

PHYSICAL PROPERTIES AND BIOACTIVITIES OF CURCUMIN-LOADED IN-SITU IMPLANT

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Received: October 02, 2024; Revised: December 30, 2024; Accepted: January 08, 2025

Abstract

Curcumin, the important active component in turmeric extracts, has gained significant attention demonstrating notable effectiveness across a wide range of human pathological conditions. This study explores the development and characterization of borneol and ibuprofen-based in situ implant (ISI) systems loaded with curcumin, designed for advanced therapeutic applications. Curcumin ISI solution enabled self-transformation into matrix at 40% w/w concentration. Ibuprofen and borneol enhanced curcumin transformation at lower concentration. These ISI systems exhibited low viscosity and swift transformation aiding for their administration by injection. Macroscopic and cross-sectional imaging provided insights into their phase inversion process. Curcumin-loaded borneol-based ISI demonstrated high antioxidant activity. Both curcumin-loaded borneol-based and ibuprofen-based ISIs showed efficient antimicrobial activities against related periodontitis pathogens. These findings underscore the potential of curcumin-loaded borneol and ibuprofen-based ISI systems as promising candidates for localized drug delivery, particularly in treating periodontitis.

Keywords: Curcumin; *In Situ* Implant; Borneol; Ibuprofen; Bioactivity Properties

Introduction

In situ-forming implant (ISI) systems have emerged as a promising technology in drug delivery, particularly for localized treatment of diseases where controlled and sustained release of therapeutic agents is essential (Phaechamud *et al.*, 2019). ISIs are designed to undergo a phase transition from a liquid to a gel or solid matrix upon administration into the body (Eldeeb *et al.*, 2022).

This transformation is typically triggered by environmental factors such as temperature, pH, or solvent exchange, leading to the gelation of the polymer or other matrix-forming substances at the target site (Chuenbarn *et al.*, 2022; Lertsuphotvanit *et al.*, 2022). These systems offer several advantages, including minimally invasive administration through injection, ease of

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application, and the ability to provide localized and prolonged drug release (Fogueri and Singh, 2009). As a result, ISIs have found extensive applications in treating various conditions, including cancer, periodontitis, and chronic inflammation (Schwach-Abdellaoui *et al.*, 2000).

Periodontitis, a severe gum infection that damages the soft tissue and destroys the bone supporting the teeth, is one of the diseases where localized drug delivery is particularly beneficial. Traditional treatment methods often involve systemic administration of antibiotics, which can lead to side effects and suboptimal drug concentrations at the site of infection. In contrast, ISIs allow for the direct delivery of antibiotics or other therapeutic agents to the periodontal pocket, ensuring higher local drug concentrations, reduced systemic exposure, and enhanced therapeutic outcomes (Schwach-Abdellaoui *et al.*, 2000; Amato *et al.*, 2023).

Curcumin, the primary bioactive component of *Curcuma longa* (turmeric), has been extensively studied for its broad spectrum of pharmacological activities. It exhibits potent anticancer, antioxidant, antinociceptive, anti-inflammatory, wound healing and antimicrobial properties (Sabira *et al.*, 2021; Urošević *et al.*, 2022; Cozmin *et al.*, 2024), making it a valuable candidate for the treatment of inflammatory diseases such as periodontitis. However, curcumin's clinical application has been limited by its poor water solubility, low bioavailability, and rapid degradation under physiological conditions (Urošević *et al.*, 2022). To overcome these challenges, researchers have explored various drug delivery systems, including nanoparticles, liposomes, and ISIs, to enhance curcumin's solubility, stability, and therapeutic efficacy (Urošević *et al.*, 2022).

The use of non-polymeric materials such as borneol (BOR) and ibuprofen (IBU) in ISI formulations represents a novel approach to improving curcumin's bioavailability and therapeutic effects. BOR, a natural monoterpene, is traditionally used in Chinese medicine and is known for its ability to enhance drug absorption and penetration (Lertsuphotvanit *et al.*, 2022). It acts as a penetration enhancer, facilitating the transport of drugs across biological membranes (Guo *et al.*, 2014). In the context of ISIs, BOR not only aids in the rapid gelation of curcumin at lower concentrations but also contributes to the overall therapeutic effect by providing additional anti-inflammatory and analgesic properties (Puyathorn *et al.*, 2023a). IBU, a widely used nonsteroidal anti-inflammatory drug (NSAID), is another promising candidate for combination with curcumin in ISI systems. IBU's anti-inflammatory action

complements curcumin's effects, potentially providing a synergistic therapeutic outcome. Moreover, IBU has been shown to assist in the gel formation process (Batool *et al.*, 2019), making it a valuable component in the design of curcumin-loaded ISIs. The incorporation of IBU into ISI systems not only enhances the anti-inflammatory efficacy of the formulation but also supports the rapid phase transition necessary for effective drug delivery (Puyathorn *et al.*, 2023b).

This study investigates the development and characterization of curcumin-loaded ISI systems based on BOR and IBU. The focus is on understanding the physicochemical properties, matrix transformation behavior, and bioactivities of these formulations, with the goal of establishing their potential as advanced therapeutic platforms for localized drug delivery, particularly in the treatment of periodontitis. Through detailed analysis of macroscopic and microscopic phase inversion, viscosity, density, and bioactivity assays, this research aims to provide insights into the design and optimization of ISI systems for enhanced curcumin delivery.

