UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

MÔNICA BEATRIZ PORTELA FERREIRA

FORMULAÇÃO DE LIDOCAÍNA INCORPORADA EM SISTEMA LÍQUIDO CRISTALINO À BASE DE ÓLEO DE SEMENTE DE UVA E ÁCIDO HIALURÔNICO PARA APLICAÇÃO TÓPICA EM LESÕES DE MUCOSITE ORAL

FORMULATION OF LIDOCAINE INCORPORATED IN LIQUID-CRYSTALLINE SYSTEM BASED ON GRAPE SEED OIL AND HYALURONIC ACID FOR TOPICAL APPLICATION IN ORAL MUCOSITIS LESIONS

PIRACICABA
2024

MÔNICA BEATRIZ PORTELA FERREIRA

FORMULAÇÃO DE LIDOCAÍNA INCORPORADA EM SISTEMA LÍQUIDO CRISTALINO À BASE DE ÓLEO DE SEMENTE DE UVA E ÁCIDO HIALURÔNICO PARA APLICAÇÃO TÓPICA EM LESÕES DE MUCOSITE ORAL

FORMULATION OF LIDOCAINE INCORPORATED IN LIQUID-CRYSTALLINE SYSTEM BASED ON GRAPE SEED OIL AND HYALURONIC ACID FOR TOPICAL APPLICATION IN ORAL MUCOSITIS LESIONS

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Odontologia, na Área de Farmacologia, Anestesiologia e Terapêutica.

Orientadora: Profa. Dra. Michelle Franz Montan Braga Leite

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA DISSERTAÇÃO DEFENDIDA PELA ALUNA MÔNICA BEATRIZ PORTELA FERREIRA, E ORIENTADA PELA PROFA. DRA. MICHELLE FRANZ BRAGA LEITE

PIRACICABA

2024

Ficha catalográfica
Universidade Estadual de Campinas (UNICAMP)
Biblioteca da Faculdade de Odontologia de Piracicaba
Marilene Girello - CRB 8/6159

F413f

Ferreira, Mônica Beatriz Portela, 1992-
Formulação de lidocaína incorporada em sistema líquido cristalino à base de óleo de semente de uva e ácido hialurônico para aplicação tópica em lesões de mucosite oral / Mônica Beatriz Portela Ferreira. – Piracicaba, SP : [s.n.], 2024.

Orientador: Michelle Franz Montan Braga Leite.
Dissertação (mestrado) – Universidade Estadual de Campinas (UNICAMP), Faculdade de Odontologia de Piracicaba.

1. Estomatite. 2. Cristais líquidos. 3. Lidocaína. 4. Produtos naturais. 5. Ácido hialurônico. I. Franz-Montan, Michelle, 1982-. II. Universidade Estadual de Campinas (UNICAMP). Faculdade de Odontologia de Piracicaba. III. Título.

Informações Complementares

Título em outro idioma: Formulation of lidocaine incorporated in liquid-crystalline system based on grape seed oil and hyaluronic acid for topical application in oral mucositis lesions
Palavras-chave em inglês:
Stomatitis
Liquid crystals
Lidocaine
Natural products
Hyaluronic acid
Área de concentração: Farmacologia, Anestesiologia e Terapêutica
Titulação: Mestra em Odontologia
Banca examinadora:
Michelle Franz Montan Braga Leite [Orientador]
Marcela Tavares Luiz
Gislaine Ricci Leonardi
Data de defesa: 21-06-2024
Programa de Pós-Graduação: Odontologia

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-8896-700X
- Currículo Lattes do autor: http://lattes.cnpq.br/3396851168843573

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Dissertação de Mestrado, em sessão pública realizada em 21 de junho de 2024, considerou a candidata MÔNICA BEATRIZ PORTELA FERREIRA aprovada.

PROFª. DRª. MICHELLE FRANZ MONTAN BRAGA LEITE

PROFª. DRª. MARCELA TAVARES LUIZ

PROFª. DRª. GISLAINE RICCI LEONARDI

A Ata da defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

DEDICATÓRIA

Dedico este trabalho à minha mãe, que bravamente lutou e venceu o câncer e ao meu filho, que superou, juntamente comigo, as adversidades para o desenvolvimento desta pesquisa. Eles são minha força e minha motivação.

AGRADECIMENTOS

À **Universidade Estadual de Campinas (UNICAMP)**, na pessoa do Magnífico Reitor Prof. Dr. Antônio José de Almeida Meirelles, à **Faculdade de Odontologia de Piracicaba (FOP)**, na pessoa de seu diretor Prof. Dr. Flávio Henrique Baggio Aguiar, à Comissão de **Pós-Graduação (CPG) da FOP-UNICAMP,** na pessoa do presidente Prof. Dr. Valentim Adelino Ricardo Barão, à Comissão do **Programa de Pós-Graduação em Odontologia**, na pessoa do coordenador Prof. Antônio Pedro Ricomini Filho.

Ao **Departamento de Biociências da FOP-UNICAMP**, na pessoa do seu Chefe Prof. Dr. Sergio Roberto Peres Line.

Aos professores da **Área de Fisiologia e Biofísica**, Profa. Dra. Fernanda Klein Marcondes e Prof. Dr. Yuri Martins Costa, por todo o aprendizado durante a minha trajetória.

Aos professores da **Área de Farmacologia, Anestesiologia e Terapêutica**, Prof. Dr. Francisco Carlos Groppo, Prof. Dr. Pedro Luiz Rosalen e Prof. Dr. Sidney Figueroba Raimundo, pelos ensinamentos durante a minha formação.

À **Divisão de Educação Infantil e Complementar da FOP-UNICAMP** (DEdIC), e às professoras da creche da UNICAMP, por toda a atenção ao filho durante o período na FOP.

Ao Prof. Dr. Marlus Chorilli Profa.e à Profa. Dra. Aline Martins dos Santos e à Faculdade de Ciências Farmacêuticas – Universidade Estadual Paulista "Júlio de Mesquita Filho, pelo auxílio no desenvolvimento dos ensaios de reologia das formulações do presente estudo.

Às professoras das bancas de qualificação de primeira fase, Profa. Dra. Jessica Bernegossi e Profa. Larissa Bueno Tofani, bem como às professoras da banca de qualificação de segunda fase, Profa. Dra. Raquel Petrilli Eloy, Profa. Dra. Francesca Victorelli e Profa. Alexsandra Conceição Apolinário, pelas valiosas e excelentes contribuições.

Ao técnico do laboratório de Fisiologia Fábio Padilha, pela parceria, auxílio e pelas viagens com muita conversa até o Frigorífico.

Ao Laboratório Cristália, pela doação do anestésico local utilizado no estudo.

Às agências de fomento à pesquisa. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – código de financiamento 001, Fundação de Desenvolvimento da UNICAMP (FAEPEX), por meio do Processo nº 3454/23 (Auxílio Ponte) e da Fundação de Amparo à Pesquisa do Estado de São Paulo, por meio do Auxílio Regular à Pesquisa (FAPESP, Processo # 2020/03786-9).

AGRADECIMENTOS ESPECIAIS

À minha orientadora, Profa. Dra. Michelle Leite, que me inspirou desde a sua escolha, sendo um exemplo de pesquisadora, professora e mãe e que com sua orientação e extrema compreensão, contribuiu imensamente para o meu crescimento profissional e pessoal.

À Profa. Dra. Giovana Calixto, a minha outra inspiração, também como pesquisadora e como mãe, a quem devo o fascínio pelos cristais líquidos, por todos os ensinamentos, orientação, bem como por todas as sugestões e suporte nas dúvidas.

Ambas serão, para sempre, minhas *professoras fascinantes*, que como aborda Augusto Cury, são aqueles professores que marcam e ensinam para a vida. Elas me mostraram que é possível conciliar sonhos, carreira e maternidade.

Aos meus pais, Tânia e Aguiar, por todo apoio nas minhas escolhas, pelo suporte que sempre ofereceram mesmo nas dificuldades para garantir meu crescimento. Devo tudo a eles.

Ao meu marido, por todo amparo, em todos os aspectos e por toda ajuda e compreensão para que eu pudesse alcançar os tão almejados e sonhados objetivos profissionais.

Ao meu filho, que tão pequeno, me acompanhou na jornada estando longe de casa, mas sendo minha melhor motivação para continuar. Sempre foi, é e continuará sendo meu fiel "companheirinho", que me dá forças e alegra meus dias. Sempre será a minha motivação!

Aos meus irmãos, Thicianne, Luana e Leonardo pelo apoio e preocupação diária, bem como pelo companheirismo da vida.

Aos colegas da pós-graduação, Aylla Pestana, Arthur Antunes, Iago Cortês, Bárbara Prohmann, Carla Arita, Suéllen Meneghin pelo convívio diário em aulas, na monitoria PED e no laboratório, bem como à graduanda Ana Luiza, pela empreitada em conjunto no estudo dos cristais líquidos.

À Aylla Pestana, pelo suporte diário, orientações, pelo auxílio imensurável para o desenvolvimento do estudo e por todo apoio, inclusive em questões pessoais.

À Arthur Antunes, por toda a contribuição inestimável para o desenvolvimento da pesquisa, pelas orientações e apoio. E a ambos pelos momentos de descontração e conversas.

À Fabio Padilha, pelas conversas, risos e pelo essencial e fundamental auxílio na execução da pesquisa.

