Drug Del Sci Tech. 57,. https://doi.org/10.1016/j.jddst.2020.101615 101615.
Chantadee, T., Santimaleeworagun, W., Phorom, Y., Chuenbarn, T., Phaechamud, T., 2020c. Saturated fatty acid-based in situ forming matrices for localized antimicrobial delivery. Pharmaceutics 12(9), article 808, https://doi.org/10.3390/pharmaceutics12090808
Chao, Y., Zhu, W., Ye, Z., Wu, P., Wei, N., Wu, X., Li, H., 2015. Preparation of metal ions impregnated polystyrene resins for adsorption of antibiotics contaminants in aquatic environment. J. Appl. Polym. Sci. 132, 1–9.
Chassot, J.M., Ribas, D., Silveira, E.F., Grünspan, L.D., Pires, C.C., Farago, P.V., Braganhol, E., Tasso, L., Cruz, L., 2015. Beclomethasone dipropionate-loaded polymeric nanocapsules: Development, in vitro cytotoxicity, and in vivo evaluation of acute lung injury. J. Nanosci. Nanotechnol. 15, 855–864.
Chaudhary, B., Verma, S., 2014. Preparation and evaluation of novel in situ gels containing acyclovir for the treatment of oral Herpes Simplex virus infection. Sci. World J. 280928. https://doi.org/10.1155/2014/280928.
Coelho, C., Nanabala, R., Ménager, M., Commereuc, S., Verney, V., 2012. Molecular changes during natural biopolymer ageing – the case of shellac. Polym. Degrad. Stabil. 97, 936–940.
Convention USP. USP 32 NF 27: United States Pharmacopeia [and] National Formulary: United States Pharmacopeial Convention; 2009.
Dadashi, S., Boddohi, S., Soleimani, N., 2019. Preparation, characterization, and antibacterial effect of doxycycline loaded kefiran nanofibers. J. Drug Deliv. Sci. Tech. 52, 979–985.
Diaf, K., Bahri, Z.E., Chafi, N., Belarbi, L., Mesli, A., 2012. Ethylcellulose, polycaprolactone, and eudragit matrices for controlled release of piroxicam from tablets and microspheres. Chemical Papers. 66, 779–786.
Do, M.P., Neut, C., Metz, H., Mäder, K., Siepmann, J., Siepmann, F., 2015. In-situ forming composite implants for periodontitis treatment: How the formulation determines system performance. Int. J. Pharma. 486, 38–51.
Eidi, H., Joubert, O., Attik, G., Duval, R.E., Bottin, M.C., Hamouia, A., Maincent, P., Rihn, B.H., 2010. Cytotoxicity assessment of heparin nanoparticles in NR8383 macrophages. Int. J. Pharm. 396, 156–165. https://doi.org/10.1016/j.ijpharm.2010.06.006.
Fu1, Y., Kao, W.J., 2010. Drug release kinetics and transport mechanisms of nondegradable and degradable polymeric delivery systems. Expert Opin. Drug Deliv. 7, 429–444.
Garala, K., Joshi, P., Shah, M., Ramkishan, A., Patel, J., 2013. Formulation and evaluation of periodontal in situ gel. Int. J. Pharm. Invest. 3, 29–41.
Grimoud, A.M., Marty, N.P., Bocquet, H., Andrieu, S., Lodter, J.P., Chabanon, G., 2003. Colonization of the oral cavity by Candida species: risk factors in long-term geriatric care. J. Oral Sci. 45, 51–55. https://doi.org/10.2334/josnusd.45.51.
Gokulanathan, S., Balan, N., Aravind, R.J., Thangavelu, K., 2014. Patient compliance and supportive periodontal therapy: study among young adults of Namakkal district. J. Pharm. Bioallied Sci. 6, S171-3. https://doi.org/ 10.4103/0975-7406.137443.
Harish, N.M., Prabhu, P., Charyulu, R.N., Gulzar, M.A., Subrahmanyam, E.V., 2009. Formulation and evaluation of *in situ* gels containing clotrimazole for oral candidiasis. Indian J. Pharm. Sci. 71, 421–427.
Hatefi, A., Amsden, B., 2002. Biodegradable injectable in situ forming drug delivery systems. J. Control Release. 80, 9–28.
Hoang-Dao, B.T., Hoang-Tu, H., Tran-Hung, L., Camps, J., Koubi, G., About, I., 2008. Evaluation of a natural resin-based new material (Shellac F) as a potential desensitizing agent. Dent Mater. 24 (7), 1001–1007. https://doi.org/10.1016/j.dental.2007.11.014.
Hoffart, V., Lamprecht, A., Maincent, P., Lecompte, T., Vigneron, C., Ubrich, N., 2006. Oral bioavailability of a low molecular weight heparin using a polymeric delivery system. J. Control. Rel. 113, 38–42.
Irimia-Vladu, M., Głowacki, E.D., Schwabegger, G., Leonat, L., Akpinar, H.Z., Sitter, H., Bauer, S., Sariciftci, N.S., 2013. Natural resin shellac as a substrate and a dielectric layer for organic field-effect transistors. Green Chem. 15, 1473–1476.