Materials and Methods

Materials

The primary matrix-forming materials used were BOR (Chareorsuk-osop Herbal Shop, Nakhon Pathom, Thailand) and IBU (lot no. 4000/1101/18/A-0150B, P.C. drug center CO. LTD, Bangkok, Thailand). Curcumin extract (curcumin content of 95.2%) was purchased from Thai-China Flavours and Fragrances Industry Co., Ltd., Phra Nakorn Si Ayutthaya, Thailand and employed as the active compound. Dimethyl sulfoxide (DMSO) (lot no. 453034) and *N*-methyl-2-pyrrolidone (NMP) (lot no. A0251390) were obtained from Fluka (New Jersey, USA) and used as aprotic solvents to dissolve the matrix former and curcumin extract.

Preparation of ISI

Curcumin-loaded ISI formulations were prepared by dissolving 5% w/w curcumin in DMSO and NMP as control groups. Solutions of 5% w/w curcumin combined with 40% w/w BOR or 40% w/w IBU solutions were prepared by dissolving BOR or IBU in DMSO and NMP, followed by the addition of curcumin. The mixtures were stirred continuously in a glass bottle using a magnetic stirrer (Benchmark H3770-HS-E-Digital Hotplate Stirrer, Benchmark Scientific Inc., Sayreville, NJ, USA) until homogeneous, clear solutions were obtained. Control formulations containing BOR and

IBU in the solvents without curcumin addition were also prepared for comparison as presented in Table 1.

Physicochemical Property Tests Density

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

Viscosity

Viscosity and shear stress of NMP and formulations were conducted by using a viscometer (Brookfield Engineering Laboratories Inc., Middleborough, MA, USA) with a CP-40 spindle ($n = 3$) at 25°C at a shear rate of 250 sec⁻¹.

Phase Transformation of ISI

Macroscopic aspect phase transformation of implant unit

The transformation from ISI solution into gel or matrix-like of formulations was observed visually as described previously (Phaechamud, 2018a). The preparations were injected from a 1-mL syringe through an 18-Gauge needle into Phosphate buffer saline (PBS) solution (pH 6.8) and recorded the change at different time intervals (1, 5, 10, 15 and 30 min)

Cross-sectional aspect phase transformation at interface under stereomicroscope

Microscopic observation of the gel formation process was performed under a stereomicroscope. Agarose gel was prepared by dissolving 0.6% w/w agarose in PBS and filling it into 9-cm diameter petri dishes, which were then cooled to form a gel. A cylindrical well was made in the agarose using

a 6-mm diameter cylindrical stainless-steel cup, and 150 µL of the ISI formulation was added to the well. The transformation process was observed under a stereomicroscope (Motic SMZ-171, Hong Kong) at 12X magnifications (Phaechamud, 2018a).

Bioactivity properties of ISI

Antioxidation activity

Free radical scavenging capacity of the curcumin-loaded ISI formula was determined by using the scavenging of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The curcumin-loaded ISI were filled into cup using micropipette and weighing on the analytical balance, then carefully placing this cup into a glass bottle containing 80 mL PBS pH 6.8. The condition at temperature of 37°C using an incubator (Model NB-205, N-Biotek, Gyeonggi-do, Korea) with rotational speed of 50 rpm. After sampling 5 mL of PBS medium for being sample. The samples tested using DPPH technique. A total of 24 milligrams of DPPH were dissolved in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 µL of samples. Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. the percentage of antioxidants was computed via equation (1) (Baliyan *et al.*, 2022):

$$\% \text{ of antioxidant activity} = [(Ac - As) / Ac] \times 100 \quad (1)$$

where: Ac is Control reaction absorbance;
As is Testing specimen absorbance.

Table 1. Percentage of components in curcumin solution, curcumin-free and curcumin-loaded ISI formulations

Formulation	Composition (%w/w)				
	Curcumin	BOR	IBU	DMSO	NMP
CD	5	-	-	95	-
CN	5	-	-	-	95
C40D	40	-	-	60	-
C40N	40	-	-	-	60
CBD	5	40	-	55	-
CBN	5	40	-	-	55
CID	5	-	40	55	-
CIN	5	-	40	-	55
BD	-	40	-	60	-
BN	-	40	-	-	60
ID	-	-	40	60	-
IN	-	-	40	-	60

Anti-inflammatory activity

The anti-inflammatory activity of the curcumin-loaded ISI formulations was tested using the thermal inhibition of protein denaturation of egg albumin (Puyathorn *et al.*, 2023c). The sample of this experiment was collected similar to antioxidation activity experiment. Briefly, a 5 mL mixture solution composed of 2.8 mL of PBS pH 6.4, 0.2 mL of egg albumin (from fresh hen's egg), and 2 mL of a sample was prepared. Double distilled water and diclofenac sodium were used as the negative and positive controls, respectively. The mixtures were first incubated at 37°C for 15 min in the incubator and then heated in the water bath at 70°C for 5 min. After cooling down to room temperature, the absorbance of the mixtures was measured at 660 nm using a UV-vis spectrophotometer (Hitachi U-2000, COAX Group Corporation Ltd., Bangkok, Thailand). The percentage inhibition of protein denaturation was calculated using equation (2):

$$\% \text{ inhibition} = [(A_{nc} - A_s) / A_{nc}] \times 100 \quad (2)$$

where: A_{nc} is a negative control absorbance; A_s is a sample absorbance.