EPÍGRAFE

*“*O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis... *”*

José de Alencar

RESUMO

A mucosite oral (MO) é uma inflamação comum entre pacientes em tratamento oncológico, como radioterapia e quimioterapia, causando desconforto significativo. A ausência de tratamento padronizado e os resultados lentos podem prejudicar e até interromper o tratamento do câncer. Por isso, há uma necessidade constante de alternativas terapêuticas mais eficazes e acessíveis. Neste contexto, os derivados da uva, com propriedades antioxidantes, anti-inflamatórias e cicatrizantes, combinados com o ácido hialurônico, que hidrata e favorece a regeneração tecidual, e a lidocaína, um anestésico local seguro, são componentes promissores. Os sistemas líquidos cristalinos (SLC) são sistemas de liberação capazes de aumentar a permeação de fármacos e apresentam alta mucoadesividade. Este estudo desenvolveu e caracterizou uma formulação contendo lidocaína, óleo de semente de uva e ácido hialurônico em SLC, para tratamento da MO. A formulação foi determinada por um diagrama de fases e caracterizada usando técnicas como microscopia de luz polarizada, estudos reológicos, cinética de liberação *in vitro*, capacidade de permeação e mucoadesão *in vitro*, além de testes de toxicidade *in vivo* em membrana corioalantóica (CAM) de embriões de galinha. A formulação que apresentou estrutura SLC hexagonal mesofásica considerada ideal para a formulação proposta apresentou a composição de óleo de semente de uva:ácido oleico (4:1), 40% PPG-5-CETETH-20 e 20% ácido hialurônico com 5% lidocaína e comportou-se como fluido newtoniano, com boa espalhabilidade e fácil aplicação, com aumento da viscosidade ao contato com a saliva, contribuindo para a mucoadesão. A cinética de liberação ajustou-se ao modelo de *Weibull*, indicando múltiplos processos de liberação. O anestésico da formulação permeou a mucosa suína mais lentamente, mas com início imediato, com fluxo em estado estacionário e tempo de latência menores comparados à formulação comercial ($p<0,05$). Sua toxicidade *in vivo* foi semelhante à formulação comercial. Em conclusão, o estudo desenvolveu uma formulação inovadora com propriedades ideais para aplicação clínica, oferecendo uma alternativa para o tratamento da MO, com componentes que apresentam potencial para promover alívio sintomático e cicatrizante, os quais, se confirmados, podem melhorar a qualidade de vida de pacientes em tratamento oncológico.

Palavras-chaves: estomatite, lidocaína, cristais líquidos, produtos naturais, ácido hialurônico, sistema de liberação de fármacos.

ABSTRACT

Oral mucositis (OM) is a common inflammation among patients undergoing oncological treatment, such as radiotherapy and chemotherapy, causing significant discomfort. The absence of standardized treatment and slow results can hinder and even interrupt cancer treatment. Therefore, there is a constant need for more effective and accessible therapeutic alternatives. In this context, grape derivatives, with antioxidant, anti-inflammatory, and healing properties, combined with hyaluronic acid, which hydrates and promotes tissue regeneration, and lidocaine, a safe local anesthetic, are promising components. Liquid crystalline systems (LCS) are drug delivery systems capable of increasing drug permeation and exhibit high mucoadhesion. This study developed and characterized a formulation containing lidocaine, grape seed oil, and hyaluronic acid in LCS for the treatment of OM. The formulation was determined by a phase diagram and characterized using techniques such as polarized light microscopy, rheological studies, *in vitro* release kinetics, *in vitro* permeation and mucoadhesion capacity, and *in vivo* toxicity tests on the chorioallantoic membrane (CAM) of chicken embryos. The formulation that presented an ideal hexagonal mesophase LCS structure had a composition of grape seed oil:oleic acid (4:1), 40% PPG-5-CETETH-20, and 20% hyaluronic acid with 5% lidocaine and behaved as a Newtonian fluid with good spreadability and easy application, with increased viscosity upon contact with saliva, contributing to mucoadhesion. The release kinetics fit the Weibull model, indicating multiple release processes. The anesthetic in the formulation permeated porcine mucosa more slowly but with immediate onset, with a steady-state flux and lower latency time compared to the commercial formulation ($p<0.05$). It's *in vivo* toxicity was similar to the commercial formulation. In conclusion, the study developed an innovative formulation with ideal properties for clinical application, offering an alternative for the treatment of OM, with components that have the potential to promote symptomatic relief and healing, which, if confirmed, can improve the quality of life of patients undergoing oncological treatment.

Keywords: Stomatitis, Lidocaine, Liquid Crystals, Biological Products, Hyaluronic Acid, Drug delivery.

SUMÁRIO

1. INTRODUÇÃO

A mucosite oral é uma inflamação aguda da mucosa oral que ocorre como consequência de terapia sistêmica para câncer e/ou radioterapia. Normalmente, pode afetar aproximadamente 40% dos pacientes em quimioterapia e cerca de 100% dos pacientes em tratamento radioterápico (INCA, 2023). O diagnóstico se baseia na história clínica e no exame físico, e geralmente, as lesões são muito dolorosas. O quadro clínico varia de eritema a ulceração irregular ou confluente com uma membrana pseudomembranosa superficial até lesões erosivas e ulceração ou, raramente, com áreas de necrose, que se manifestam em toda a cavidade bucal (Lalla et al., 2021).

Dependendo da gravidade das lesões, essa condição pode resultar em uma indesejada redução na dose e/ou uma interrupção na terapia contra o câncer (Lalla et al., 2021; Robijns et al., 2022), além de prejudicar o estado nutricional, diminuir a qualidade de vida do paciente e predispor o paciente a maior risco para infecções sistêmicas, conforme abordado a seguir (Fernandes et al., 2019).

Para melhor explicar o mecanismo de desenvolvimento da mucosite, Sonis (2004) propôs um modelo teórico que divide a evolução da lesão em cinco estágios: iniciação, sinalização, amplificação de sinal, ulceração e cicatrização. A fase de ulceração é conhecida por ser a mais morosa e intensa, pois ativa macrófagos a produzirem e liberarem citocinas pró-inflamatórias adicionais – IL-1, IL-6, IL-8 e TNF (Logan, Stringer, 2018). Dessa forma, especialmente durante a fase de ulceração, a mucosite oral provoca dor e grande dificuldade durante a mastigação e a deglutição e, consequentemente, resulta em uma nutrição extremamente deficiente. Além disso, a condição dolorosa também impacta na alteração do padrão de higiene oral e interfere nas funções orais básicas, podendo predispor a infecções oportunistas, como a candidose (Glenny et al., 2010; Walsh, 2010). Esse conjunto de complicações pode acarretar um prognóstico desfavorável para a doença com debilidade do paciente e, inclusive, interrupção do tratamento oncológico (Fernandes et al., 2019).

O tratamento atual da mucosite envolve várias abordagens, focando tanto na prevenção quanto no manejo dos sintomas. No entanto, ainda não há padrão estabelecido na literatura (Silva et al., 2023). Como medida preventiva, a crioterapia é utilizada durante a administração da quimioterapia para reduzir a absorção do agente quimioterápico pela mucosa oral, diminuindo o risco de mucosite (Liu et al., 2021). Outro método preventivo é o uso da palifermina, um fator de crescimento de queratinócitos utilizado principalmente para prevenir a mucosite em pacientes submetidos a transplante de células-tronco hematopoiéticas e

tratamento com altas doses de quimioterapia.Para o tratamento da mucosite já em desenvolvimento normalmente, são administradas várias opções terapêuticas, incluindo (i) fármacos antifúngicos: embora eficazes, apresentam uma resposta tardia e podem causar resistência antifúngica (Berto, Hermes, 2023). (ii) soluções de clorexidina: embora utilizadas, podem causar ardência e disgeusia e tem pouca efetividade, especialmente em casos de mucosite radioinduzida (Costa et al., 2023); (iii) laser de baixa potência: utilizado tanto na prevenção quanto no tratamento, promove uma melhora considerável das lesões em menor tempo. No entanto, é um recurso caro e que exige habilitação do profissional, o que limita seu acesso para todos os pacientes (Liu et al., 2021; Silva et al., 2023); (iv) suplementos e fitoterápicos: inclui a glutamina oral, usada para reduzir a incidência e severidade da mucosite em alguns pacientes; (v) Enxaguantes bucais com lidocaína (Colella et al., 2023) ou com sulfato morfina 0,2 % (Leal et al., 2024), ambos para o alívio da dor. (vi) benzidamina, um anti-inflamatório não esteroidal, é utilizada como enxaguante bucal para reduzir a inflamação e dor da mucosite em pacientes submetidos a radioterapia para câncer de cabeça e pescoço. No entanto, esses métodos apresentam resolução demorada (Colella et al., 2023).

Assim, torna-se necessário a busca de novas alternativas para o tratamento e alívio sintomático dessa doença com medicamentos eficazes e seguros. Nesse contexto, produtos naturais tem se destacado para diversos tratamentos, pois são recursos importantes na descoberta de novos metabólitos bioativos e em sua maioria, apresentam poucos efeitos colaterais (Zhou et al., 2022; Wang et al., 2024).

Dentre eles as uvas (*Vitis vinífera*) são consideradas uma das espécies frutíferas mais benéficas para saúde (Zhou et al., 2022), principalmente, por apresentarem ação antioxidante, cicatrizante, antialérgica, anti-inflamatória, anticancerígena, anti-hipertensiva e antimicrobiana devido à presença de polifenóis (Garavaglia et al., 2016; Jambi et al., 2019). Os principais polifenóis identificados no óleo de semente de uva são as catequinas, epicatequinas, trans-resveratrol, e procianidina B1 (Garavaglia et al., 2016), que são importantes metabólitos secundários da videira, os quais podem ser eficazes para o tratamento de diversas condições (Jambi et al., 2019; Rodriguez-Lopez et al., 2022).

Muitos estudos relataram que sua atividade anti-inflamatória, antibacteriana, antifúngica, antiviral ocorre por meio da ação direta contra patógenos orais ou pela inibição de fatores de virulência tais como a produção de toxinas, principalmente, aderência e invasão das células hospedeiras (Rahman et al., 2019), agindo diretamente sobre a membrana celular bacteriana, impedindo a divisão mitótica, causando desidratação nas células e impedindo a

sobrevivência de bactérias patogênicas (Idris et al., 2022). Esta ação ocorre por diversos mecanismos, incluindo a interrupção de processos metabólicos, como a formação da parede celular, levando à lise, a inibição da síntese de proteínas, o comprometimento do transporte de nutrientes, a interrupção da respiração celular e a interferência na comunicação celular das bactérias. No caso de fungos, pode afetar sua reprodução e, em vírus, pode interferir na sua replicação, impedindo sua multiplicação (Rahman et al., 2019; Singh et al., 2019).

Em relação às propriedades anti-inflamatórias, os polifenóis bloqueiam a ação de enzimas específicas, especialmente a isoforma COX-2 (cicloxigenase-2), no organismo humano, que causam inflamação na biossíntese dos eicosanóides (Idris et al., 2022). Em acréscimo, modificam as rotas metabólicas das prostaglandinas; protegem a agregação plaquetária e inibem a ativação de carcinógenos justificando sua capacidade de modular a resposta inflamatória e apresentar propriedades anticarcinogênicas (Idris et al., 2022; Singh et al., 2019; Franco, 2023; Santos et al., 2023).

