

DEVELOPMENT OF BIOPLASTIC *IN-SITU* IMPLANT FOR ANTIBIOTIC DRUG LOADING

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Abstract

Typically, solvent removal-induced *in-situ* implants start in a solution state and undergo phase inversion to form depot formulations. In this research, the development and evaluation of a bioplastic *in-situ* implant for antibiotic drug loading were conducted. Two types of cellulose acetate butyrate (CAB) and cellulose acetate propionate (CAP) were used as bioplastic polymers for the *in-situ* implant. These polymers, at a concentration of 15% w/w, were dissolved in various solvents, including dimethyl sulfoxide (DMSO), glycerol formal (GF), *N*-methyl pyrrolidone (NMP), 2-pyrrolidone (PYR), and tetraglycol (TG). The polymers did not completely dissolve in TG, whereas all the other solvents were able to dissolve the polymers fully. Notably, the lowest apparent viscosity of these polymeric solutions was achieved when NMP was used as the solvent. The *in-situ* implants loaded with 1% w/w levofloxacin hemihydrate (Lv) were prepared using these polymers dissolved in NMP. The low viscosity of these solutions, combined with their rapid transformation into a depot matrix, facilitates administration via injection. The use of CAP as a polymer in this drug delivery system enabled self-transformation into a matrix in an aqueous medium due to solvent removal. Nonetheless, its depot hardness and adhesion were slightly lower than some *in-situ* implant prepared from CAB. Microscopic observations under both stereoscope and microscope confirmed swift phase inversion into a matrix implant of the Lv-loaded CAP-based *in-situ* implant after exposure to aqueous phase of agarose gel. This implant prolonged the release of the antibiotic drug for five days. Additionally, the developed *in-situ* implant demonstrated effective antimicrobial activity against *Phophyromonas gingivalis*, *Staphylococcus aureus* and *Candida albicans*. These findings highlight the potential of Lv-loaded CAP-based *in-situ* implant systems as promising candidates for localized depot delivery systems, particularly after exposure to physiological fluids such as crevicular fluid for the treatment of periodontitis.

Keywords: Bioplastic; *In-Situ* implant; Antibiotic; Drug delivery syste

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Introduction

Periodontal disease is an infection affecting the tissues supporting the teeth, starting with symptoms like gum inflammation, redness, and bleeding. If untreated, it can advance to the bone, causing pain, and potentially leading to loosen teeth or extraction. The disease progresses from gingivitis, a mild inflammation of the gums, to periodontitis, which involves severe inflammation and the loss of bone and tissue. Periodontitis is characterized by deep periodontal pockets, often inhabited by harmful bacteria, and is prevalent worldwide, particularly in older adults (Tonetti *et al.*, 2018). Treatment includes non-surgical methods like scaling and root planning, with emerging interest in local drug delivery systems that conform to the shape of periodontal pockets for more effective treatment (Vigliani *et al.*, 2023).

The solvent exchange-induced *in-situ* implant (ISI) is a current drug delivery system widely used in modern pharmaceutical research, known for its ability to transition from a liquid to a semisolid or solid state. It is favored for the site-specific targeting, minimal adverse effects, prolonged drug release, and improved patient compliance (Swain *et al.*, 2019). Practically, the drug of ISI is dissolved in an aqueous insoluble-polymer solution, which solidifies when exposed to an aqueous environment due to phase inversion. This solidification allows the drug to be released gradually as the polymer degrades (Senarat *et al.*, 2023).

Biodegradable polymers such as polycaprolactone, poly(lactide-co-glycolide), polylactide, and poly(trimethylene carbonate) are of interest and have been reported for use in fabricating solvent exchange-induced ISI (Zhang *et al.*, 2019; Ramos *et al.*, 2024). Cellulose esters are regarded as potentially valuable biodegradable polymers. Cellulose acetate butyrate (CAB) and cellulose acetate propionate (CAP) are bioplastics. CAB is an aqueous insoluble bioplastic polymer that typically employed as binders and additives in coating applications for a diverse range of substrates. In the structure of cellulose esters, the hydroxyl groups within the glycoside rings are partially or fully replaced by acetyl and butyryl groups (Peter, 2008). Film coated tablet fabricated using cellulose acetate butyrate as a polymer could prolong phenylpropanolamine release (Sprockel and Prapaitrakul, 1988). In pharmaceutical field, bioplastic such as CAP has been employed as a matrix former, drug carrier, and film former for controlled-release systems including microspheres (Shukla and Price, 1991). These two bioplastics are used to encapsulate drugs and develop of

controlled-release membranes incorporated with ketotifen. They are effective in modulating of drug delivery owing to their controlled drug release. Notably, ketotifen release is generally higher from CAB membranes, while CAP membranes provide a more uniform release, showcasing the distinct release characteristics of each polymer in drug delivery (Sobral *et al.*, 2008). However, these two bioplastics, CAP and CAB, have not previously been reported as polymers used *in-situ* implants (ISI).

Typical solvents used for fabricating *in-situ* forming systems, including *in-situ* implants, are aqueous-miscible vehicles like *N*-methyl pyrrolidone (NMP), tetraglycol (TG), dimethyl sulfoxide (DMSO), 2-pyrrolidone (PYR), and glycerol forma (GF) (Voigt *et al.*, 2012). Due to their water miscibility, high solvation power for polymeric materials, and safety, these solvents are well-suited for ISI systems (Brodbeck *et al.*, 1999). Practically, the low viscosity of the solvent is beneficial for ensuring easy injection of the formulations, enhancing the injectability of the *in-situ* forming systems (Chen and Singh, 2005).