Antimicrobial activity

The antimicrobial activities of curcumin-loaded ISI formulations were evaluated against standard microbes (*Staphylococcus aureus* ATCC 6538, Methicillin-resistant *Staphylococcus aureus* (MRSA) (*Staphylococcus aureus* ATCC 4430), *Candida albicans* 10231, *Porphyromonas gingivalis* ATCC 33277, and *Aggregatibacter actinomycetemcomitans* ATCC 29522) using the agar cup diffusion method (Phaechamud *et al.*, 2018b). The bacteria inocula were incubated for 36 h in tryptic soy broth (TSB), whereas the bacteria and fungi were incubated in SDB. The turbidity of the broth suspensions of organisms was checked using the 0.5 McFarland standard. Then, the prepared broth suspensions of *S. aureus* ATCC 6538 and MRSA, were swab spread on the tryptic soy agar plates, whereas sheep blood and chocolate agar were used as the media for the antimicrobial testing of *P. gingivalis* and *A. actinomycetemcomitans*, respectively. The inoculum of calibrated *C. albicans* was swab spread on SDA. Sterilized cylindrical cups were carefully placed on the surface of the swabbed agar. A 200 μ L aliquot of the prepared ISGs was added to the cup and incubated at 37°C for 24 h. The antimicrobial test against these two anaerobic bacteria was conducted in an anaerobic incubator (Forma Anaerobic System, Thermo Scientific, OH, USA) at 37°C for 72 h. The antimicrobial activities

were measured as the diameter (mm) of the inhibition zone using a ruler ($n = 3$).

Statistical analysis

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

Results and Discussion

Appearance of curcumin extract and ISI solution

Curcumin solution at 5%w/w and ISI solution was easily prepared into amber solutions by mixing in short time during preparation using DMSO and NMP as solvents. Increasing concentration of curcumin to 40%w/w the preparation was harder than 5%w/w because the solution was supremely higher viscous, their color was dark yellowish almost dark brown solution owing to the color characteristic of curcumin extract.

Physicochemical properties

Density

The density values for all the ISI formulations were measured to assess their suitability for injection (Figure 1a). The C40D and C40N formulations, containing 40% curcumin, had densities of $1.1613 \pm 0.0010 \text{ g/cm}^3$ and $1.1210 \pm 0.0009 \text{ g/cm}^3$, respectively, which were significantly higher than the lower concentration formulations CN ($1.08 \pm 0.02 \text{ g/cm}^3$) and CD ($1.07 \pm 0.01 \text{ g/cm}^3$) ($p < 0.05$). This indicates that increasing curcumin content contributes to higher density, which could impact the retention of the ISI at the injection site, as denser formulations are less likely to migrate.

Formulations without curcumin (BN, BD, IN, ID) showed lower densities overall, with BN and BD having densities of $1.0068 \pm 0.0011 \text{ g/cm}^3$ and $1.0438 \pm 0.0016 \text{ g/cm}^3$, respectively (Li *et al.*, 2013). These non-curcumin formulations, while exhibiting good density for injectability, may not offer the same level of stability and localized retention as their curcumin-containing counterparts.

In comparison, BOR-containing formulations, such as CBN ($1.05 \pm 0.01 \text{ g/cm}^3$) and CBD ($1.06 \pm 0.02 \text{ g/cm}^3$), had densities lower than IBU-containing systems like CIN ($1.0399 \pm 0.0005 \text{ g/cm}^3$) and CID ($1.0716 \pm 0.0002 \text{ g/cm}^3$). This difference is statistically significant ($p < 0.05$), indicating that IBU-based formulations may be better suited for localized applications due to their

slightly higher density, ensuring that the matrix remains stable at the application site.

Viscosity

The viscosity of each formulation was measured to evaluate their injectability and ease of administration (Figure 1b). Higher curcumin concentrations (C40N and C40D) exhibited viscosities of 222.30 ± 1.22 and 123.63 ± 0.61 cPs, significantly higher than the lower concentration formulations (CN and CD), which had viscosities of 5.99 ± 0.80 and 4.72 ± 0.59 cPs, respectively ($p < 0.05$). This increase in viscosity at higher curcumin concentrations aligns with previous studies on polymer-based ISG systems (e.g. polylactic acid (PLA), polylactic acid-co-glycolic acid (PLGA), Eudragid RS, and nitrocellulose) (Senarat *et al.*, 2023a,b; Gad *et al.*, 2008), which demonstrated that higher matrix former agents loading leads to increased viscosity due to the interaction between these loaded-molecules and the solvents (Khaing *et al.*, 2024).