Ademais, os derivados de uvas exercem um importante papel quimiotáxico para macrófagos, sendo fundamental na expressão de componentes do sistema fibrinolítico (regulação da produção de colagenase); favorece o debridamento autolítico no leito da ferida por contribuir com a produção de metaloproteinases, podendo acelerar o processo de cicatrização (Mirza et al., 2023).

Outrossim, essas fontes naturais permitem que estratégias farmacotécnicas possam ser aplicadas para melhorar a biodisponibilidade de seus metabólitos bioativos (Esteban-Fernández et al., 2017). Desta forma, o emprego do óleo de semente de uva como fase oleosa dos sistemas líquidos cristalinos (SLC) se destaca como uma possibilidade inovadora para o tratamento tópico da mucosite oral.

Sistemas líquido-cristalinos (SLCs), descritos pela primeira vez por Lehmann em 1889 e denominados "estado mesomórfico" por Friedel em 1922, representam um estado intermediário da matéria que exibe propriedades tanto de sólidos quanto de líquidos. Eles possuem ordem estrutural e rigidez típicas de sólidos e mobilidade e regiões desordenadas características dos líquidos. Os SLCs são divididos em duas classes principais: termotrópicos, que são formados sob a influência da temperatura e são menos estáveis, e liotrópicos, que consistem em misturas de compostos anfifílicos em solventes, geralmente água, formando mesofases como lamelar, hexagonal e cúbica (Chorilli et al., 2009).

Estes sistemas apresentam geleificação *in situ*, visto a absorção de água, quando em contato com a saliva, e no caso dos SLCs liotrópicos, os sistemas são formados pela mistura de

uma molécula anfifílica (tensoativo), como o polioxipropileno (5) poloxietileno (20) cetil éter, e solventes, como óleos e água, que dependendo de sua concentração, podem se organizar em mesofases do tipo lamelar, hexagonal ou cúbica (Benergossi et al., 2015; Salmazi et al., 2015; Chorilli et al., 2016; Calixto et al., 2021; Victorelli et al., 2023). Os SLC apresentam várias vantagens para utilização como meio de liberação de fármacos pois possuem área de superfície aumentada, maior biointeração com o tecido-alvo, mantém o efeito do fármaco no tecido, diminui o número de aplicações e doses e há redução de efeitos colaterais (Apolinário et al., 2020).

Nesse mérito, este sistema de liberação surge como uma valiosa estratégia, pois podem ser administrados em mesofase com baixa viscosidade que facilitam a sua aplicação, por exemplo, por seringa. Contudo, ao entrar em contato com o ambiente bucal, o SLC tem a capacidade de incorporar água da saliva, se tornando um SLC mais viscoso *in situ*, com alta capacidade mucoadesiva, permanecendo por mais tempo no sítio de aplicação intrabucal (Chorilli et al., 2016, Calixto et al., 2016a; Cintra et al., 2016; Dos Santos Ramos et al, 2016; Fonseca-Santos et al., 2017; Calixto et al., 2018; Rodero et al., 2018; Calixto et al., 2021; Miyashiro et al., 2023).

Portanto, os SLC podem superar as limitações que a administração tópica de alguns medicamentos na mucosa bucal apresenta devido à mastigação, fala, deglutição e principalmente, à secreção contínua da saliva (0,5 a 2 L/dia) (Salamat-Miller et al., 2005; Hearnden et al., 2012; Franz-Montan et al., 2017a; Franz-Montan et al., 2017b) que podem levar a remoção da formulação do seu sítio de aplicação.

Em acréscimo, a alta capacidade de mucoadesão dos SLC também podem aumentar a permeação de fármacos, superando as características da barreira de revestimento de mucosa oral que limitam muito a permeação de fármacos (Franz-Montan et al., 2017a; Calixto et al., 2018; Rodero et al., 2018; Miyashiro et al., 2020; Calixto et al., 2021; Pestana et al., 2024).

Nesse contexto, uma possível estratégia para aumentar a mucoadesão dos SLC é utilizar como fase aquosa uma dispersão de sal de ácido hialurônico, que é um biopolímero formado por ácido d-glucurônico e N-acetil-d-glucosamina que apresenta elevada capacidade mucoadesiva (Bernkopschnürch, 2005; Kumar & Sinha, 2013). Além disso, o ácido hialurônico promove eventos celulares auxiliares ao processo de reparação tecidual e renovação epitelial pois aumenta a migração de neutrófilos e macrófagos, mediadores no processo de reparação tecidual, para o local da lesão, aumentando a capacidade fagocítica de ambos. Ademais, acentua a migração, proliferação e atividade de miofibroblastos e fibroblastos. Paralelamente, é capaz

de aumentar a proliferação de células endoteliais, favorecendo a angiogênese e, consequentemente, melhorando as condições de aporte sanguíneo à área lesada. Esta etapa é fundamental na reparação do tecido, pois proporciona uma maior circulação de células necessárias ao processo e novos vasos que participam da formação do tecido de granulação provisório, suprindo o novo tecido com nutrientes e oxigênio (Bortolazzo, 2018).

Além disso, tem a capacidade de promover alta hidratação, promovendo uma ação hidratante adicional na superfície da mucosa oral, evitando as infecções oportunistas causadas por fungos e bactérias, mantendo íntegra a mucosa e favorecendo a cicatrização de feridas e/ou o não aparecimento destas (Huang et al., 2014; Nascimento, Lombello, 2016; Mohammed et al., 2022).

Os anestésicos locais (AL) são fármacos capazes de inibir reversivelmente a percepção das sensações, sobretudo a dor, por meio do bloqueio dos canais de sódio regulados por voltagem, impedindo, assim, a propagação dos impulsos nervosos ao longo dos neurônios (Yagiela, et al., 2011; Parise, Grando, 2017). Dentre os AL, a lidocaína, pertencente ao grupo das amino-amidas, é um dos mais utilizados na prática odontológica, sendo considerada o padrão ouro devido à sua ação rápida e eficaz, com raro risco de reações alérgicas. Além disso, é segura para crianças, gestantes e idosos, o que a torna adequada para diferentes situações clínicas (Franz-Montan, 2017b; Malamed, 2021). Em sua forma de sal mais empregada, o cloridrato de lidocaína, apresenta uma alta solubilidade em água, alcançando cerca de 1000 mg/mL a 20°C, como descrito por Malamed (2021). Essa alta solubilidade facilita seu uso em formulações injetáveis e tópicas, permitindo uma dissolução eficiente e rápida em solventes aquosos, o que possibilita sua incorporação na fase aquosa do SLC (Vilas Boas de Almeida et al., 2023).

Assim, o objetivo deste estudo foi desenvolver e caracterizar uma formulação contendo a combinação do óleo de semente de uva, ácido hialurônico e lidocaína em um sistema precursor de cristal líquido como futura estratégia para alívio sintomático e cicatrizante de lesões de mucosite oral. Além disso, avaliar se esse sistema de liberação é eficiente em promover permeação lenta e sustentada do anestésico, com alta capacidade mucoadesiva e reduzida toxicidade.

A hipótese deste estudo foi que seria possível desenvolver uma formulação contendo a combinação do óleo de semente de uva, ácido hialurônico e lidocaína em sistema precursor de cristal líquido de geleificação *in sito* com propriedades reológicas e mecânicas ideais para

aplicação tópica em cavidade bucal, ainda apresentando alta capacidade mucoadesiva, permeação lenta do anestésico local, e reduzida toxicidade.

Esta dissertação será apresentada em formato alternativo, de acordo com Art. 2º da Instrução Normativa CCPG Nº 002/2021 e será composta de um artigo científico, que será submetido no periódico *Journal of Pharmaceutical Sciences* após a defesa da dissertação (comprovante de submissão será anexo na versão final a ser encaminhada para homologação). As normas de formatação do artigo a seguir, estão de acordo com aquelas preconizadas pela revista.

2. ARTIGO

Title: Mucoadhesive Liquid Crystalline System Based on Grape Seed Oil, Hyaluronic Acid, and Lidocaine for Topical Treatment of Oral Mucositis

Authors:

Mônica Beatriz Portela Ferreira[1], MSc Student

Giovana Maria Fioramonti Calixto[1], PhD

Aylla Mesquita Pestana[1], PhD Student

Arthur Antunes Costa Bezerra[1], PhD Student

Michelle Franz-Montan[*1], Associate Professor[1]

[1]Department of Biosciences, Piracicaba Dental School, University of Campinas, Piracicaba, São Paulo, Brazil

***Corresponding author:**

Michelle Franz-Montan

901 Limeira Avenue, Bairro Areião, 13414-903, Piracicaba, Sao Paulo, Brazil

Tel.: +55 19 2106 5306; FAX: 55 19 2106 5306;

E-mail: mfranz@unicamp.br

ABSTRACT

Oral mucositis is a complication of antineoplastic treatment resulting in painful ulcerated lesions in the oral cavity and difficulty in drug administration. Liquid crystalline precursor systems (LCPS) emerge as a promising approach due to their high mucoadhesive capacity when in contact with saliva. This study aimed to develop an innovative formulation for the treatment of oral mucositis using a mucoadhesive liquid crystalline precursor system composed of grape seed oil, PPG-5-CETETH-20 as a surfactant, and hyaluronic acid, with the incorporation of lidocaine as a therapeutic alternative for oral mucositis. The developed formulation, as determined in a preliminary study, consisted of PPG-5-CETETH-20 as a surfactant, 40% of a mixture of grape seed oil and oleic acid (ratio 4:1), 40% of PPG-5-CETETH-20, and 20% of hyaluronic acid with the addition of 5% lidocaine. This formulation was characterized by polarized light microscopy (PLM), texture profile analysis (TPA), and rheology. The swelling capacity in artificial saliva, release kinetics, *in vitro* permeation and mucoadhesion in porcine buccal mucosa, and *in vivo* toxicity in the hen's egg test - chorioallantoic membrane (HET-CAM) model were evaluated with the liquid crystalline system (LCS) formulation (bulk gel). The developed formulation presented a stable hexagonal liquid crystalline phase, with increased viscosity and hardness after dilution with artificial saliva, showed a Newtonian fluid behavior, a high mucoadhesive capacity, immediate and sustained permeation, and toxicity similar to the commercial formulation. In conclusion, the LCS formulation demonstrated promising properties for the topical treatment of oral mucositis, exhibiting desirable physical-chemical characteristics as a novel therapeutic alternative.