Levofloxacin hemihydrate (Lv), a fluoroquinolone antibiotic, works by interfering with bacterial DNA gyrase and topoisomerase IV, which are essential enzymes for DNA replication and repair. This interference leads to bactericidal effects, giving Lv a broad therapeutic potential against various bacterial infections (Islam *et al.*, 2022). Its local application reduces systemic side effects. The advantage of local delivery lies in targeted administration, minimizing systemic exposure and potential adverse effects.

This study aims to develop a Lv-loaded ISI using CAB and CAP as polymers. The study examined the physical properties and bioactivity of the implant, including viscosity, matrix formation, interface interaction, drug release, and antimicrobial properties.

Materials and Methods

Materials

The two CAB and two types of CAP with different substitution patterns were used as the polymer of ISI. CAB (grade CAB 551 - 0.01 with a butyryl content of 52% w/w, acetyl content of 2% w/w, and a molecular weight of 17.8 kDa (A), CAB 381 - 0.1 (B) with a butyryl content of 38% w/w, acetyl content of 13.5% w/w, CAP 482 - 0.5 (C) with a butyryl content of 45% w/w, acetyl content

of 1.5% w/w, and CAP 504 - 0.2 (D) with acetyl and propionyl contents of 1.0% w/w and 40-45% w/w, respectively was procured from Eastman Chemical Sdn. Bhd. (Kuantan, Malaysia), and employed as the gel-forming agents. Levofloxacin hemihydrate (Lv) (Lot No. ZF07U2308002) was supplied by T.O. Chemicals (1979) Co. Ltd., Thailand, and used as an antimicrobial drug in the study. NMP (Lot No. 144560-118, QReC, Auckland, New Zealand), DMSO (lot no. 453035, Fluka, Switzerland), 2-pyrrolidone (PYR) (lot no. BCBF5715V, Fluka, Germany), tetraglycol (TG) (Lot No. T3396) and glycerol formal (GF) (Lot # BCCD7726, Sigma-Aldrich, Overijse, Belgium) were used as the solvents. Potassium dihydrogen orthophosphate (Lot No. E23W60) and sodium hydroxide (Lot No. AF310204) from Ajax FineChem, New South Wales, Australia, were dissolved in water to prepare a phosphate-buffered saline (PBS) solution with a pH of 6.8. Agarose (Lot No. H7014714, Vivantis, Selangor Darul Ehsan, Malaysia) was obtained for the preparation of an agarose gel.

Strains of *Candida albicans* ATCC 10231 and *Staphylococcus aureus* ATCC 6538, were received from the Ministry of Public Health, Mueang, Nonthaburi District, Thailand. *Phophyromonas gingivalis* ATCC 33277 (Microbiologics Inc., St Cloud, MN, USA) was obtained from Thai Can Biotech Co., Ltd., Bangkok, Thailand, and used as a main test microbe pathogen of periodontitis. Tryptic soy agar (TSA) and tryptic soy broth (TSB) (Difco™, Detroit, MI, USA) were utilized as media for antimicrobial test of *S. aureus* ATCC 6538. Sabouraud dextrose broth (SDB) and Sabouraud dextrose agar (SDA) from Difco, Detroit, MI, USA were prepared as media for the antifungal test against *C. albicans* ATCC 10231. Sheep blood agar procured from M&P Impex, Bangkok, Thailand was used as the medium for *P. gingivalis*.

Preparation of *in-situ* implant systems

Two types of CAB and CAP at concentration of 15% w/w were accurately weighed and dissolved individually in DMSO, GF, NMP, PYR and TG. They were combined using a simple mixing technique with the aid of a magnetic stirrer (Benchmark H3770-HS-E-Digital Hotplate Stirrer, Benchmark Scientific Inc., Sayreville, NJ, USA) for 24 hours. The components of the formulations are detailed in Table 1. The 1% w/w Lv was added in the selected polymeric solutions as shown in Table 2.

Evaluations

Appearance

The physical emergences including colour, transparency and precipitation of prepared ISI systems were noticed visually.

Table 1. Composition of CAB 551 - 0.01 (A), CAB 381- 0.1 (B), CAP 482 - 0.5 (C), and CAP 504 - 0.2 (D) solutions in five different solvents

Formulation	%w/w				
	DMSO	GF	NMP	PYR	TG
A15D	15				
A15G		15			
A15N			15		
A15P				15	
A15T					15
B15D	15				
B15G		15			
B15N			15		
B15P				15	
B15T					15
C15D	15				
C15G		15			
C15N			15		
C15P				15	
C15T					15
D15D	15				
D15G		15			
D15N			15		
D15P				15	
D15T					15

Table 2. Compositions of Lv-loaded *in-situ* forming gel formulations

Formulation	% w/w		
	Polymer	Lv	NMP
LvA15N	15	1	84
LvB15N	15	1	84
LvC15N	15	1	84
LvD15N	15	1	84

*CAB 551 - 0.01 (A), CAB 381 - 0.1 (B), CAB 482 - 0.5 (C) and CAP 504 - 0.2 (D)

Macroscopic Phase Transformation

The matrix-forming behavior of drug-free and Lv-loaded *in-situ* implant (ISI) systems using these bioplastics as the polymers were investigated using two methods. In the first method, a 1 mL aliquot of the Lv-loaded ISI system was injected into 5 mL of PBS (pH 6.8) through an 18-gauge stainless steel needle in a glass test tube, and the phase transformation was monitored by taking photographs at 1, 3, 5, and 10-minute intervals. In the second method, a 0.6% agarose gel, prepared with PBS (pH 6.8) was poured into petri dishes to

a depth of 1 cm and allowed to set into a solid agar. The 150 μ L test sample was dropped into a cylindrical hole created in the agarose gel. The morphology of phase separation was photographed using a stereomicroscope (SZX10, Olympus Corp., Tokyo, Japan) at intervals of 0, 1, 5, 10, 20, and 30 minutes.