Javali, M.A., Vandana, K.L., 2012. A comparative evaluation of atrigel delivery system (10% doxycycline hyclate) Atridox with scaling and root planing and combination therapy in treatment of periodontitis: A clinical study. J. Indian Soc. Periodontol. 16, 43–48.
Jouyban, A., Fakhree, M.A.A., Shayanfar, A., 2010. Review of pharmaceutical applications of N-methyl-2-pyrrolidone. J. Pharm. Pharm. Sci. 13, 524–535.
Kelly, H.M., Deasy, P.B., Ziaka, E., Claffey, N., 2004. Formulation and preliminary in vivo dog studies of a novel drug delivery system for the treatment of periodontitis. Int. J. Pharm. 274, 67–83.
Khairuzzaman, A., Ahmed, S.U., Savva, M., Patel, N.K., 2006. Zero-order release of aspirin, theophylline and atenolol in water from novel methylcellulose glutarate matrix tablets. Int. J. Pharm. 318, 15–21.
Larsen, T., 2002. Susceptibility of *Porphyromonas gingival*is in biofilms to amoxicillin, doxycycline and metronidazole. Oral Microbiol. Immunol. 17, 267–271.
Liu, H., Venkatraman, S.S., 2012. Cosolvent effects on the drug release and depot swelling in injectable in situ depot-forming systems. J. Pharm. Sci. 101 (5), 1783–1793.
Lwin, W.W., Puyathorn, N., Senarat, S., Mahadlek, J., Phaechamud, T., 2020. Emerging role of polyethylene glycol on doxycycline hyclate-incorporated Eudragit RS in situ forming gel for periodontitis treatment. J. Pharm. Invest. 50 (1), 81–94. https://doi.org/10.1007/s40005-019-00430-6.
Marco, I.D., Baldino, L., Cardea, S., Reverchon, E., 2014. Production of ethyl cellulose scaffolds by supercritical CO_2 phase separation. Chem. Eng. Trans. 38, 265–270.
Martin, A., 1993. Physical pharmacy, fourth ed. Philadelphia.
Mathur, V., Nagpal, K., Singh, S.K., Mishra, D.N., 2013. Comparative release profile of sustained release matrix tablets of verapamil HCl. Int. J. Pharm. Investi. 3, 60–65.
Maulvi, F.A., Lakdawala, D.H., Shaikh, A.A., Desai, A.R., Choksi, H.H., Vaidya, R.J., Ranch, K.M., Koli, A.R., Vyas, B.A., Shah, D.O., 2016. In vitro and in vivo evaluation of novel implantation technology in hydrogel contact lenses for controlled drug delivery. J. Control. Release. 226, 47–56.
McBurney, L.F., 1949. Oxidative stability of cellulose derivatives - heat stability of ethylcellulose. Ind. Eng. Chem 41, 1251–1256.
Pahwa, R., Piplani, M., Garg, V.K., Rao, R., Lamba, H.S., 2011. Formulation and evaluation of orally disintegrating tablets: comparison of natural and synthetic superdisintegrants. Der Pharmacia Lettre 3, 407–418.
Parent, M., Nouvel, C., Koerber, M., Sapin, A., Maincent, P., Boudier, A., 2013. PLGA in situ implants formed by phase inversion: critical physicochemical parameters to modulate drug release. J Control Release 172, 292–304.
Papich, M.G., 2016. Saunders Handbook of Veterinary Drugs. Fourth ed., 274–277
Perioli, L., Ambrogi, V., Rubini, D., Giovagnoli, S., Ricci, M., et al., 2004. Novel mucoadhesive buccal formulation containing metronidazole for the treatment of periodontal disease. J. Control. Release 95, 521–533.
Phaechamud, T., Jantadee, T., Mahadlek, J., Charoensuksai, P., Pichayakorn, W., 2016a. Characterization of antimicrobial agent loaded Eudragit RS solvent exchange-induced in situ forming gels for periodontitis treatment. AAPS PharmSciTech 18, 494–508. https://doi.org/10.1208/s12249-016-0534-y.
Phaechamud, T., Lertsuphotvanit, N., Praphanwittaya, P., 2018. Viscoelastic and thermal properties of doxycycline hyclate-loaded bleached shellac *in situ* forming -gel and -microparticle. J Drug Del Sci Tech 44, 448–456. https://doi.org/10.1016/j.jddst.2018.01.021.
Phaechamud, T., Mahadlek, J., Charoenteeraboon, J., Choopun, S., 2013. Characterization and antimicrobial activity of *N*-methyl-2-pyrrolidone-loaded ethylene oxide-propylene oxide block copolymer thermosensitive gel. Ind. J. Pharm. Sci. 74, 498–504.
Phaechamud, T., Mahadlek, J., Chuenbarn, T., 2016b. *In situ* forming gel comprising bleached shellac loadedwith antimicrobial drugs for periodontitis treatment. Mater. Des. 89, 294–303.