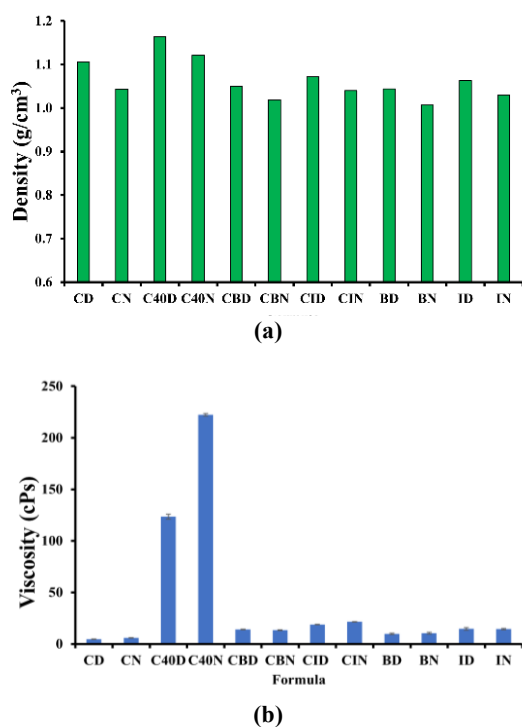


Figure 1. Density (a) and viscosity (b) property of curcumin solution, curcumin loaded and unloaded ISI

Additionally, the curcumin-free formulations, BN, BD, IN and ID, also showed slightly low viscosity of 10.38 ± 0.30 , 9.88 ± 0.50 , 14.64 ± 0.38 and 14.45 ± 0.67 cPs, respectively, further

confirming BOR and IBU's role in maintaining fluidity in the ISI system.

BOR-based systems such as CBN (13.43 ± 0.61 cPs) and CBD (14.09 ± 0.57 cPs) exhibited significantly lower viscosities compared to IBU-based formulations CIN (21.56 ± 0.68 cPs) and CID (18.85 ± 0.63 cPs, $p < 0.05$). However, both of curcumin-loaded IBU and BOR-based ISI presented remarkably low viscosity (< 20 cPs) because of the low density, small size, and unsophisticated structure suggests that BOR and IBU enhances injectability, making the formulations easier to administer without compromising the gelation process (Grimling *et al.*, 2015; Irvine *et al.*, 2018; Chen *et al.*, 2019).

Phase transformation of ISIs

Macroscopic level

At the macroscopic level, the phase transformation of various curcumin-loaded and non-curcumin-loaded ISI formulations was evaluated after exposure to PBS (pH 6.8). At higher curcumin concentrations, such as 40% w/w (C40D and C40N), gel matrix formation occurred within 5 minutes. This transformation is likely driven by curcumin's hydrophobic nature, which promotes phase separation in aqueous environments. In contrast, the 5% w/w curcumin formulations, CN and CD, did not exhibit noticeable matrix formation, emphasizing that a higher curcumin concentration was necessary for self-initiated phase transitions driven by hydrophobic interactions.

The inclusion of BOR and IBU in the lower concentration curcumin systems played a critical role in facilitating matrix formation. BOR-containing formulations, such as CBN and CBD, achieved faster gelation within 5 minutes whereas IBU-based systems, CIN and CID, took around 10 minutes for full gelation. BOR's hydrophobic properties appear to enhance solvent exchange (Phaechamud *et al.*, 2018c), resulting in quicker gel formation compared to IBU, which likely slowed down matrix formation due to its higher viscosity. The difference in solvent exchange rates between BOR and IBU can be explained by the viscosity and hydrophobic nature of the additives, with BOR promoting faster solvent interaction than IBU (Lertsuphotvanit *et al.*, 2022; Puyathorn *et al.*, 2023a).

The viscosity data provides additional insight into this process. BOR-based formulations exhibited significantly lower viscosity, which allowed faster and more uniform solvent exchange, leading to more homogeneous gel formation. In contrast, IBU-based formulations (CID and CIN) displayed higher viscosities, resulting in slower solvent exchange and less uniform matrices. This

suggests that the choice of matrix forming agent greatly influences gelation speed and uniformity.

Interestingly, the curcumin-free formulations (BN, BD, IN, ID) showed faster gelation compared to their curcumin-containing counterparts, implying that the presence of curcumin slows the phase transformation. This delay may be due to curcumin increasing the overall viscosity of the ISI system, which hinders solvent exchange to some extent.

When comparing different solvents, formulations in DMSO exhibited faster matrix transformation than those in NMP. This is likely due to DMSO's better miscibility with aqueous media, as reflected in its higher LogP value (-1.35) compared to NMP (-0.38) (Brayton, 1986 ; Jouyban *et al.*, 2010), facilitating more rapid solvent exchange.

Although the density does not directly affect the matrix formation rate, it influences the behavior of ISI systems at the administration site. Formulations with higher densities, such as C40D and C40N, tended to sink more quickly in PBS, which suggests they might remain stable at the application site. Conversely, lower density formulations may float, potentially affecting their positioning in biological environments (Chantadee *et al.*, 2021). Interestingly, despite the lower density of CBD and CID, these formulations did not float because they quickly transformed into solids and adhered to the target site. This provides valuable

insights into predicting how these ISI formulations behave post-administration.

Overall, matrix formation dynamics are heavily influenced by the properties of solvent exchange, the choice of additives, and solvent type. BOR's hydrophobicity and lower viscosity allowed for faster, more efficient gelation (Chantadee *et al.*, 2020), while IBU promoted a slower, less uniform matrix formation. The concentration of curcumin and its interaction with the matrix further added complexity to the phase transition process.