Viscosity

The viscosity measurement was conducted using a cone-plate viscometer (RM 100 CP2000 plus, Lamy Rheology Instruments Co., Champagne-au-Mont-d'Or, France).

Microscopic Phase Transformation

The interfacial interaction between Lv-loaded ISI systems and an aqueous environment was studied to understand the phase transition phenomena. A 0.6% agarose solution was poured onto a glass slide, forming a 2 mm thick layer once set. This layer was carefully cut to create a smooth edge located 2 cm from one side of the slide. The agarose gel was then placed adjacent to a 20 μ L sample, positioned near the edge of the incision. Morphological changes due to phase inversion at the interface when exposed to PBS within the agarose gel were observed and recorded using an inverted stereomicroscope (Nikon Eclipse TE2000S, Nikon, Kawasaki, Japan) at various time intervals.

Determination of Hardness and Adhesion

The Lv-loaded ISI systems using these bioplastics as the polymers were left to form within the agarose hole for 3 days to ensure complete phase inversion and matrix formation. After this period, the mechanical properties of the resulting matrix were evaluated. The mechanical properties of the levofloxacin hemihydrate-loaded matrix were evaluated using a texture analyzer (TA.XT plus, Stable Micro Systems, Godalming, UK) with a 5 mm diameter stainless-steel spherical probe. The probe was inserted into the matrix at a constant speed of 0.5 mm/sec. Upon reaching a penetration depth of 1.5 mm, the probe was held stationary for 60 seconds before being retracted at a speed of 10 mm/sec. The force recorded at the maximum penetration depth was defined as the maximum deformation force, indicating the matrix's hardness. The remaining force during the 60-second hold represented the matrix's resilience, while the force required to lift the probe from the matrix surface was recorded as the adhesion force. All measurements were conducted in triplicate at room temperature.

Drug Content and In Vitro Drug Release Test

A cylindrical porcelain cup, measuring 1 cm in diameter and 1.2 cm in height, was filled with 0.3 g of the formulation and immersed in 80 mL of PBS (pH 6.8) at 37°C. The setup was agitated at 50 rpm to simulate drug release from a crevicular pocket. At predetermined intervals, 5 mL samples of the release medium were withdrawn and replaced with fresh PBS. The drug content in formulation and concentration of the released drug were quantified using a UV-visible spectrophotometer (Cary 60 UV-Vis, Model G6860A, Agilent, Selangor, Malaysia) at a wavelength of 287 nm. They were filtered through 0.45 μ m nylon membrane before analyzed. All experiments were performed triplicately. The drug release mechanism was analyzed by fitting the dissolution data to various mathematical models, including zero order, first order, Higuchi's, and Korsmeyer–Peppas. The analysis involved calculating the coefficient of determination (r^2) to assess the fit of each model. This mathematical analysis was conducted using the DD-Solver[®] software, an add-in program for Microsoft Excel (Redmond, WA, USA) developed in Visual Basic for Applications.

Scanning Electron Microscope (SEM)

After the matrix formation was complete, the remnant samples were washed multiple times with distilled water to remove any residual impurities. They were then carefully dried in a desiccator for one week to prevent potential melting or structural collapse. Once dried, the ISGs were coated with a thin layer of gold to prepare them for examination using a field-emission scanning electron microscope (SEM) (TESCAN MIRA3, Brno-Kohoutovice, Czech Republic) operating at an accelerating voltage of 15 kV. This preparation ensured the samples maintained their integrity and allowed for detailed analysis of their morphology and structure.

Antimicrobial Activity Test

The antimicrobial properties of the Lv solution, drug-free polymeric solution, and Lv-loaded ISI solutions were tested against microbial strains such as *P. gingivalis* 33277, *S. aureus* 6538 and *C. albicans* 10231. *S. aureus* 6538 inocula were cultivated for 36 hours in TSB, and turbidity of broth was adjusted to correspond to 0.5 McFarland standard. These prepared bacterial suspensions were then swabbed and spread onto TSA plates. Sheep blood agar was employed in antimicrobial tests against *P. gingivalis* ATCC 33277. *C. albicans* ATCC 10231 inoculum was also calibrated and then

spread onto SDA. Sterilized cylindrical cups were carefully positioned on surface of swabbed agar, and 200 μ L of test solutions were introduced into the cups. The plates were then incubated at 37°C for 24 hours, with anaerobic bacteria requiring 72 hours in anaerobic tank. After the incubation period, the inhibition zones were measured with a calibrated ruler ($n = 3$).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate statistical significance. A significance level of $p < 0.05$ or $p < 0.01$ was applied to assess the results.

Results and Discussion

Physical Appearance

All four polymers were found to be soluble in DMSO, GF, NMP, and PYR within 48 hours. The polymer solutions in DMSO and PYR appeared transparent with a slight yellowish tint, while those in NMP and GF were completely transparent. In contrast, a lumpy polymeric mass was observed in systems prepared using TG as the solvent, specifically in A15T, B15T, C15T, and D15T. The physical appearances of these systems are shown in Figure 1. Based on these observations, TG was not a suitable solvent for these polymers, despite its previous use as a solvent to reduce the burst drug release and extend drug release *in-situ* forming biodegradable PLGA microspheres (Jain *et al.*, 2000). The ideal solvent for *in-situ* forming systems should exhibit several key properties: appropriate water affinity, low viscosity with good injectability, high solubility for the active compound or polymer, and safety with biocompatibility (Rein *et al.*, 2022).

