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Doxycycline hyclate-loaded bleached shellac *in situ* forming microparticle for intraperiodontal pocket local delivery

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ABSTRACT

Bleached shellac (BS) is a water-insoluble polyester resin made up of sesquiterpenoid acids esterified with hydroxy aliphatic acids. In this study, BS dissolved in *N*-methyl pyrrolidone (NMP), dimethyl sulfoxide (DMSO) and 2-pyrrolidone was used as the internal phase of oil in oil emulsion using olive oil emulsified with glyceryl monostearate (GMS) as the external phase of *in situ* forming microparticles (ISM). Doxycycline hyclate (DH)-loaded BS ISMs were tested for emulsion stability, viscosity, rheology, transformation into microparticles, syringeability, drug release, surface topography, *in vitro* degradation and antimicrobial activities against *Staphylococcus aureus*, *Streptococcus mutans* and *Porphyromonas gingivalis*. All emulsions exhibited pseudoplastic flow and notably low syringeability force. Slower transformation from emulsion into microparticles of ISM prepared with 2-pyrrolidone was owing to slower solvent exchange of this solvent which promoted less porous structure of obtained BS matrix microparticles. The system containing 2-pyrrolidone exhibited a higher degradability than that prepared with DMSO. Developed DH-loaded BS ISMs exhibited a sustainable drug release for 47 days with Fickian diffusion and effectively inhibited *P. gingivalis*, *S. mutans* and *S. aureus*. Therefore a DH-loaded BS ISM using olive oil containing GMS as the external phase and 2-pyrrolidone as a solvent was a suitable formulation for periodontitis treatment.

1. Introduction

The *in situ* forming microparticle (ISM) system is an injectable emulsion with the internal phase containing drug dissolved in polymer solution whereas the continuous phase consists of oil with stabilizer (Voigt et al., 2012). The internal phase of this system consists of an active ingredient and polymer (such as poly(D,L-lactide-*co*-glycolide), poly(D,L-lactide)) dissolved in a biocompatible solvent (such as *N*-methyl-2-pyrrolidone (NMP), 2-pyrolidone, dimethyl sulfoxide (DMSO), triacetin or low molecular weight polyethylene glycol) (Rungseevijitprapa and Bodmeier, 2009). The two phases were mixed using two syringe connectors before administration (Voigt et al., 2012). After injection, the inner polymer phase hardened and formed into ISM. An ISM comprising poly (lactide-*co*-glycolide) in NMP as the internal phase and peanut oil with 2%w/w span 80 as the external phase was used as the injectable implant, which was more easily injectable with a smaller needle size and thus expected to be less painful and give better patient comfort (Rungseevijitprapa and Bodmeier, 2009). Leuprolide acetate loaded-ISM using aluminum monostearate as the emulsion stabilizer, was used as the injectable implant while the initial burst release was decreased when increasing the polymer concentration (Luan and Bodmeier, 2006; Yapar et al., 2012). ISM showed advantages over the *in situ* forming gel, such as decreased cytotoxicity, greater reproducibility, minimized burst release and better injectability, because the drug and solvent did not directly contact the cell, and the external phase (oil) performed as a lubricant (Luan and Bodmeier, 2006). It is interesting to use ISM for treatment of periodontitis with an intra-pocket drug delivery system which promotes high drug concentration in the gingival crevicular fluid, lower side effects, improved drug efficacy and enhancement of patient compliance (Jain et al., 2008).

Shellac is a resin consisting mainly of polyesters made up of sesquiterpenoid acids esterified with hydroxy aliphatic acids (Czarnocka and Alhnan, 2015) (Fig. 1). A major sesquiterpene in the structure is jalaric acid along with a smaller proportion of laccijalaric acid (Sutherland and del Río, 2014; Limmatvapirat et al., 2007). It is a nontoxic, physiologically harmless, edible biodegradable resin and classified as a generally recognized as safe (GRAS) material therefore it is used as a glazing agent on pills and candies (Irimia-Vladu et al., 2013; Farag and Leopold, 2011; Okamoto and Ibanez, 1986). Shellac-coated tablets have been used for a timed enteric or colonic release. Bleached shellac (BS) is obtained by dissolving shellac in some alkaline solution and treating it with sodium hypochlorite for removal of some pigments. It is a well-known water-insoluble polymer dissolved in an organic

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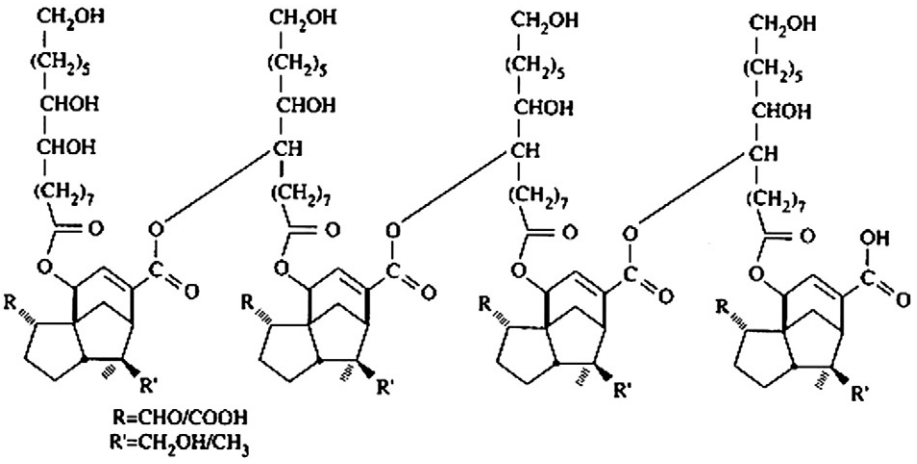