Microscopic level

At the microscopic level, cross-sectional images (Figure 3) clearly demonstrated the differing matrix behaviors between BOR- and IBU-based formulations. The colored regions in these cross-sectional images represent different stages of solvent exchange and matrix solidification including yellow, orange, and clear brown areas. In the curcumin solution, the yellow gradient region showed the low concentration of curcumin diffusing out to the environment. At the interface region, the presentation of light turbid yellow represents the formation of a solid matrix and entrapped curcumin inside both BOR- and IBU-containing systems, while the clear brown-orange solution at the center showed untransformed ISI solution.

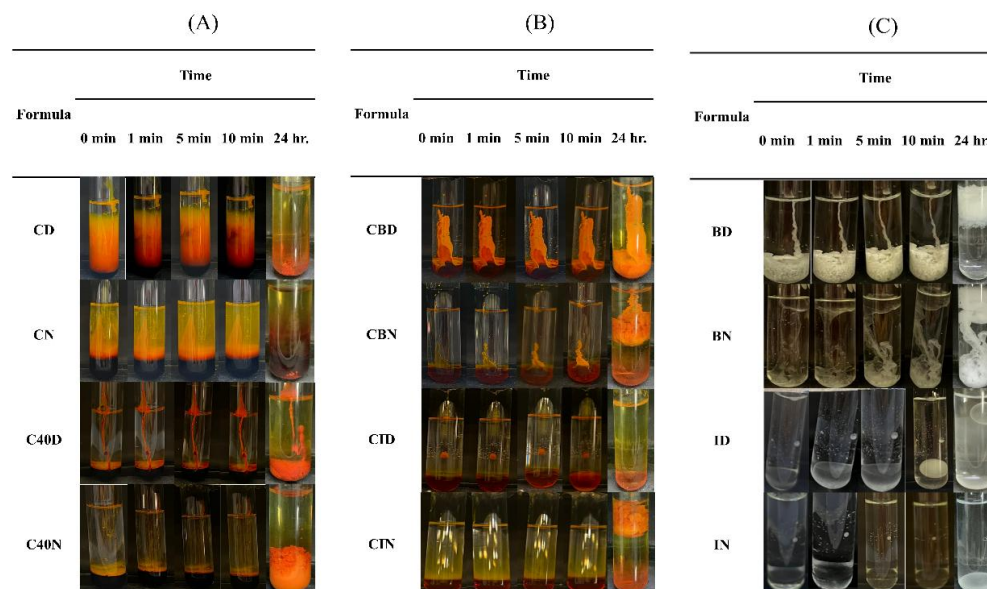
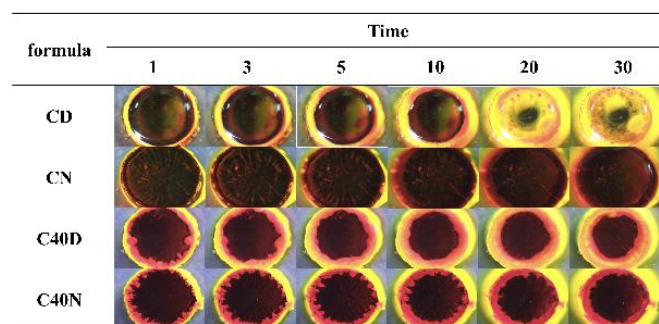
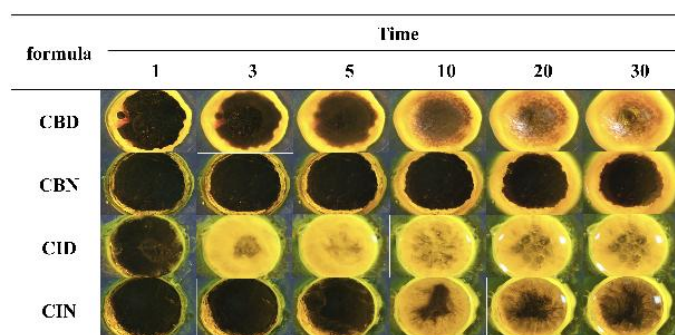


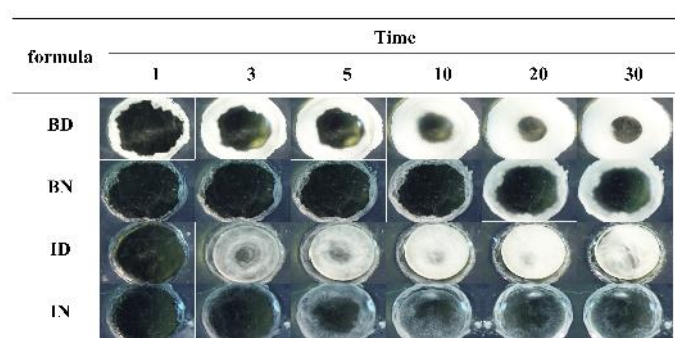
Figure 2. Phase transformation behavior of curcumin solution and ISIs system in PBS pH 6.8



(a)



(b)



(c)

Figure 3. Cross-sectional view of matrix formation of each ISI formula in cylindrical agarose well

BOR-containing systems (CBN and CBD) produced more uniform and denser matrices with well-defined interfaces between the gel and surrounding media. This can be attributed to BOR's enhancement of solvent exchange, which results in quicker and more cohesive matrix formation. In contrast, IBU-based systems (CIN and CID) exhibited slower and less uniform gelation, with more irregular matrices, likely due to IBU's higher viscosity and slower solvent diffusion.