Macroscopic Phase Transformation

All prepared drug-free bioplastic solutions transformed from their solution state to opaque mass after injection into PBS pH 6.8 at 5 min (Figure 2). The solutions of CAB type A and B dissolved in NMP and DMSO remained some their parts as solution state. All of CAP solutions formed into opaque matrix in this PBS. The rather less whiteness matrix of bioplastics in NMP indicated their slower matrix formation owing to slower solvent exchange (Ramos *et al.*, 2024).

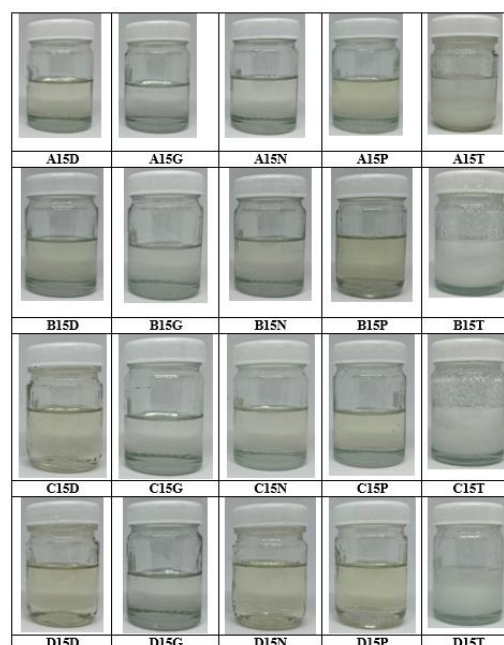


Figure 1. Physical appearance of 15% CAB and CAP ISI systems in different solvents

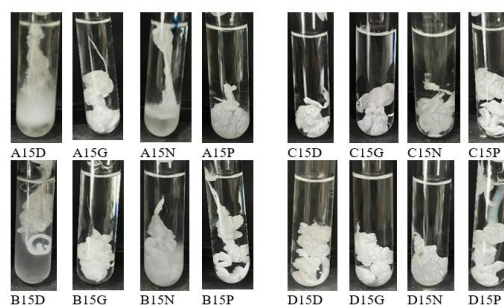


Figure 2. Physical appearance of 15% CAB and CAP ISI systems dissolved in different solvents after injection into PBS pH 6.8 at 5 min

Viscosity

Table 3 presents the viscosity values of the prepared ISI systems prepared using these bioplastics as the polymers and their respective solvents. Upon comparison, NMP exhibited a lower viscosity than DMSO, PYR, GF, and TG, respectively. Additionally, the viscosity of CAB 551-0.01 (A) solutions was lower than that of CAB

381-0.1 (B) and CAP 482-0.5 (C). This indicates that a higher molecular weight of polymer significantly contributes to the increased viscosity of their solutions. Notably, CAP 504-0.2 (D) demonstrated a viscosity lower than that of CAP 482-0.5 (C). Prominently, the use of NMP as a solvent for all polymer solutions led to a significantly lower viscosity ($p < 0.05$), except for CAP 482-0.5 (C), which the viscosity in NMP was not significantly different from that in DMSO. Moreover, the viscosity values were primarily influenced by the solvent used, except in the cases of CAB 381-0.1 (B) and CAP 504-0.2 (D) in GF and PYR.

Normally, an effective solvent should exhibit strong interactions with the polymer. Furthermore, substituting some solvents with the drug may increase the system's viscosity in drug-loaded formulations, as previously reported (Phaechamud *et al.*, 2018). Therefore, the different in substitute group of butyrate with propionate affected the solubility and viscosity of prepared these polymeric solutions. Typically, crucial parameters such as molecular weight should be considered and directly related to viscosity behavior. Unfortunately, only CAB 381-0.1 has had its molecular weight reported, which is 16.0 kDa (Sobral *et al.*, 2008). The highly viscous CAP 482-0.5 (C) solutions in GF (C15G) and PYR (C15P) were not measured. The viscosity of the used solvent can directly affect the formulation's viscosity (Khaing *et al.*, 2024), as well as influence the diffusion rate, the nature of solvent exchange, and the injectability of the *in-situ* drug delivery system. Typically, a low-viscosity formulation facilitates easier solvent exchange and subsequent matrix formation (Jain *et al.*, 2000; Rein *et al.*, 2022). However, the ability to form a matrix depends on the nature of the polymer upon exposure to an aqueous environment. The notably low viscosity values observed in these polymer solutions when using NMP as a solvent made them particularly interesting for further study.

The viscosity of Lv-loaded bioplastic polymer ISI solutions is shown in Table 4. The viscosity of LvA15N was significantly lower compared to LvD15N, LvB15N, and LvC15N ($p < 0.01$). The addition of the drug appears to reduce the viscosity of A15N and D15N. Mechanical property checks

were performed to access the properties of the resulting matrices after phase transformation. The hardness of the LvC15N matrix, after being placed in an agarose hole, was significantly higher than that of LvD15N, LvB15N, and LvA15N ($p < 0.01$) (Table 4). The inclusion of a high proportion of plasticizer in the borneol-based *in-situ* gel formulation has been reported to reduce the firmness of the matrix (Lertsuphotvanit *et al.*, 2023). Typically, the hardness of the transformed *in-situ* system tends to increase in correlation with the polymer concentration (Khaing *et al.*, 2024). Therefore, the differences in hardness among these developed systems likely arise from the inherent properties of the polymer, such as its polymeric chain stiffness and the rate of solvent exchange (Lertsuphotvanit *et al.*, 2023). The adhesion force in Table 4 shows that LvB15N was significantly higher than LvC15N, LvA15N, and LvD15N ($p < 0.01$). Thus, the type of polymer influenced the mechanical characteristics of the resulting matrix following solvent exchange.