Keywords: Oral mucositis, lidocaine, liquid-crystalline system, grape seed oil, hyaluronic acid, mucoadhesion, drug delivery system, topical administration.

1. INTRODUCTION

Oral mucositis (OM) is a serious complication of antineoplastic therapy. It is an inflammatory process caused by the cytotoxic action of chemotherapeutic agents and ionizing radiation, causing discomfort such as burning, erythema, pain, difficulty in eating, and oral care. Normally, epithelial cells have good regeneration capacity; however, due to treatment with antineoplastic agents, there may be delay and inhibition of cellular processes, resulting in lesions such as aphthae, ulcers, and peeling of the buccal tissue (Yarom et al., 2020).

The condition impacts patients' quality of life of and often requires partial or complete interruption of oncological treatment. This can lead to systemic complications, hospitalization, and, not infrequently, progression to death (Oganesyan et al., 2023).

It is estimated that about 60 to 85% of patients undergoing hematopoietic stem cell transplantation, 40% of patients undergoing conventional chemotherapy, and 100% of patients undergoing radiotherapy will manifest some degree of OM during and/or after treatment (INCA, 2023; Leal et al., 2024). The complications associated with this condition include changes in treatment protocols, nutritional impairment, predisposition to systemic infections, which can worsen the disease prognosis, and reduce quality of life, highlighting the urgent need for effective and safe therapeutic strategies (Leal et al., 2024).

Currently, the treatment of OM is challenging, involving cryotherapy, antifungal and anti-inflammatory drugs, chlorhexidine solutions, mouthwashes with anesthetics and analgesics, and low-level laser therapy. However, these approaches have limitations such as delayed effectiveness, burning sensation, and dysgeusia, as well as high costs and the requirement for specific technical skills (Elad et al., 2020; Oganesyan et al., 2023; Leal et al., 2024).

Liquid-crystalline systems (LCSs) emerge as a promising strategy for drug delivery in the oral cavity, offering advantages such as increased mucoadhesion capacity, improved drug bioavailability, which can reduce application frequency and minimize side effects (Miyashiro et al., 2020). This system is composed of aqueous and lipid phases, as well as a surfactant. We hypothesized that it would be possible to prepare a LCPS with a functionalized composition, including grape seed oil as the lipid phase, and hyaluronic acid and lidocaine hydrochloride as the aqueous phase. This formulation aims to combine healing, hydration, and analgesic properties with prolonged action, while also achieving ideal characteristics such as appropriate viscosity for topical application in the oral cavity, high mucoadhesive capacity, immediate but sustained permeation of the local anesthetic, and low toxicity.

Grape seed oil has therapeutic properties with phenolic compounds having antioxidant, anti-inflammatory, healing, and antimicrobial actions, showing promising potential in the treatment of OM (Jambi et al., 2019; Singh et al., 2019; Santos et al., 2023). Hyaluronic acid, not only promotes mucoadhesion but also contributes to essential cellular events in tissue repair and epithelial renewal (Griesser et al., 2018). Additionally, lidocaine, is a safe and effective local anesthetic, and has the potential to provide immediate symptomatic relief (Malamed, 2021).

Thus, the objective of this study was to develop and characterize an innovative formulation for the treatment of OM using a LCPS composed of grape seed oil, hyaluronic acid with the incorporation of lidocaine hydrochloride with improved mucoadhesive capacity, sustained permeation ability, and reduced toxicity.

In the supplementary material, detailed test results for a developed conventional lipid formulation, composed of oleic acid, PPG-5-CETETH-20 surfactant, and water, are available to better evaluate and compare the results of the formulation under study. These additional data are provided to offer a comparison between the mucositis formulation and a conventional formulation, allowing for a more comprehensive assessment of the efficacy and rheological properties of the new formulation in relation to established standards.

2. MATERIAL AND METHODS

2.1. Development of liquid crystal systems

Firstly, a phase diagram using 40% grape seed oil, 40% PPG-5-CETETH-20 (Procetyl®), and 20% hyaluronic acid dispersion (5%) was constructed following the method described by Calixto et al. (2018). All formulations remained at room temperature (25°C) to allow stability and air removal.

None of the combinations resulted in the formation of LCPS as observed in Figure S1. Therefore, it was decided to include a oleic acid in the lipid phase in order to stabilize the liquid-crystalline system structure, ensuring the formation of a hexagonal phase (Calixto et al., 2016; 2018).

New tests were conducted to define the proportion of oleic acid in the oil phase. After testing different proportions of the oil phase containing grape seed oil and oleic acid since oleic acid is present in grape seed oil, acts as a stabilizer (Otto et al., 2009) , and is a recognized component used in drug delivery systems (Calixto et al., 2018) with grape seed oil varying between 50-95% and oleic acid between 50-5%. Therefore, the proportion of 4:1 (80% grape

seed oil to 20% oleic acid) was chosen to ensure the required properties. The visual classification and MLP analysis for determining the best proportion are detailed in the Supplementary Material.

A new phase diagram was constructed using grape seed oil and oleic acid as the oil phase, a 5% (w/w) hyaluronic acid dispersion as the aqueous phase, and PPG-5-CETETH-20 (Procetyl®) as the surfactant. At 25 ± 5 ºC, 54 different proportions varying by 10% (w/w) for each phase of the systems were used, resulting in the construction of a phase diagram with 54 points.

The oil phase was prepared by manually mixing its components. Subsequently, it was mixed with the surfactant. Then, the oil phase was slowly poured into the aqueous phase. The formulation without drugs was defined as F. The formulation with 5% lidocaine hydrochloride (FL), was prepared in the same way, with the lidocaine being incorporated into the aqueous phase. All formulations were then stirred using a vortex and a homogenizer and left to rest for 48 hours to stabilize and eliminate bubbles.

After this period, they were visually classified as transparent liquid system (TLS), transparent viscous system (TVS), translucent liquid system (TLTr), translucent viscous system (TVTr), opaque system (OS), and phase separation (PS).

The grape seed oil used in this research was acquired from the company Mapric Produtos Farmacêuticos, Brazil (Batch numberAUTO308147).

2.2. Physicochemical characterization of formulations

2.2.1. Polarized light microscopy (PLM)

After visual classification, the systems were analyzed by PLM. For this, a small aliquot of the selected systems was placed on a slide, covered with a coverslip, and analyzed under a polarized light microscope (Leica DM 2000 Optical Microscope) with a 20x magnification lens. The homogeneity of the dispersion was evaluated and the presence of anisotropy areas indicative of the presence of LCSs was observed. The analysis was conducted at room temperature (25 ± 2 ºC). Liquid-crystalline systems (LCS) containing Maltese cross were classified in lamellar, hexagonal, or cubic phases.

After this classification, the phase diagram was redefined in LCS regions, and a LCS from the diagram was selected for further studies, located in a transition area between lamellar and hexagonal or cubic liquid-crystal phases, aiming to obtain a proportion that increases

viscosity upon water addition. Phase diagrams were plotted using SigmaPlot® version 10.0 (Systat Software, USA).

After defining the proportion, artificial saliva was added. Artificial saliva was added (w/w) to F and FL at 30% (F30 and FL30) and 100% (F100 and FL100). Previous studies tested various concentrations of saliva to investigate its action in the phase transition of LCS, showing that phase transitions occurred with 30% and 100% saliva (Dos Santos Ramos et al., 2016). The artificial saliva (pH 6.8) was composed of 8 g/L of sodium chloride (NaCl), 0.19 g/L of potassium monobasic phosphate (KH_2PO_4), and 2.28 g/L of disodium phosphate (Na_2HPO_4) (Marques et al., 2011). After the addition of saliva, the systems were visually classified and analyzed by PLM.

2.2.2. Texture profile analysis

The texture profile of the formulations (F, F30, F100, FL, FL30 e F100) was analyzed using a TA-XT plus texture analyzer (Stable Micro Systems, UK), from which mechanical properties such as hardness, compressibility, adhesiveness, and cohesiveness were extracted. For the test, the viscous formulations (10 g) were placed in conical centrifuge tubes of 50 mL (Falcon BD®, Franklin Lakes, USA) and centrifuged at 4000 rpm for 3 minutes to remove air bubbles and smooth the surface. These tubes were then placed under the analytical probe (10 mm diameter) of the texture analyzer programmed to compress the sample at a speed of 0.5 mm/s to a predefined depth (4 mm) and return to the sample surface at the same speed. After a 5-second rest, a second compression was performed under the same conditions. All analyses were conducted in decaplicate at room temperature (± 25 °C).

2.2.3. Rheological behavior

Rheograms of the systems were obtained using a Discovery HR-1 rheometer - Trios TA Instruments, using a cone/plate geometry with a 40 mm diameter, 2° angle, and a gap of 52 µm. A sample of three grams of the systems was carefully placed on the lower plate of the rheometer, and after a 3-minute rest, the analysis began.

2.2.3.1. Continuous rheological analysis

The shear rate used was 0 to 100 s^{-1} for the ascending curve and 100 to 0 s^{-1} for the descending curve over 120 seconds each at 37 °C. Consistency and flow indices were determined by Equation 1 for quantitative flow behavior analysis: $\tau = k \cdot \gamma^{\eta}$ (Equation 1), where “τ” is the shear stress, “γ” is the shear rate, “k” is the consistency index, and “η” is the

flow index (Calixto et al., 2015). The analysis was performed with all six (six) independent samples.

2.2.3.2. Oscillatory rheological analysis

The oscillatory analysis was conducted using the same rheometer model and the same plate and gap conditions. First, a stress sweep test was performed to determine the viscoelastic region. For this test, a shear stress range of 0 to 50 Pa and a frequency of 1 Hz was used. After determining the 1 Pa stress of the viscoelastic region, a frequency sweep test was conducted to determine the elastic modulus (G') and viscous modulus (G''). For this test, a frequency range of 0 to 10 Hz was used at a stress of 1 Pa at 37 °C (Calixto et al., 2018). The analysis was conducted with all 6 (six) independent samples.