These findings align with the viscosity measurements, where BOR-based formulations had lower viscosities, promoting quicker and more homogeneous solvent exchange. The slower matrix formation observed in IBU-based systems, due to

their higher viscosities, resulted in less consistent gel structures.

Furthermore, previous studies on the water sensitivity profiles of BOR- and IBU-based ISI systems reported %water tolerance values. For 40% w/w IBU in DMSO and NMP, the values were 16.06% and 18.43%, respectively (Puyathorn *et al.*, 2023c), while 40% w/w BOR in NMP exhibited a value of 12.76% (Puyathorn *et al.*, 2023a). These results suggest that IBU-based formulations are less sensitive to water, delaying the phase transformation process, especially in NMP-based systems, which further supports the slower gelation observed in our study.

In summary, BOR significantly accelerates matrix formation by promoting faster and more uniform solvent exchange, making it an ideal additive for efficient gelation in curcumin-loaded ISI systems. IBU, while still effective in promoting gel formation, leads to slower and less uniform matrices, which may affect the consistency of drug release.

The feasibility of these ISI systems for practical applications appears promising, particularly for localized drug delivery. Both BOR- and IBU-based systems demonstrated rapid and uniform gelation, making them suitable for applications requiring quick matrix formation and sustained release, such as in the treatment of periodontitis or localized infections. The lower viscosity of these formulations allows for easy injection (Rungseevijitprapa and Bodmeier, 2009), while rapid matrix formation ensures the efficient creation of drug depots at the target site.

These findings reinforce the feasibility of using these ISI systems for therapeutic applications where controlled and localized drug release is essential. Further studies on long-term stability and drug release kinetics would help confirm their suitability for clinical use.

Bioactivity Study

Anti-inflammatory Activity

The anti-inflammatory effects of the ISI formulations were evaluated using the protein denaturation assay, as shown in Figure 4a. All curcumin-loaded ISI formulations significantly induced protein denaturation, evidenced by the absorbance values of denatured albumin increasing by approximately 100% compared to the negative control. This suggests that curcumin-loaded ISIs may promote albumin denaturation, potentially due to the properties of the ISI components or the high concentration of ISI used during the assay.

Previous studies on IBU-based in situ gels have demonstrated effective anti-inflammatory activity, particularly in preventing protein denaturation (Puyathorn *et al.*, 2023a). Additionally, NMP, BOR and curcumin are known to enhance the anti-inflammatory effects of active compounds by inhibiting pro-inflammatory cytokines, such as TNF- α and IL-6 (Jin *et al.*, 2020; Peng *et al.*, 2021; Chaiya *et al.*, 2022; Urošević *et al.*, 2022). To fully validate these initial findings, further research involving cytokine inhibition assays (*in vitro*) or *in vivo* models is necessary to confirm the anti-inflammatory potential of curcumin-loaded ISIs.

Antioxidation Activity

The antioxidant activity of the curcumin-loaded ISI formulations was measured using the DPPH radical scavenging assay, as depicted in Figure 4b. The CBN formulation exhibited significantly higher antioxidant activity ($15.64 \pm 1.76\%$) compared to the CIN formulation ($10.54 \pm 2.31\%$), with the difference being statistically significant ($p < 0.5$). This elevated antioxidant activity in the CBN formulation can be attributed to BOR's inherent antioxidant properties, which work synergistically with curcumin to enhance free radical scavenging. These findings align with previous research by Lei *et al.* (2011), which showed similar increases in antioxidant activity of D-borneol against DPPH scavenging assay.

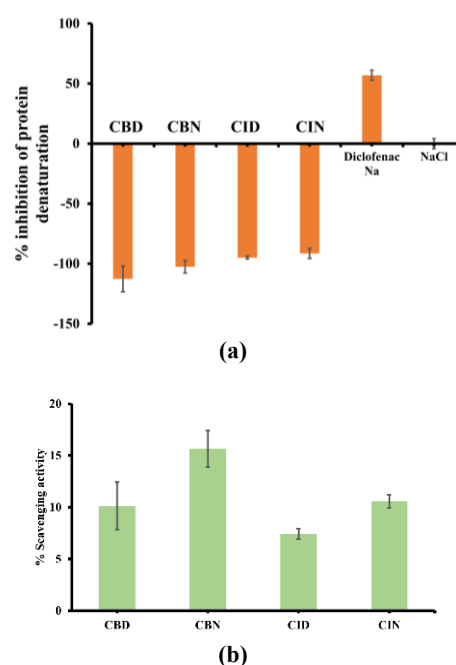


Figure 4. Anti-inflammation and antimicrobial activities of curcumin-loaded ISI systems

The strong antioxidant effect of borneol-based formulations is particularly beneficial for conditions like periodontitis, where oxidative stress contributes significantly to tissue damage. The marked difference in antioxidant efficacy between CBN and CIN further emphasizes BOR's role as an effective enhancer of curcumin's antioxidant activity, solidifying its potential in ISI systems designed to mitigate oxidative stress-related conditions.