Table 3. Viscosity values of 15% CAB and CAP ISI systems in different solvents (mean $\pm$ S.D., n=3)

Formulation	Viscosity (cP)
A15D	43.54 $\pm$ 1.61 ^a
A15G	463.23 $\pm$ 11.74 ^b
A15N	37.07 $\pm$ 1.53 ^{a,b,c}
A15P	359.63 $\pm$ 11.79 ^c
B15D	324.11 $\pm$ 4.87 ^d
B15G	3327.87 $\pm$ 10.85 ^e
B15N	300.01 $\pm$ 12.29 ^{d,e,f}
B15P	3760.28 $\pm$ 12.16 ^f
C15D	909.70 $\pm$ 11.97
C15G	NA
C15N	888.38 $\pm$ 12.81
C15P	NA
D15D	952.26 $\pm$ 10.56 ^g
D15G	2091.19 $\pm$ 15.67 ^h
D15N	157.69 $\pm$ 3.9 ^{g,h,i}
D15P	2343.09 $\pm$ 15.32 ⁱ
DMSO	4.52 $\pm$ 0.01
GF	17.20 $\pm$ 0.20
NMP	4.88 $\pm$ 0.20
PYR	13.76 $\pm$ 0.20
TG	19.27 $\pm$ 0.82

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

Table 4. Viscosity values of 15% CAB and CAP ISI systems in different solvents (mean $\pm$ S.D., n=3)

Formula	Viscosity (cP)	Hardness (N)	Adhesion (N)
LvA15N	28.76 $\pm$ 1.20 ^{a,b,c}	5.242 $\pm$ 1.15 ^d	0.014 $\pm$ 0.008 ^e
LvB15N	230.89 $\pm$ 17.12 ^a	17.62 $\pm$ 0.78 ^c	0.091 $\pm$ 0.064 ^{g,h,i}
LvC15N	977.1 $\pm$ 95.21 ^b	28.79 $\pm$ 1.72 ^{d,e,f}	0.039 $\pm$ 0.03 ^h
LvD15N	189.49 $\pm$ 15.70 ^c	20.97 $\pm$ 2.57 ^f	0.012 $\pm$ 0.004 ⁱ

The superscripts a-i indicate a significant difference ($p < 0.01$)

Matrix Formation Behavior

The phase transformation observed after exposing the four Lv-loaded ISIs to PBS at pH 6.8 in a glass test tube is illustrated in Fig. 3. PBS at pH 6.8 was used as the immersion fluid to simulate cervical fluid in the periodontal pocket and aqueous fluid in the oral cavity (Lertsuphotvanit *et al.*, 2023). The developed ISI is intended for application as a drug delivery system for periodontitis therapy through administration into the periodontal pocket.

In comparison, LvD15N promptly transformed into an opaque bioplastic mass and moved to the bottom of the test tube. In contrast, LvA15N exhibited incomplete transformation, forming a dispersed phase. Therefore, CAB 551-0.01 (A) at this concentration was not suitable for generating a sufficiently dense matrix for controlled drug release. Increasing its concentration, similar to the PLA used in the commercial product Atridox[®], to nearly 37.5% (Ramos *et al.*, 2024) might promote the formation of a dense polymer matrix adequate to moderate drug release. LvB15N gradually changed into an opaque mass, starting from the outer skin and moving inward. The phase transformation of LvC15N presented as falling in line polymeric mass. Initially transparent, LvB15N, LvC15N, and LvD15N became translucent masses and eventually transformed into more opaque masses over time. This gradual change indicates solvent exchange, inducing a phase transformation from a solution state to a gel, and finally to a solid polymeric mass with drug entrapment inside (Senarat *et al.*, 2023). LvD15N demonstrated rapid phase inversion, and its viscosity was acceptable for use as an injectable dosage form for periodontitis treatment. Furthermore, CAP has been successfully used in coating materials for controlled drug delivery applications, showing a favorable cytotoxicity profile in cellular adhesion experiments (Sobral *et al.*, 2008). Therefore, LvD15N solution was selected and applied as the ISI system for further evaluations.

The cross-sectional phase inversion of LvD15N is shown in Figure 4. The PBS pH 6.8 aqueous phase facilitated the phase transformation of the formulation from the agarose rim into the deeper inner formulation over time, indicating more extensive solvent exchange within the formulation. The Lv solution in NMP was fully miscible with PBS in the test tube, and no cross-sectional matrix formation was observed (data not shown) due to the absence of polymer loading. Its hydrophilic nature enabled it to dissolve in the aqueous phase. The high solvation power of NMP made it an ideal solvent for ISI systems (Brodbeck *et al.*, 1999). The movement of this solvent from the formulation into agarose was previously confirmed by tracking the diffusion

of amaranth dye. A high rate of dye diffusion was observed initially, followed by a gradual decline over time (Phaechemud *et al.*, 2019).