2.2.4. Lidocaine analysis by high performance liquid chromatography (HPLC)

The analytical method used for lidocaine hydrochloride (LDC) quantification in *in vitro* release and permeation assays was validated using the parameters: linearity, intra-run and intermediate precision, accuracy, specificity/selectivity, detection limit, and quantification limit. The mobile phase used was a 60:40 (v/v) mixture of acetonitrile and 25 mM NH_4OH adjusted to pH 7.0 with H_3PO_4 solution at a flow rate of 1.2 mL/min. A reverse-phase column (150 x 4.6 mm, 5 µm, Phenomenex Gemini C18 – Torrance, California, USA) was used, with an injection volume of 20 µL and a detector wavelength of 220 nm (Franz-Montan et al., 2015). The method's linearity was confirmed by a triplicate calibration curve on three different days with five different lidocaine solution concentrations. The detection and quantification limits were 0.24 and 0.80 mg/mL, respectively.

2.2.5. In vitro lidocaine release kinetics evaluation

The LDC release study in solution (S-LDC), and LDC incorporated in LCS, i.e., the teste formulation (FL), was conducted using the Phoenix DB-6 Manual Transdermal System (Teledyne Hanson® - Chatsworth, California, USA) consisting of Franz diffusion cells (average size with a permeation diameter of 15 mm and receptor compartment volume of 25 mL). A synthetic cellulose acetate membrane with a molar mass of 12–14 kDa (Sigma-Aldrich®) and a receptor solution composed of phosphate-buffered saline (PBS) at pH 7.4 at 37 ºC and stirring at 300 rpm were used. The formulations were placed in the ring positioned in the donor compartment containing approximately 300 mg of formulation. Samples (400 µL)

were collected from the receptor compartment at predetermined times (15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 720, and 1440 min). The released LDC was quantified by HPLC using the described method. Different mathematical models were applied to select the LDC release kinetics (defined by the highest R^2 value). The data processing software used was KinetDS 3.0 (Jagiellonian University Medical College, Krakow, Poland).

2.2.6. Preparation of porcine buccal mucosa for in vitro mucoadhesion and permeation assays

Porcine maxilla was obtained from five-month-old Landrace pigs (*Sus scrofa domestica*) weighing between 75 and 80 kg, acquired from Frigorífico Angelelli® Ltda (certified by the Secretaria da Agricultura e Abastecimento do Estado de São Paulo – SIF 2259). The pieces were transported to the laboratory in phosphate-buffered saline (PBS) (pH 7.4) within 30 minutes after slaughter. The processing of the buccal mucosa followed the methodology described by Franz-Montan et al. (2016).

Initially, the cheeks were carefully removed from the adjacent tissues using a scalpel blade, then washed with PBS. The mucosae were subjected to heat treatment in distilled water at 60 ºC for 2 minutes. As reported by Nicolazzo et al. (2003), this thermal treatment is gentle enough not to alter the histological characteristics of the samples. After this treatment, the epithelium was meticulously separated from the underlying connective tissue (lamina propria) using a Molt elevator. Each sample was visually inspected to identify and discard any tissue presenting injuries.

2.2.7. *In vitro permeation assay through porcine buccal epithelium*

The *in vitro* LDC permeation assay of the described formulations (FL; FC) was conducted using the same equipment described for the release assay.

The prepared porcine buccal epithelium was gently placed on the upper part of the Franz cell, with the epithelium facing the donor compartment. The formulations were placed in the dosing ring positioned in the donor compartment of the Franz diffusion cell. Samples were collected from the receptor compartment at predetermined times (15, 30, 45, 60, 90, 120, 150, 180 min). The permeated LDC was quantified by HPLC using the validated analytical method.

After preparing the tissue and visual inspection, the electrical resistivity (ER) of the mucosa epithelia was measured by placing them in the Franz cells of the Phoenix DB-6 Manual Transdermal System (Teledyne Hanson® - Chatsworth, California, USA) at 37 ºC.

The basal layer of the epithelium was placed in contact with the receptor compartment, and both the donor and receptor compartments were filled with degassed phosphate-buffered saline (pH=7.4) at 37 ºC.

Ag/AgCl electrodes connected to a Keysight 33220a signal generator (Agilent Technologies, Barueri, São Paulo, Brazil) and a digital multimeter ET-2053DMM (Minipa, São Paulo, Brazil) were positioned in the donor and receptor compartments, respectively, allowing the membrane to be positioned between the electrodes. An alternating current of 100 mV (rms) and a frequency of 10 Hz was applied, and the ER was calculated according to Ohm's law (Equation 1):

$$ER = \frac{\left(\frac{DP}{I}\right)}{A} \quad (1)$$

where DP is the system's potential difference (mV), I is the measured current (A), and A is the area (cm^2).

Only mucosae with ER ≥ 3 kΩ/cm^2 were used in the *in vitro* permeation assays (de Araújo et al., 2021). After ER measurement, the buffer solution in the donor compartment was replaced with the evaluated formulations. 0.5 g of FL and 0.7 g of FC were placed in the dosing ring positioned in the donor compartment of the Franz diffusion cell. The permeated LDC was quantified by HPLC using the validated analytical method.

2.2.8.3 In vitro mucoadhesion study

This assay used a TA.XT Plus texture analyzer – Stable Micro Systems® (Surrey, UK) to evaluate *in vitro* the force required to detach the selected systems from the surface of the porcine buccal mucosa. The mucosa, prepared as described previously, was fixed in a glass device under the A/MUC probe in contact with artificial saliva at a controlled temperature of 37 ºC. The formulations were placed at the lower end of the equipment probe. The test was performed by lowering the probe at a constant speed (1 mm/s) until the mucosa contacted the sample. The mucosa and sample were kept in contact for 600 seconds, applying a contact force of 0.552 N during this time (Pestana et al., 2024). Then, the probe rose at a constant speed (1 mm/s) until detachment between the mucosa and sample occurred. The force required to detach the mucosa from the sample was obtained and considered as F_{max}. The analyses were performed in 10 replicates.

The work of mucoadhesion (W_{muc}), referring to the force necessary for mucosa/formulation detachment over time, was calculated from the area under the force *versus* time curve (N.s) using the Exponent 6.1.18 software (Stable Micro Systems, Surrey, UK).

2.2.9 *In vivo toxicity assay using the hen's egg test - chorioallantoic membrane (HET-CAM) model*

The toxicity test using the CAM (chorioallantoic membrane of the chicken embryo) was conducted according to protocol and methodology described by the research group using the HET-CAM model (Bezerra, 2023). This method is based on the use of chicken embryos at the 10th day of embryonic development when the nervous system is not yet formed, thus providing a relevant and ethical experimental model to evaluate substance toxicity.

After previous visual inspection, weighing, and incubation, the eggs were evaluated by ovoscopy (a technique for observing the egg by placing it against a light source, which favors the visualization of its internal content). Those that did not develop embryos were discarded from the study. The 90 eggs with embryos had their air chambers marked with a graphite pencil during ovoscopy.

A window was recorded in each egg within the air chamber area, with a diameter of 1.8 cm. The window was then opened with curved iris scissors. The eggshell membrane covering the chorioallantoic membrane was moistened with approximately 1 mL of saline solution and gently removed with conical tweezers to expose the CAM.

The CAM of the chicken embryo and its vascular system were visually evaluated, and only eggs with intact systems were used. Each substance was applied to 18 eggs for the toxicological analysis of the following formulations:

- *Test formulation:* lidocaine incorporated in a liquid-crystalline system based on grape seed oil and hyaluronic acid: **"FL"**
- *Control formulation:* test formulation without the incorporation of lidocaine hydrochloride: **"F"**
- *Positive control I:* commercial Lidocaine® ointment 50 mg/g (EMS Pharma): **"Lidocaine®"**
- *Positive control II:* sodium hydroxide 1 mol/L solution: **"positive control"**
- *Negative control:* 0.9% sodium chloride solution: **"negative control"**

Using a stereomicroscope (OPTIKA model SZX-T), the CAM was recorded before and after treatments, followed by evaluation. The toxicity assessment of the formulations by the HET-CAM model was conducted using the "endpoint evaluation" method, which analyzes the

effects of substances over a period of time. In this case, after contact with the substances for 180 seconds, they were removed, and the CAM was analyzed 30 seconds after this process.

Toxic effects were evaluated according to protocol No. 96 of the European Centre for the Validation of Alternative Methods (ECVAM) titled: Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM).

Each substance was applied to six eggs for final formulation scoring, with each substance evaluated in triplicate (n=3), totaling 18 (eighteen) eggs per formulation. The score for each triplicate was calculated by summing the scores obtained in the six evaluated eggs of each triplicate. Based on the final score, the substance was classified according to the table below:

Table 1: Classification of substances using the endpoint evaluation method (Brantom et al., 1997).

Score	***Classification***
0	Non-irritant
<6	Slightly irritant
6≤S≤12	Moderately irritant
12<S<16	Irritant
≥16	Severely irritant

All eggs excluded during the experimental period or at the end of the experiment were discarded following AVMA *Guidelines for the Euthanasia of Animals* (2020) through freezing at -20 ºC, placed in white plastic bags, and taken to the biological material disposal site at FOP-Unicamp.

2.3 Statistical analysis

To evaluate the efficacy and safety of the new liquid-crystalline formulation in the treatment of oral mucositis, rigorous statistical analysis of the data obtained in the assays was conducted. Analyzed variables included peak mucoadhesive force, mucoadhesion work, hardness, compressibility, cohesiveness, adhesiveness, and rheological properties. Data were presented as mean ± standard deviation, and statistical analysis was performed using parametric and non-parametric techniques appropriate for data distribution.

One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test was used to evaluate differences between formulations with a 95% confidence level and $p < 0.05$ indicating statistically significant differences. For non-parametric data, the Kruskal-Wallis test followed by a two-stage linear step-up procedure (Benjamin, Krieger, and Yekutieli) was applied to control the type I error rate in multiple comparisons.

Rheological data analysis was conducted through two-factor independent analysis of variance with post hoc Bonferroni test, adjusting the significance level for multiple comparisons, maintaining the error rate at 5%.