Antimicrobial study

The antimicrobial efficacy of the various ISI formulations was tested against key pathogens involved in periodontitis, including *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *Candida albicans*, *P. gingivalis*, and *A. actinomycetemcomitans* (Figures 5 and 6). Interestingly, the 40% curcumin formulations (C40D and C40N) exhibited no clear inhibition zones, except for C40N, which showed activity against *A. actinomycetemcomitans*. This contrasts with the 5% curcumin formulations (CN and CD), which demonstrated inhibition zones against all tested pathogen strains. For example, the CN formulation exhibited inhibition zones of 18.33 ± 0.6 mm against *P. gingivalis* and 47.00 ± 2.65 mm against *A. actinomycetemcomitans*, while CD showed inhibition zones of 13.33 ± 0.5 mm and 26.33 ± 1.5 mm against the same pathogens, respectively. The reduced antimicrobial activity observed in the 40% curcumin formulations is

likely due to the extremely high viscosity, which restricts the diffusion of active components into the environment, thereby limiting their bactericidal effects. This finding supports the concept that excessive viscosity can hinder the release and diffusion of curcumin, reducing its overall antimicrobial potential.

Among the BOR- and IBU-based systems, CBN and CBD formulations exhibited lower antimicrobial activity compared to CIN and CID. For example, CBN exhibited inhibition zones of 15.67 ± 0.5 mm and 13.67 ± 1.15 mm against *S. aureus* and MRSA, respectively, while CIN showed larger inhibition zones (18.67 ± 0.6 mm for *S. aureus* and 19.00 ± 1.00 mm for MRSA, $p < 0.05$). This difference in activity may be attributed to two factors: (1) IBU allows faster diffusion of curcumin and other components compared to BOR, and (2) IBU itself has inherent antimicrobial activity, which is stronger than that of borneol.

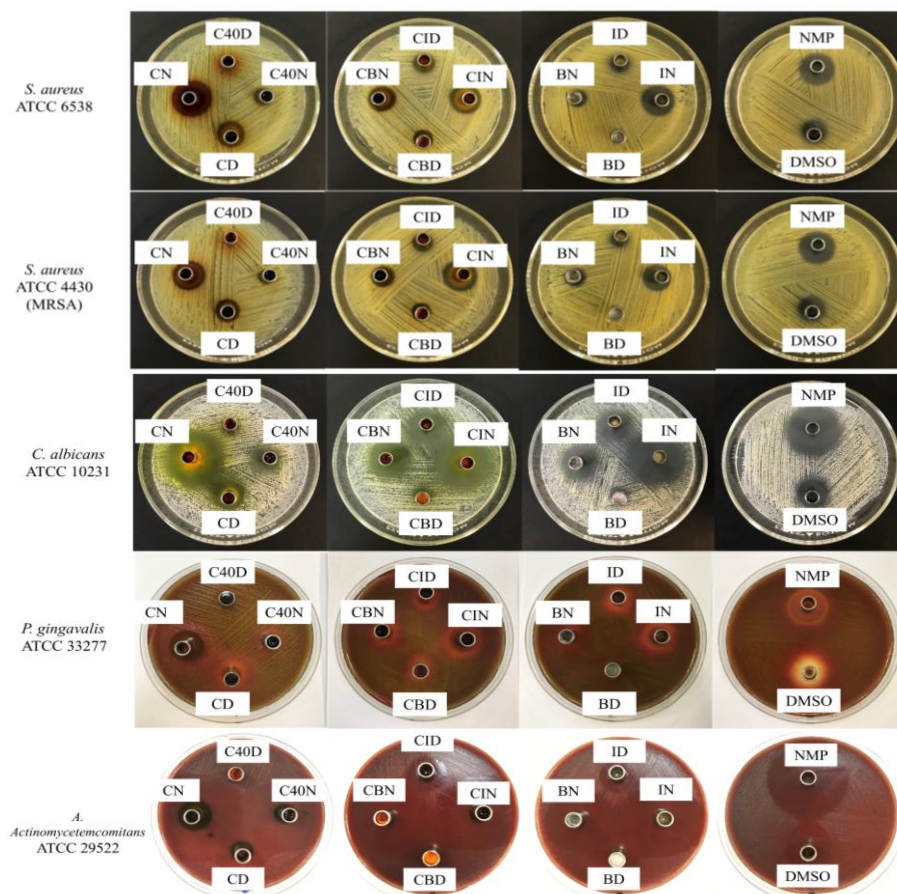


Figure 5. Inhibition zone of curcumin-loaded ISI systems against *S. aureus* (ATCC 6538), MRSA (ATCC 4430), *C. albicans* (ATCC 10231), *P. gingivalis* (ATCC 33277) and *A. actinomycetemcomitans* (ATCC 29522)

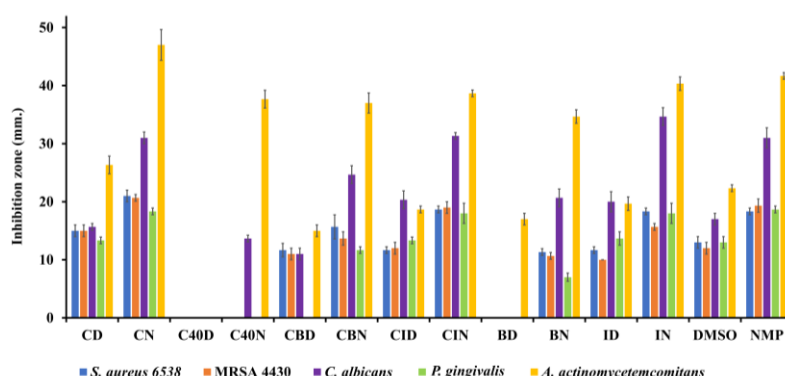


Figure 6. Clear zone diameter value of curcumin-loaded ISI systems against *S. aureus* (ATCC 6538), MRSA (ATCC 4430), *C. albicans* (ATCC 10231), *P. gingivalis* ATCC 33277 and *A. actinomycetemcomitans* ATCC 29522