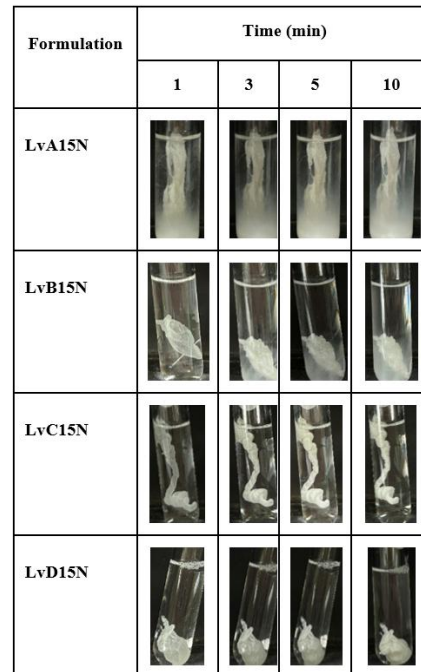


Figure 3. Matrix formation of Lv-loaded CAB and CAP ISI systems in NMP after injection into PBS pH 6.8 at different times

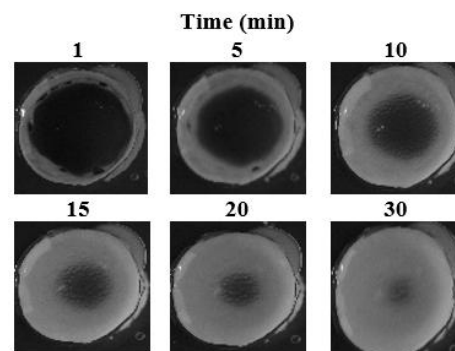


Figure 4. Cross-sectional matrix formation of LvD15N

Interface Interaction

The microscopic phase transformation of LvD15N against a thin agarose plate, observed under a microscope, is shown in Figure 5. The polymeric mass formation is seen expanding more toward the right side of the formulation. Although the expanded mass matrix at 5 minutes appeared less dense than at 3 minutes, its expansion distance was greater. This suggests that longer exposure to

agarose induces more extensive phase transformation into a solid mass. No phase transformation was observed at the interface between the Lv solution in NMP, without polymer addition, was fully miscible with agarose (photo not shown). This miscibility is attributed to the polar characteristics of both the NMP and the aqueous phase of agarose (Brodbeck *et al.*, 1999). Previous studies have reported similar microscopic matrix formation resulting from interface interactions between agarose and nitrocellulose or borneol-based *in-situ* forming systems (Lertsuphotvanit *et al.*, 2023; Khaing *et al.*, 2024). An apparent crystal-like structure was observed in the borneol-based *in-situ* formulation, while an opaque polymeric mass was found in nitrocellulose-based formulation.

In the case of the CAP-based ISI, a transparent, irregular thin film expanded over time, indicating its unique morphology. The solvent exchange-induced matrix formation is related to the scaffold morphology of polymer after its phase separation, which directly influences drug release behavior from ISI. The denser scaffold structure was evident as Eudragit® L concentration increased *in-situ* forming system (Senarat *et al.*, 2023). Microscopic examination of interface interactions is beneficial for gaining a deeper understanding of the phase inversion in the developed ISI system.

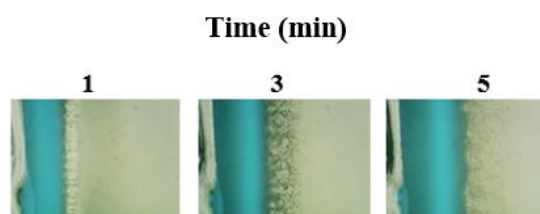


Figure 5. Interface interaction between agarose gel (left) and LvD15N (right) observed under an inverted microscope at 40× magnification

Drug Content and Drug Release

After preparing the formulations, their drug content was determined. The drug content in samples LvN and LvD15N was found to be $99.82 \pm 1.84\%$ and $100.03 \pm 0.88\%$, respectively. These formulations were prepared through a simple mixing process without the need for heating or complicate procedures, resulting in Lv content levels close to 100%. To evaluate the ability to release the entrapped drug from the polymer matrix,

the Lv release was measured at different time intervals, as shown in Fig. 6. The drug release from LvD15N was compared with the Lv solution (LvN). To simulate the drug release behavior from the periodontal pocket, the cup method, as previously reported by Senarat *et al.* (2023), was used in the experimental design. The target site of this dosage form for periodontitis therapy is the infected periodontal pockets in which it is small space created by detaching inflamed gum tissue. The *in-situ* forming formulation is intended for injection directly into the periodontal pocket, where solvent exchange predominantly occurs at the contact surface in a one-dimensional manner, primarily at the upper part of the pocket. The gingival fluid pH in patients with chronic generalized periodontitis is approximately 6.8 (Koppolu *et al.*, 2022). Consequently, PBS at pH 6.8 was used as the release fluid in this drug release test.

A swift release of Lv from LvN was observed (Figure 6) due to the free diffusion of this hydrophilic salt compound from the solution into the release medium. In contrast, LvD15N demonstrated a more prolonged drug release, extending to 72 hours before reaching a plateau state at 120 hours. Therefore, the CAP polymeric matrix effectively sustained the release of Lv from this transformed dosage form. The release rate of doxycycline hyclate from the Eudragit L-based system (Senarat *et al.*, 2023) or ibuprofen-based system (Puyathorn *et al.*, 2023) in a solvent exchange-induced *in-situ* forming system, using the membrane-less method (cup method), was significantly reduced when the concentration of the matrix material was increased. Consequently, the crucial factor is the denser transformed matrix, which more efficiently sustains drug release due to its effective barrier effect against solvent exchange and drug release into release fluid.