The software used for these analyses were GraphPad Prism 9 and IBM® SPSS® Statistics, ensuring the accuracy and reliability of the results.

3. RESULTS

3.1. Development and structural characterization of formulations

The constructed ternary diagram presents the proportions of the three components: a mixture of grape seed oil and oleic acid (4:1), PPG-5-CETETH-20, and 5% hyaluronic acid dispersion, demonstrating the structures formed at different proportions.

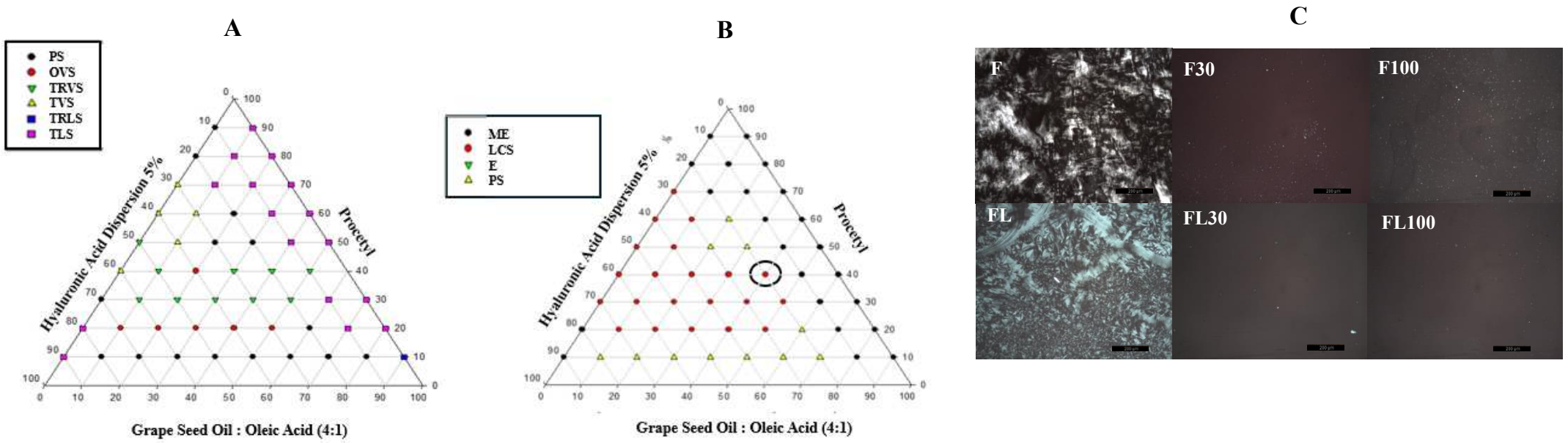
Regarding visual classification, a ternary diagram was constructed, demonstrating the formed systems: transparent and liquid systems were considered TLS, while more viscous transparent systems with little or no fluidity were termed TVS. Points in the diagram where translucency and liquidity were observed were termed TRLS, and translucent systems with low or no fluidity were classified as TRVS. Viscous and opaque systems were termed OVS. Systems that exhibited phase separation upon mixing specific proportions of surfactant, oil, and water were termed PS.

Through the construction of the diagram composed of Procetyl®, grape seed oil plus oleic acid, and 5% hyaluronic acid dispersion (Figure 1A and 1B), the formulation F was selected, located in a transparent viscous system area and close to a transition region maintaining the viscous system with increasing aqueous phase, with a proportion of 40% oil phase, 40% surfactant, and 20% aqueous phase.

The incorporation of lidocaine in F maintained this behavior and visual appearance; i.e., the diluted formulations FL30 and FL100 were more viscous than FL. According to the microscopic images (Figure 1C), F was classified as liquid-crystalline due to the streaks visualized by polarized light microscopy. This characteristic of F was maintained with the incorporation of lidocaine (FL). Moreover, after dilution with 30% artificial saliva, F30 became

a cubic liquid-crystalline system, evidenced by a dark field, as well as F diluted with 100% artificial saliva (F100).

The microscopic structure of FL30 and FL100 was not altered after lidocaine incorporation, as the photomicrographs of FL30 and F100 also showed a dark field. Thus, FL30 and F100 were also classified as cubic liquid-crystalline systems. The composition and classification of all formulations are in Figure 1D.



D

Formulation	Surfactant	Oil Phase	Aqueous Phase	Dilution Medium	Drug	Visual Classification	Microscopic Classification
	Procetyl®	Grape Seed Oil + Oleic Acid	Hyaluronic Acid Dispersion 5%	Artificial Saliva	Lidocaine		
F	40	40	20	-	-	SVT	Hexagonal
F30	40	40	20	30	-	SVO	Cubic
F100	40	40	20	100	-	SVO	Cubic
FL	40	40	20	-	5	SVTr	Hexagonal
FL30	40	40	20	30	5	SVO	Cubic
FL100	40	40	20	100	5	SVO	Cubic

Figure 1. A) Phase diagram with 54 different proportions of the test formulation composition, discriminating the visual classification. **B)** Phase diagram with 54 different proportions of the test formulation composition, discriminating the formed structures with classification by PLM. **C)** Photomicrographs of test formulations: F; F30; F100; FL; FL30; FL100. 20x magnification. **D)** Composition (% w/w), visual, and microscopic classification of test formulations. F is the liquid-crystalline system without lidocaine incorporation. FL is the liquid-crystalline system with 5% lidocaine hydrochloride. F30 and FL30 are F and FL with 30% artificial saliva, and F100 and FL100 are F and FL with 100% artificial saliva, respectively.

3.2. Texture profile and rheological analysis

The texture analysis profiles (F, F30, F100, FL, FL30 e FL100) are shown in Figure 2A. The test formulation (F) is characterized as a viscous formulation, with its viscosity increasing with dilution with 30% (F30) and 100% saliva (F100), with F30 being the most viscous among the three conditions ($p<0.05$), resulting in greater hardness compared to F100 and even more so than the base formulation F. Similarly, F30 resulted in greater hardness ($p<0.05$) among the studied samples. These values are consistent with the compressibility study of the samples, with F30 requiring greater force incidence ($p<0.05$) for its compression, followed by FL.

The highest cohesion value were found in the samples of the formulation with 100% saliva, with the highest value ($p<0.05$) obtained in the analysis of F100 and FL100, respectively. F and FL are the formulations with the lowest viscosity ($p>0.05$) among those studied. However, this parameter increased with the increasing concentration of artificial saliva, with formulations diluted by 30% being the most viscous, with F30 showing the highest value. Finally, the addition of lidocaine decreased the average values ($p>0.05$) of mechanical properties compared to the formulation without the drug.

The qualitative analysis of the rheograms illustrated in Figure 4B and 4C. In the continuous rheology (Figure 4B), the test formulation with lidocaine incorporated at its original concentration (FL) demonstrated Newtonian fluid behavior. This suggests that the formulation has constant viscosity regardless of shear rate, which is important to ensure uniform application to the oral mucosa.

When the test formulation was diluted with 30% saliva (FL30), the Newtonian fluid behavior was maintained. This is a positive indication, suggesting that the formulation maintains its rheological stability even when exposed to a simulated oral mucosa environment.

When the test formulation was diluted with 100% saliva (FL100), a change in rheological behavior was observed, indicating pseudoplastic fluid. This means that the viscosity of the formulation decreases with increasing shear rate, which can be beneficial for more effective application and spreading on the oral mucosa.

Thus, FL and FL30 exhibited characteristic Newtonian flow curves ($\eta=10$), while FL100 exhibited a characteristic pseudoplastic flow curve ($\eta<10$). Regarding FL, based on data analysis, it is important to highlight that this sample demonstrates Newtonian behavior at low to intermediate shear rates, where the relationship between stress and shear rate is linear. However, at higher shear rates, the formulation begins to exhibit pseudoplastic behavior,

suggesting that its viscosity decreases as the shear rate increases. This indicates that FL can be classified as Newtonian under normal application conditions, but with pseudoplastic tendencies under higher stress conditions. This rheological profile offers significant benefits to the patient, providing an initial smooth and controlled application, ensuring that the product uniformly covers the oral mucosa without dripping. As pressure or movement increases, the decrease in viscosity facilitates the spreading of the product with less effort, reducing discomfort and minimizing irritation. Therefore, it is recommended that the patient initially apply the product gently on the mucosa to ensure even coverage, and then lightly rub it to further facilitate spreading, taking advantage of the shear-thinning effect provided by its pseudoplastic behavior.

The formulation without lidocaine (F) initially showed pseudoplastic fluid behavior; however, with increasing shear rate, it exhibited constant deformation like a Newtonian fluid. F30 exhibits non-Newtonian fluid behavior. The curve of F100 shows a decrease in shear stress after a certain point, indicating a thixotropic, non-Newtonian behavior. The saliva dilutions presented plastic behavior.

The data showed that both the formulation and the artificial saliva, as well as the interaction of the two variables, had a significant effect on all continuous rheological parameters ($p<0.00001$, two-factor ANOVA). In general, dilution with 30% saliva increased ($p<0.00001$) the consistency index, yield stress, and thixotropy of the formulations, followed by dilution with 100%. Thus, the continuous rheological analysis of the formulations showed that both formulation and saliva concentration significantly impact continuous rheological parameters ($p<0.0001$). The test formulations exhibited Newtonian behavior at the original concentration and with 30% saliva, while the formulation with 100% saliva showed pseudoplastic behavior.

The oscillatory rheological analysis (Figure 4C) is essential to understand the viscoelastic behavior of fluids under different conditions, particularly relevant for formulations intended for oral mucosa. The test formulation with lidocaine (FL) demonstrated viscoelastic liquid behavior (G”>G’). This indicates that the formulation exhibits both liquid and solid properties, being able to deform and recover its initial structure.

When the formulation was diluted with 30% saliva (FL30), the viscoelastic liquid behavior was maintained. This suggests that it retains its viscoelastic properties even when exposed to a simulated oral mucosa environment. When diluted with 100% saliva, the formulation (FL100) continued to show viscoelastic liquid behavior. This is advantageous for effective application and spreading on the oral mucosa. However, shortly after, it exhibited a

change in its rheological behavior, presenting as a viscoelastic solid (G'>G"). This suggests a structural transition in the formulation, which can be more resistant to deformation.