Interestingly, in NMP-based formulations, both BOR-based and IBU-based ISIs showed greater potency against *C. albicans* than their DMSO counterparts. The inhibition zones for CBN, CIN, CBD, and CID against *C. albicans* were 24.67 ± 1.53 mm, 31.33 ± 0.58 mm, 11.00 ± 1.00 mm, and 20.33 ± 1.53 mm, respectively. These trends align with previous studies, which suggest that NMP enhances cell permeability, thereby boosting antifungal activity (Saw *et al.*, 2007; Phaechamud *et al.*, 2012; Rein *et al.*, 2020).

Curcumin-free BOR-ISI formulations (BN and BD) exhibited reduced antimicrobial activity against *S. aureus* and MRSA, confirming that the antimicrobial effects in CBD and CBN formulations are primarily due to curcumin. Conversely, the IBU-based formulations (IN and ID) displayed antimicrobial activity similar to CID and CIN, indicating that IBU's intrinsic antimicrobial properties dominate over curcumin's activity. This is consistent with findings from Puyathorn *et al.* (2023a), which also highlighted the strong antimicrobial effects of IBU.

These results suggest that both BOR and IBU slow the diffusion of curcumin and other solvents, which helps sustain antimicrobial activity over time. This indicates the potential of ISI systems to control curcumin release, supporting the feasibility of using these systems for sustained drug delivery, particularly in targeting pathogens involved in periodontitis. Future studies should focus on conducting drug release experiments to further validate the controlled release properties of these formulations.

This controlled antimicrobial activity also demonstrates the feasibility of using ISI systems for sustained drug release in clinical settings. Exploring the drug release profiles further could help optimize these formulations for more effective treatment of

periodontal pathogens and other localized infections.

Conclusions

This study investigated the potential of curcumin-loaded ISI systems incorporating BOR and IBU, focusing on their physicochemical properties, phase transformation behavior, and bioactivity. The results demonstrated that these ISI systems offer promising platforms for localized drug delivery, with significant advantages in controlling drug release and promoting therapeutic effects.

The phase transformation studies revealed that the solvent exchange process and the viscosity of the formulations are critical factors influencing matrix formation. At higher curcumin concentrations (40% w/w), the gelation process was slower and less uniform, likely due to increased viscosity hindering solvent diffusion. In contrast, 5% w/w curcumin formulations displayed faster matrix formation. BOR-containing ISI systems demonstrated quicker and more uniform gelation compared to IBU-based formulations, attributed to borneol's lower viscosity and enhanced solvent exchange properties. The solvent type also influenced phase transformation, with DMSO-based formulations showing faster gelation compared to NMP-based systems. These findings highlight the need to optimize viscosity and solvent compatibility for efficient gel formation and stability in clinical applications. The physicochemical properties of the formulations were closely linked to their phase transformation behavior. Lower viscosity formulations promoted faster solvent exchange, resulting in more homogenous matrix formation. Density played a key role in the stability of the ISI systems at the

application site, with higher density formulations sinking more quickly, ensuring stability in the target area. Importantly, both borneol and ibuprofen helped modulate the phase transformation process, with borneol facilitating faster and more uniform matrix formation and ibuprofen contributing to slower, controlled gelation.

In terms of bioactivity, curcumin-loaded ISIs demonstrated induced protein denaturation, effects, while BOR enhanced curcumin's antioxidant properties, making it suitable for addressing oxidative stress in conditions such as periodontitis. The antimicrobial studies revealed that lower curcumin concentrations (5% w/w) were more effective, with high-viscosity formulations at 40% w/w limiting the diffusion of active agents. IBU-based ISIs exhibited greater antimicrobial activity than BOR-based systems, likely due to IBU's inherent antimicrobial effects, and NMP-based formulations further enhanced efficacy through improved cell permeability. Overall, the curcumin-loaded ISI systems, particularly those incorporating BOR and IBU, offer significant promise for localized drug delivery. Their ability to control drug release, combined with favorable physicochemical properties and bioactivity, suggests strong potential for treating localized infections and inflammatory conditions, including periodontitis. Future studies should focus on optimizing the balance between viscosity, solvent exchange, and curcumin concentration to achieve sustained release and enhanced clinical outcomes. *In vivo* studies and long-term stability assessments will be essential for confirming the clinical viability of these formulations.

Acknowledgements

The authors would like to express their gratitude to the Faculty of Pharmacy, Chiang Mai University, the Faculty of Pharmaceutical Sciences, Burapha University and the Faculty of Pharmacy at Silpakorn University, for facility and support. This research was funded by the Thailand Science Research and Innovation (TSRI) National Science, Research, and Innovation Fund (NSRF), as the Fundamental Fund (Fiscal Year 2024), Grant No. 195979.

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