Fig. 1. The chemical structure of shellac.

solvent or solvent mixture for producing water-insoluble films (Bodmeier and Paeratakul, 1994; Madan et al., 2009). Shellac is widely used as a moisture barrier coating for tablets and pellets due to its notably low water vapor and oxygen permeability (Wei et al., 2015 Chitravathi et al., 2014). Recently the *in situ* forming gel prepared using BS as the polymer in NMP has been reported for periodontitis treatment (Phaechamud et al., 2016). *N*-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO) and 2-pyrrolidone (Fig. 2) have been reported as vehicles for an *in situ* forming gel (Parent et al., 2013) because they are thermally stable and biocompatible (Sanghvi et al., 2008). *In situ* forming gel systems containing PLGA as the polymer dissolved in these solvents transform into gel by solvent exchange therefore they are used as injectable implant (Kempe et al., 2008). However the application of these solvents for dissolving BS to prepare it for ISM has not been reported previously and this polymeric system should exhibit its potential use as the internal phase of an o/o emulsion of ISM.

This investigation aimed to develop a doxycycline hyclate (DH)-loaded BS ISM for periodontitis treatment. BS ISMs with and without DH-loading formula using different solvents (NMP, DMSO and 2-pyrrolidone) were prepared and tested for their physical properties and biological actions including emulsion stability, viscosity, rheology, transformation into microparticles, syringeability, drug release, surface topography, degradation and antimicrobial activities against *Staphylococcus aureus*, *Streptococcus mutans* and *Porphyromonas gingivalis*.

2. Materials and methods

2.1. Materials

Bleached shellac (BS) (Ake shellac Co. Ltd., Lumpang, Thailand) with an acid value of 70–95 mg KOH/g, loss on drying <3.5% and colour index of 2 was used as received. DH (Batch No. 20071121, Huashu Pharmaceutical Corporation, Shijiazhuang, China), was used as the model drug. Olive oil (Lot no. L4418R, Bertolli, Italy) was used as the medium for the external phase. Glyceryl monostearate (GMS) (PC Drug, Bangkok, Thailand) was used as an emulsion stabilizer. *N*-methyl-2-pyrrolidone (NMP) (lot no. A0251390, Fluka, New Jersey, USA), dimethyl sulfoxide (lot no. 453035, Fluka, Switzerland) and 2-pyrrolidone (lot no. BCBF5715V, Fluka, Germany) were used as the solvents for BS. Brain Heart Infusion (BHI) (lot no. 0270845, Bacto™, USA), Brain Heart Infusion Agar (BHA) (lot no. 0298038, Bacto™, USA), Mitis Salivarius Agar (MSA) (lot no. 0118681, Difco™, USA), Tryptic Soy Agar (TSA) (lot no. 7341698, Difco™, USA), Tryptic Soy Broth (TSB) (lot no. 8091999, Difco™, USA) were used as media for the antimicrobial tests. *Staphylococcus aureus* ATCC 6853P, *Streptococcus mutans* ATCC 27175 and *Porphyromonas gingivalis* ATCC 33277 were used as the test bacteria. Antimicrobial susceptibility test discs containing 10 μg ampicillin (Becton Dickinson & Company, USA) was used as positive control for antibacterial tests. A syringe connector (Qosina, USA) was used as received. Potassium dihydrogen orthophosphate (lot no. E23W60, Ajax Finechem, Australia) and sodium hydroxide (lot no. AF 310204, Ajax Finechem, Australia) acted as components in a phosphate buffer (PBS) pH 6.8 to simulate gingival crevicular fluid (Kulkarni et al., 2012).

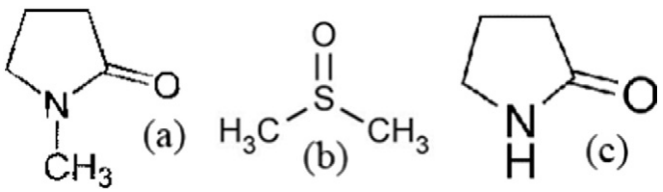

Fig. 2. The chemical structure of NMP (a), DMSO (b) and 2-pyrrolidone (c).

2.2. Preparation of ISM from BS

The internal phase was prepared by dissolving 30% w/w BS as a polymer in solution using NMP, DMSO and 2-pyrrolidone as the solvents. 10% w/w DH was mixed and stirred for 24 h then a clear solution was formed and used as the internal phase of ISM. The external phase was prepared by mixing olive oil and GMS. The GMS amount and the internal/external phase ratios were varied. The prepared two phases were mixed by back-and-forth movement of the syringe plungers of 50 cycles in a two-syringe/connector system. The formulae containing the

Table 1
Composition formulae of ISM prepared from BS using different solvents.

Formula	Amount (%w/w)						
	Internal phase					External phase	
	BS	DH	Solvent				
			NMP	DMSO	2-pyrrolidone	Olive oil	GMS
MShe-1	15	–	qs to 100	–	–	47.5	2.5
MShe-2	15	–	–	qs to 100	–	47.5	2.5
MShe-3	15	–	–	–	qs to 100	47.5	2.5
MSheD-1	15	5	qs to 100	–	–	47.5	2.5
MSheD-2	15	5	–	qs to 100	–	47.5	2.5
MSheD-3	15	5	–	–	qs to 100	47.5	2.5