For all functional formulations, increasing saliva concentration significantly increased G' and G'' values ($p \leq 0.0001$). Samples with lidocaine incorporation had lower values compared to samples without lidocaine ($p \leq 0.0001$) For the formulation with lidocaine, in general, adding 30% and 100% saliva increased the average values of loss modulus G''.

In this regard, the oscillatory rheological analysis of the formulations demonstrated that both formulation and saliva concentration significantly affected storage and loss moduli ($p < 0.0001$). The original (F) and 30% saliva formulation (F30) exhibited viscoelastic liquid behavior, while the 100% saliva formulation showed a transition to viscoelastic solid behavior. High G' and G'' values in lidocaine and saliva formulations indicate a greater capacity to store and dissipate energy, reflecting better adhesion and mechanical resistance.

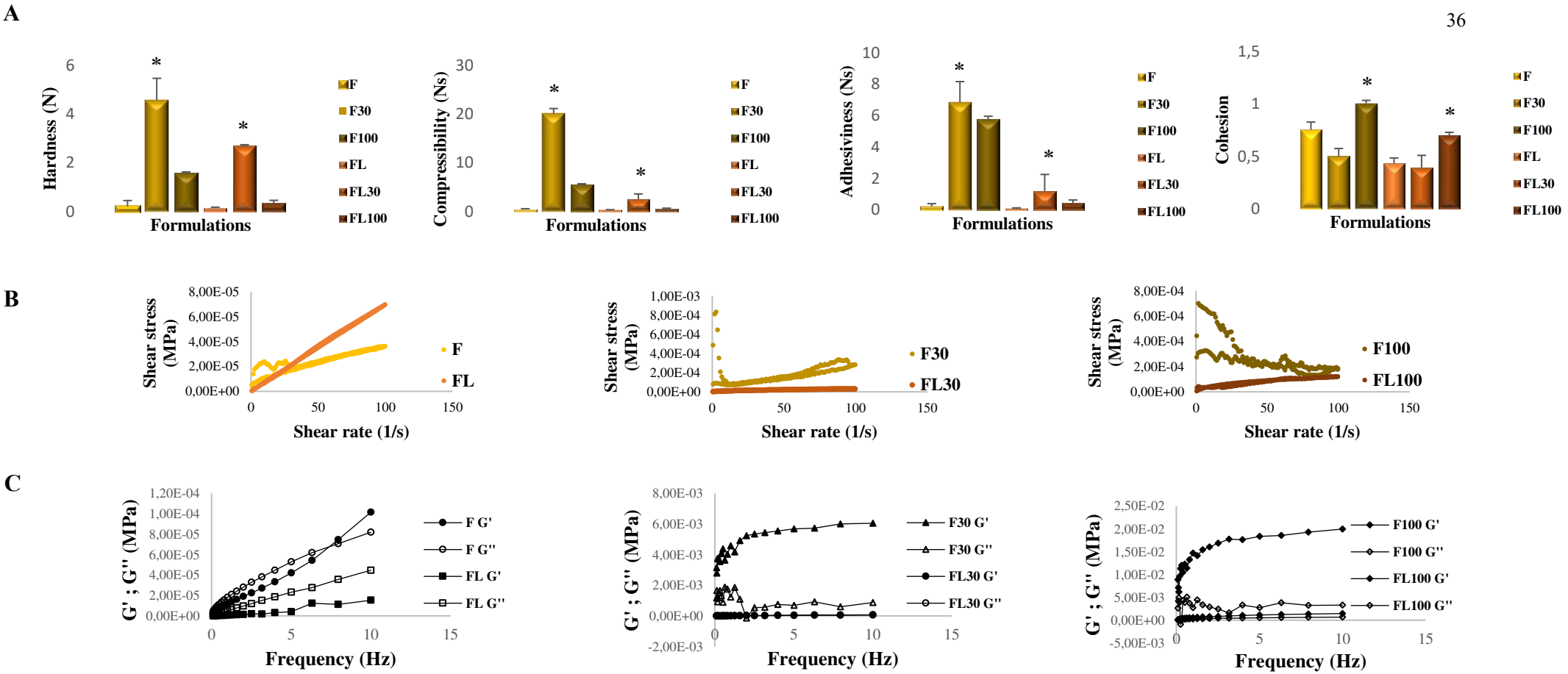


Figure 2. A) Texture Profile. Mechanical properties: Hardness, Compressibility, Adhesiveness, Cohesion. Test formulation (F, F30, F100 – FL, FL30, FL100). *($p<0.05$) (One-way ANOVA test with Tukey post-test at 0.01% significance level, n=10). **B)** Flow curves (shear stress vs. shear rate) of the formulations. Standard deviations were omitted for clarity. Data were collected at 37 ± 0.5 ºC. The coefficient of variation for triplicate analyses was less than 10%. (ANOVA with Games Howell post-test. The significance level was 0.05). **C)**Variation of storage modulus G' and loss modulus G'' as a function of frequency for all formulations. Standard deviations were omitted for clarity. Data were collected at 37 ± 0.5 ºC. The coefficient of variation for triplicate analyses was less than 10%. (ANOVA with Games Howell post-test. The significance level was 0.05). F is the liquid-crystalline system without lidocaine incorporation. FL is the liquid-crystalline system with 5% lidocaine hydrochloride. F30 and FL30 are F and FL with 30% artificial saliva, and F100 and FL100 are F and FL with 100% artificial saliva, respectively.

3.3. *In Vitro* release kinetics, permeation and mucoadhesion assays (LDC)

The analytical curve proved to be linear over the proposed range (5,0 - 200,0 μg.mL^{-1}), as shown by the linear regression coefficient (R^2) of 0.9998, demonstrating an acceptable data fit to the regression line (y = 13012 x - 14785). The method showed precision with a relative standard deviation of less than 2% and accuracy within the range of 98% to 102%, ensuring that the measured values are close to the true or nominal values. The method's sensitivity for lidocaine quantification was expressed by the analytical curve slope and the LQ and LD values of 0.313 μg/mL and 0.094 μg/mL, respectively.

The release profiles illustrated in Figure 3A were determined by the percentage relationship of released LDC concentrations over time. The results show that when in solution (S-LDC) lidocaine exhibited rapid and nearly 100% complete lidocaine release within the first 24 hours. This is typical of solutions where the drug is readily available for release without significant physical barriers.

When incorporated in the liquid-crystalline system, lidocaine exhibited the slowest and most gradual release rate. The initial release was significantly lower but steadily increased over time, indicating controlled and prolonged lidocaine release. This result can be particularly beneficial for treating chronic conditions where maintaining stable therapeutic lidocaine levels is desired over longer periods without frequent re-administration.

Mathematical modeling was employed to fit the formulations' kinetics using different models (zero-order, first-order, Korsmeyer-Peppas, Higuchi, and Weibull) as described in Figure 3B. For this case, the Weibull model was the best fit with the highest R^2 value, presenting a b value greater than 1.

This suggests that the release system does not follow simple first-order kinetics but rather a more complex behavior where the release rate is initially slow and accelerates as time progresses. This can be advantageous in applications where a gradual increase in drug release is necessary to achieve effective therapeutic concentrations in a controlled and sustained manner.

This pattern can be associated with several physical phenomena, such as the erosion of the polymeric matrix, where the material degrades exponentially, increasing the surface area available for drug diffusion or swelling, as shown by the formulation. This behavior suggests that the developed liquid-crystalline formulation has the potential to provide sustained symptom relief, meeting the clinical needs of this scenario.

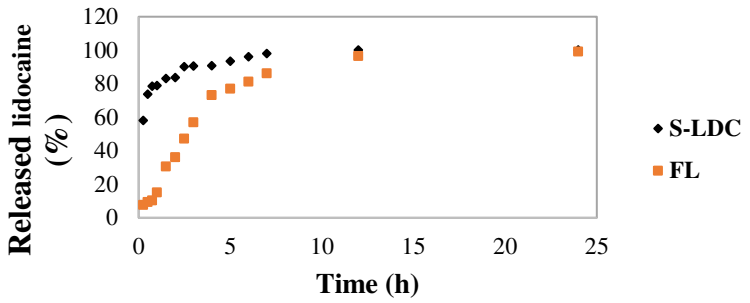
The permeation profiles of the commercial (FC) and the liquid-crystalline system (FL) through porcine buccal mucosa and the permeation parameters are reported in Figure 3C and 3D, respectively. The results show that FL exhibited a lower flux with a shorter lag time ($p<0.05$), suggesting immediate and sustained release.

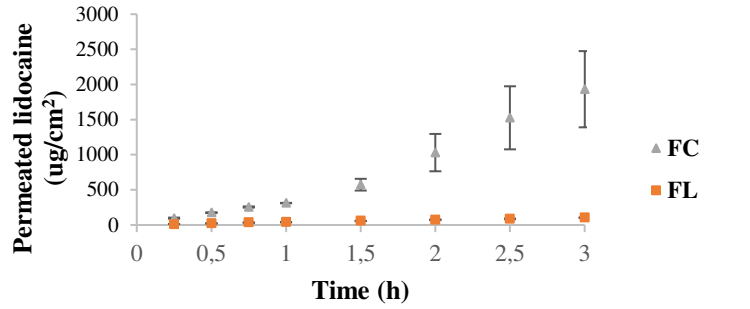
The results of the mucoadhesive properties can be observed in Figures 3E and 3F. Regarding mucoadhesive work (Figure 3E), the formulation with lidocaine (FL) presented the highest mucoadhesive work value. The formulations with lidocaine and added saliva (FL30 and FL100) showed slightly reduced values compared to FL, but still significantly higher than the formulations without lidocaine. The formulations without lidocaine (F, F30, F100) had significantly lower mucoadhesive work values ($p>0.05$). Additionally, the addition of saliva to the formulation with and without lidocaine also slightly reduced the mucoadhesive work values, with the lowest values attributed to the 30% sample (F30 and FL30) ($p>0.05$).