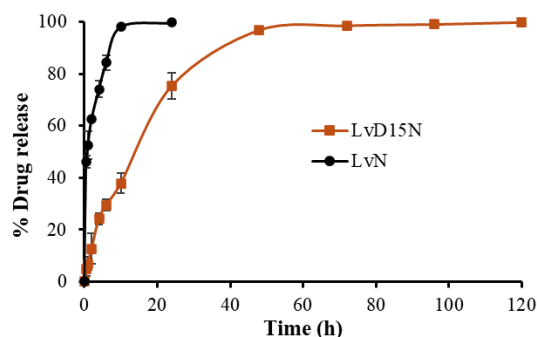


Figure 6. Release profile of Lv from Lv-loaded CAP ISI system in NMP formulations (n = 3)

The goodness of fit is typically indicated by a high regression coefficient (R^2) value. The R^2 values for the release profiles mentioned above, fitted to four different release mathematical equations, are presented in Table 5. Additionally, the diffusion exponent values (n) obtained from fitting the profiles to the Korsmeyer-Peppas equation were determined. For non-swellable cylindrical matrices, the values of n in the Korsmeyer-Peppas equation are 0.45 for Fickian transport and 1.0 for case-II transport (Ritger and Peppas, 1987). When the n value is greater than 0.45 but less than 1.0, the release is characterized as non-Fickian (Ritger and Peppas, 1987). A value of n equal to 1 indicates a zero-order release (Shefeeq and Ahmad, 2012).

Table 5. Estimate r^2 value from profile fitting to different release mathematical models

Equation	Formulation	
	LvN	LvD15N
Zero-order	0.4726	0.9157
First-order	0.7025	0.9845
Higuchi	0.2502	0.9333
Korsmeyer-Peppas	0.9947	0.9949

The exponent value (n) from profile fitting to Korsmeyer-Peppas model of LvN and LvD15N was 0.691 and 0.700, respectively.

The first-order kinetic model best aligned with the release profiles, suggesting that drug release was proportional to the remaining amount of the drug. The calculated n values indicated a non-Fickian drug release mechanism. Consequently, the release of Lv from the transformed LvD15N was primarily driven by the diffusion of the medium into the CAP composite matrix, with the dissolved drug diffusing through the porous CAP structure. In practice, the rate of drug release was dependent on the interconnectivity and size of the porous structure. The remnant LvD15N exhibited a relatively smooth surface with various pores, while the cross-sectional view elicited its scaffold topography, as shown in Fig. 7. Consequently, the release of this hydrophilic drug such as Lv through the system was driven by the porosity of the CAP bioplastic matrix.

The formation of scaffolds in polymer-based *in-situ* forming systems, following phase inversion induced by solvent exchange, has been previously described (Zhang *et al.*, 2019; Senarat *et al.*, 2023). The removal of NMP from LvD15N, along with

the phase separation of its CAP due to the limited solubility of bioplastic CAP in more polar mixed solvents over time, significantly contributed to the formation of the polymeric scaffold. Despite this, the relatively homogeneous skin surface of this bioplastic matrix scaffold is expected to serve as an effective barrier, extending the release of Lv. Additionally, the more viscous nature of the crevicular fluid in the periodontal pocket may further prolong the drug release at the actual target site. Atridox[®] is a commercially available product that contains an antibiotic compound, utilizing poly(dl-lactide) as the polymer dissolved in NMP. This formulation is prepared as an *in-situ* forming system designed for controlled drug release over one week for the treatment of periodontitis (Puyathorn *et al.*, 2023). Typically, experimental drug release tests design the release medium as a sink condition to allow for the free diffusion of the active compound, thereby ensuring a better understanding of the role of the developed controlled drug delivery systems (Weng *et al.*, 2020).

The rapid phase inversion from solution to solid state of CAP in LvD15N resulted in a skin-like topography, as observed in the SEM surface morphology. This skin texture acted as a barrier to further aqueous penetration into the inner part of LvD15N, gradually promoting scaffold formation within the dosage form. The initial burst in drug release was attributed to the rapid dissolution of the drug deposited on the skin surface. The dissolved drug molecules within the scaffold could then diffuse through its pores, along with NMP, into the PBS release fluid, following first-order kinetic. However, previous research indicated that the solvent and drug release kinetics of shellac-based *in-situ* forming microparticle formulations are best described by the Higuchi's model, with a release mechanism controlled by Fickian diffusion through denser scaffold matrix (Phaechemud *et al.*, 2019). The oil phase *in-situ* forming microparticle formulations could influence the diffusion behavior of both the solvent and drug molecules. Additionally, the size and density of the pores in the formed shellac scaffold matrix increased over time, which was reflected in the corresponding increase in the release rate of both the solvent and the drug. In contrast, drug release through the CAP scaffold of LvD15N is likely to diffuse more freely and follow first-order release kinetics.