Phaechamud, T., Setthajindalert, O., 2018. Antimicrobial in-situ forming gels based on bleached shellac and different solvents. J. Drug Deliv. Sci. Tech. 46, 285–293.
Philippot, P., Lenoir, N., D'Hoore, W., Bercy, P., 2005. Improving patients'compliance with the treatment of periodontitis: a controlled study of behavioural intervention. J. Clin. Periodontol. 32, 653–658.
Porfiryeva, N.N., Nasibullin, S.F., Abdullina, S.G., Tukhbatullina, I.K., Khutoryanskiy, V.V., 2019. Acrylated Eudragit® E PO as a novel polymeric excipient with enhanced mucoadhesive properties for application in nasal drug delivery. Int. J. Pharm. 562, 241–248.
Poursamar, S.A., Azami, M., Mozafari, M., 2011. Controllable synthesis and characterization of porous polyvinyl alcohol/hydroxyapatite nanocomposite scaffolds via an in situ colloidal technique. Colloid Surface B. 84 (2), 310–316.
Quadir, M.A., Chanda, E., Haider, S.S., Reza, M.D., Datta, B.K., 2005. Evaluation of ethylcellulose as matrices for controlled release drug delivery. Pak. J. Pharm. Sci 18, 29–34.
Rowe, R.C., Sheskey, P.J., Quinn, M.E., 2009. Handbook of pharmaceutical excipients, sixth ed. London.
Rył, A., Owczarz, P., 2020. Injectability of thermosensitive, low-concentrated chitosan colloids as flow phenomenon through the capillary under high shear rate conditions. Polymers 12, 2260. https://doi.org/10.3390/polym12102260.
Sánchez-Lafuente, C., Faucci, M.T., Fernández-Arévalo, M., ÁlvarezFuentes, J., Rabasco, A.M., Mura, P., 2002. Development of sustained release matrix tablets of didanosine containing methacrylic and ethylcellulose polymers. Int. J. Pharm. 234, 213–221.
Sanghvi, R., Narazaki, R., Machatha, S.G., Yalkowsky, S.H., 2008. Solubility improvement of drugs using N-methyl pyrrolidone. AAPS PharmSciTech. 9, 366–376.
Saw, C.L., Olivo, M., Wohland, T., Fu, C.Y., Kho, K.W., Soo, K.C., Sia Heng, P.W., 2007. Effects of *N*-methyl pyrrolidone on the uptake of hypericin in human bladder carcinoma and co-staining with DAPI investigated by confocal microscopy. Technol Cancer Res. Treat. 6, 383–394.
Senarat, S., Charoenteeraboon, J., Praphanwittaya, P., Phaechamud, T., 2020. Phase behavior of doxycycline hyclate-incorporated bleached shellac *in-situ* forming gel/microparticle after solvent movement. Key Eng Mater. 859, 21–26.
Siepmann, J., Siepmann, F., 2008. Mathematical modeling of drug delivery. Int. J. Pharm. 364, 328–343.
Srichan, T., Phaechamud, T., 2017. Designing solvent exchange-induced in situ forming gel from aqueous insoluble polymers as matrix base for periodonttitis treatment. AAPS PharmSciTech. 18 (1), 194–201. https://doi.org/10.1208/s12249-016-0507-1.
Sweetman, S.C., 2009. Martindale: The Complete Drug Reference, Thirty sixth ed. London.
Van Dyke, T.E., Sima, C., 2020. Understanding resolution of inflammation in periodontal diseases: Is chronic inflammatory periodontitis a failure to resolve?. Periodontol. 82, 205–213. https://doi.org/10.1111/prd.12317.
Vaquettea, C., Babak, V.G., Baros, F., Boulanouar, O., Dumas, D., Fievet, P., Kildeeva, N. R., Maincent, P., Wang, X., 2008. Zeta-potential and morphology of electrospun nano- and microfibers from biopolymers and their blends used as scaffolds in tissue engineering. Mendeleev Commun. 18, 38–41.

Watt, R.P., Khatri, H., Dibble, A.R., 2019. Injectability as a function of viscosity and dosing materials for subcutaneous administration. Int. J. Pharm. 554, 376–386.
Wischke, C., Schwendeman, S.P., 2008. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. Int. J. Pharm 364, 298–327.
Xiong, W., Gao, X., Zhao, Y., Xu, H., Yang, X., 2011. The dual temperature/pH-sensitive multiphase behavior of poly (N-isopropylacrylamide-co-acrylic acid) microgels for potential application in in situ gelling system. Colloids Surf. B 84, 103–110.
Yamamoto, S., Saeki, T., Inoshita, T., 2002. Drying of gelled sugar solutions-water diffusion behavior. Chem. Eng. J. 86, 179–184.
Zoubari, G., Ali, R., Dashevskiy, A., 2019. Water-soluble and -insoluble polymers as binders for pellet preparation by extrusion/spheronization. J. Drug Deliv. Sci. Tech. 49, 1–5.