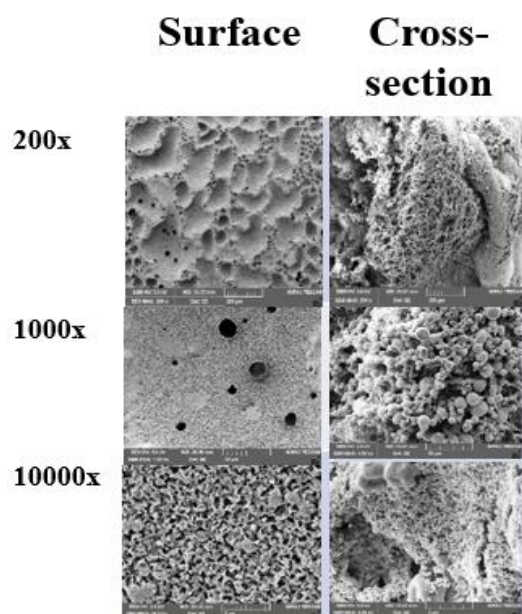


Figure 7. SEM image of dried remnant of Lv-loaded CAP-based ISI after release at different magnifications

Antimicrobial Activities

The antimicrobial activities of NMP, LvN, and LvD15N were assessed by measuring the inhibition zone diameters against three microbial strains: *S. aureus* 6538, *C. albicans* 10231, and *P. gingivalis* 33277. These particular microbes were selected due to their association with peri-implantitis and periodontitis, especially pathogens like *P. gingivalis* (Puyathorn *et al.*, 2023). Additionally, a drug-free 15% CAP 504 - 0.2 (D) solution in NMP (D15N) was tested. The results, summarized in Table 6, revealed that the formulations containing Lv exhibited the highest antimicrobial activity against *S. aureus* 6538 and *P. gingivalis* 33277. Specifically, both LvN and LvD15N formulations produced significantly larger inhibition zones compared to NMP and D15N, indicating that Lv substantially enhances antibacterial activity against these bacteria.

LvD15N exhibited a similar inhibition zone to LvN, likely because the porous CAB matrix formed after exposure to the aqueous phase of the agar medium did not hinder Lv diffusion, thereby allowing effective inhibition of bacterial growth. In contrast, this matrix appeared to slow down the diffusion of NMP, reducing its antibacterial effectiveness. The notable antimicrobial activity of NMP, particularly against *Candida* species, has been reported previously (Phaechemud *et al.*, 2013; Khaing *et al.*, 2024). NMP has been reported to enhance the anti-inflammatory activity of

doxycycline hyclate-loaded, solvent removal-induced *in-situ* forming systems (Puyathorn *et al.*, 2023). Additionally, NMP has been shown to reduce inflammation by activating Krüppel-like factor 2 (Roche-Molina *et al.*, 2020) and inhibiting nuclear factor kappa B signaling (Ghayor *et al.*, 2015). This anti-inflammatory property of NMP could further improve the therapeutic potential of the developed ISI formulation. Moreover, NMP has been used as a solvent in commercial products for periodontitis treatment, such as Atridox[®], and its low toxicity has been documented, with oral and dermal LD50 values in rodents ranging from 3600 to 7700 mg/kg (Poet *et al.*, 2009).

The inhibition zones for *C. albicans* were relatively consistent across the various formulations. The NMP and LvN formulations generated similar inhibition zones, suggesting strong antifungal activity attributed to NMP diffusion (Puyathorn *et al.*, 2023). Although D15N and LvD15N also demonstrated considerable activity, they exhibited slightly smaller inhibition zones, potentially due to a reduction in NMP diffusion caused by the matrix polymer. Previous reports have noted the retardation of NMP diffusion due to matrix material loading in solvent exchange-induced *in-situ* forming systems, which subsequently led to a decrease in the diameter of the antimicrobial inhibition zones (Senarat *et al.*, 2023; Puyathorn *et al.*, 2023).

The large inhibition zones observed with the LvN and LvD15N formulations indicate that Lv possesses strong broad-spectrum antimicrobial properties. The relatively consistent inhibition zones for *C. albicans* suggest that the antifungal activity of the formulations remains effective, regardless of the polymer used. However, the slightly reduced inhibition zone observed with LvD15N may indicate a slower diffusion of Lv from the bioplastic matrix polymer. Overall, these findings underscore the potential of Lv-loaded bioplastic CAP-based ISI as effective antimicrobial agents, particularly against pathogens associated with periodontitis.

Table 6. Inhibition zone diameters of control samples and Lv-loaded CAP-based ISI against *S. aureus*, *C. albicans*, and *P. gingivalis* (n=3)

Formula	Inhibition zone diameter (mm. $\pm$ S.D.)		
	<i>S. aureus</i> 6538	<i>C. albicans</i> 10231	<i>P. gingivalis</i> 33277
NMP	19.0 $\pm$ 1.0	34.0 $\pm$ 1.7	18.7 $\pm$ 0.6
LvN	39.0 $\pm$ 1.0	34.0 $\pm$ 0.0	34.0 $\pm$ 0.0
D15N	17.0 $\pm$ 1.0	33.7 $\pm$ 1.5	16.7 $\pm$ 0.6
LvD15N	39.3 $\pm$ 0.6	31.3 $\pm$ 1.5	34.7 $\pm$ 0.6

Conclusions

In this research, the bioplastic *in-situ* ISI drug delivery systems for antibiotic drug loading were developed and evaluated. CAB and CAP were used as bioplastic polymers dissolved in various solvents. The polymers fully dissolved in all solvents except TG, with NMP achieving the lowest viscosity. Type of bioplastic polymer influenced on matrix formation of Lv-loaded systems in simulated crevicular fluid. The use of CAP formed a swift depot bioplastic matrix upon injection due to solvent removal. Microscopic analysis confirmed swift phase inversion into a matrix implant upon exposure to an aqueous medium. Lv-loaded bioplastic CAP-based ISI also showed strong antimicrobial activities against *S. aureus*, *C. albicans*, and *P. gingivalis*, making it a promising candidate for localized drug delivery, particularly in the treatment of periodontitis.

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