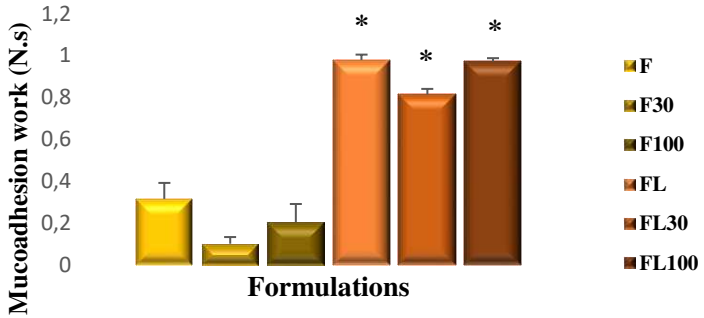
The analysis of variance (ANOVA) revealed that both the formulation and the saliva concentration had a significant effect on the mucoadhesive work values ($p<0.05$). However, the interaction between formulation and saliva was not significant ($p>0.05$), suggesting that the effect of saliva on mucoadhesive work is consistent across different formulations.

Regarding mucoadhesive peak force (Figure 3F), the formulations without lidocaine (F, F30, F100) again presented significantly lower values ($p>0.05$). The formulation with lidocaine (FL) had the highest mucoadhesive peak force value. The formulations with lidocaine and added saliva (FL30 and FL100) showed a slight reduction in peak force compared to FL, but still maintained higher values compared to the formulations without lidocaine. The analysis of variance (ANOVA) also showed that both the formulation and the saliva concentration had a significant effect on mucoadhesive peak force ($p<0.05$). The interaction between formulation and saliva was significant ($p<0.05$), indicating that the mucoadhesive peak force response varies according to the specific combination of formulation and saliva concentration.

Observing the formulation without lidocaine (F), the mucoadhesive peak force and mucoadhesive work values were significantly lower, suggesting that the incorporation of lidocaine may have significantly altered the mucoadhesive properties. This is evident when comparing the samples with lidocaine (FL) to the formulations without the drug. These results suggest that the presence of lidocaine substantially increases mucoadhesion, while the addition of saliva slightly decreases this property, but not to the point of equaling the formulations without lidocaine.







Mathematical Models	*Formulation*
	FL
Korsmeyer-Peppas	
R^2	0,8936
N	0,2872
Higuchi	
R^2	0,9031
K	26,7834
1ª ordem	
R^2	0,9915
K	0,2653
Weibull	
R^2	**0,9968**
b	**3,5006**

	Formulations	
	FC	**FL**
Flux ($\mu g.cm^{-2}.h^{-1}$)	700,0 ± 19,1	33,528 ± 1,4*
Lag time (h)	0,311 ± 0,014	-*

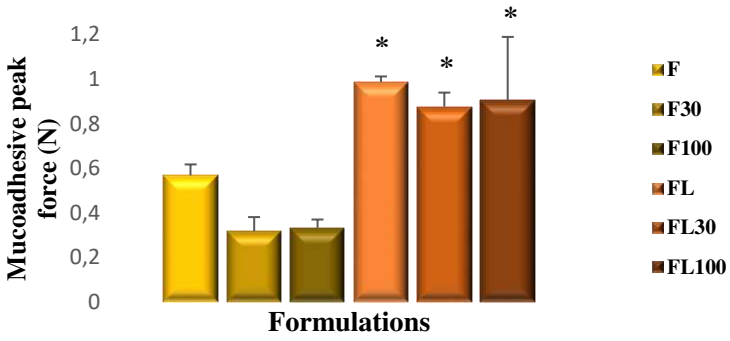


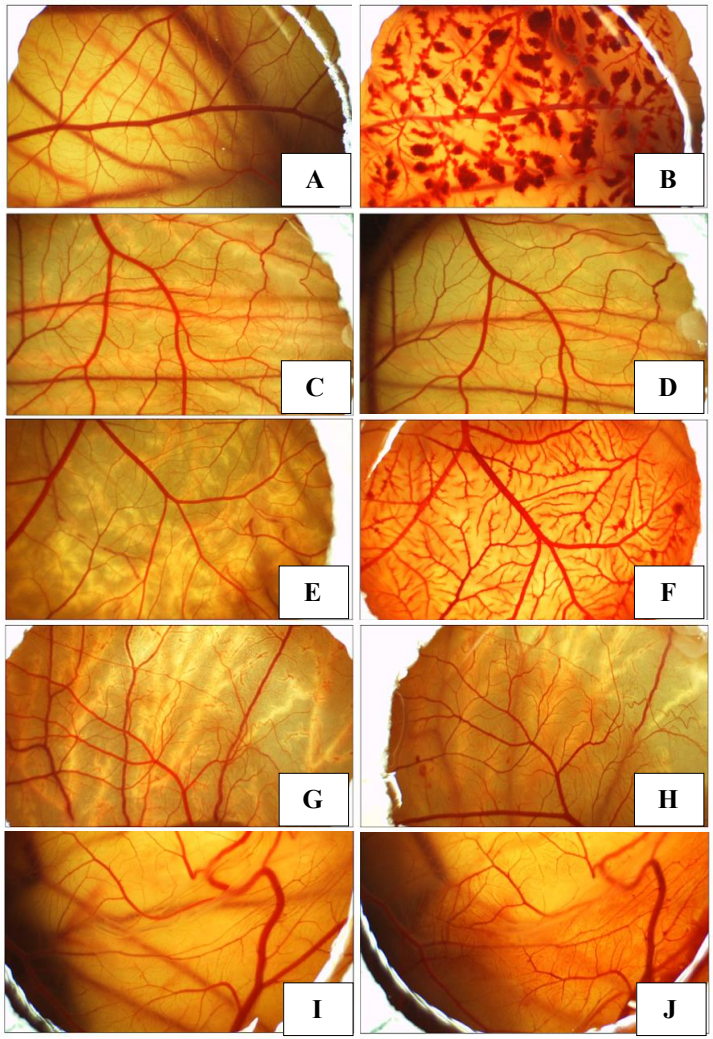
Figure 3. A) Lidocaine release profile in solution (S-LDC) and LDC incorporated in LCS – Test formulation (FL). **B)** Adjusted parameters of kinetic models used in lidocaine release. **C)** Lidocaine permeation profile through porcine buccal epithelium incorporated in FL and FC. **D)** Calculated kinetic parameters from lidocaine permeation profiles. FC is the commercial lidocaine formulation; FL is the test formulation of the liquid-crystalline system with 5% incorporated lidocaine (*p<0,05). **E)** Mucoadhesion work. **F)** Mucoadhesive peak force (F, F30, F100, FL, FL30, FL100, Fao, Fao30, Fao100, FLao, FLao30, FLao100) (Two-factor ANOVA). F is the liquid-crystalline system without lidocaine incorporation. FL is the liquid-crystalline system with 5% lidocaine hydrochloride. F30 and FL30 are F and FL with 30% artificial saliva, and F100 and FL100 are F and FL with 100% artificial saliva, respectively. FC is the commercial lidocaine formulation 50mg/g EMS ointment.

3.2.8 *In Vivo Biological Assays Using the Hen's Egg Test - Chorioallantoic Membrane (CAM) Model*

The results from the experiments are shown in the Figure 4. Left images show micrographs (Figure 4A to J) aspects of chorionallantoic membrane before (left side) and after (right side) treatments. Formulation toxicity classification using the CAM model, according to ECVAM protocol N◦96 is illustrated in Figure 4K.

The lidocaine formulation (FL) presented a degree of irritation, showing a distinct response compared to the negative control group, where no signs of irritation were observed. However, this irritation was significantly lower compared to the positive control group, which, as expected, showed an irritant response. In the FL formulation, there was no evidence of significant irritation or damage to the membrane. Only mild signs of irritation were observed, such as increased blood flow and small areas of hemorrhage. These effects were minor compared to those observed in the positive controls.

It is noteworthy that the tested formulation (FL) was classified as "moderately irritant," similar to the commercial Lidocaine® formulation. This suggests that the observed irritant effects are possibly attributable to the components of the liquid crystalline system (LCS), such as the surfactant used (Procetyl), and not exclusively to the lidocaine



Formulation	Replica	Final Replica Score	Substance Toxicity Classification
Positive control	1	13	Irritant
	2	15	
	3	16	
Negative control	1	0	Non-irritant
	2	0	
	3	0	
Lidocaine®	1	12	Moderately irritant
	2	7	
	3	10	
F	1	7	Moderately irritant
	2	7	
	3	7	
FL	1	6	Moderately irritant
	2	7	
	3	7	

K

Figure 4. Left images- Initial photograph of the CAM before application. Right images - Final photograph of the CAM 3 minutes after application of treatments, showing the effects of the substance on the CAM. (A/B) Positive control. (C/D) Negative control. (E/F) Lidocaine®. (G/H) Formulation "F". (I/J) Formulation "FL". K) Formulation toxicity classification using the CAM model, according to ECVAM protocol N◦96. Positive control is the 1 mol/L sodium hydroxide solution; Negative control is the 0.9% sodium chloride solution; Lidocaine® is the commercial Lidocaine® ointment 50 mg/g (EMS Pharma); F is the test formulation of the liquid-crystalline system without lidocaine, and FL is the test formulation of the liquid-crystalline system with 5% lidocaine hydrochloride.

4. DISCUSSION

The present study successfully described for the first time a liquid crystalline system based on grape seed oil, hyaluronic acid and lidocaine as an innovative approach for the treatment of oral mucositis. The inclusion of grape seed oil in nanostructured systems, such as nanoemulsions and nanoemulgels, has been described for its rejuvenating and healing properties (Vilas Boas de Almeida et al., 2023). These benefits are attributed to its high content of omega-6 fatty acids and vitamin E, which are well known for their anti-inflammatory and antioxidant properties (Oliveira et al. 2024).

However, it was not possible to obtain a liquid crystalline system using only these components. This difficulty could be attributed to the high lipophilic load of grape seed oil, which may have hindered the formation of the system (Miyashiro et al., 2020). It is important to note that the structures formed are highly dependent on the chemical composition of the solvents and surfactants (Kunieda et al., 1998). In this case, the characteristics of the added emollients, such as grape seed oil, promote changes in the formed micelles, influencing the formation of liquid crystal geometries (Terescenco et al., 2018). The presence of highly lipophilic molecules can alter the solubility of the components in the system, affecting the distribution of water and the organization of lipids. If the solubility of the components is compromised, the orderly structure of liquid crystals may not form adequately (Kunieda et al., 1998; Terescenco et al., 2009).

To address this issue, oleic acid was then introduced. Oleic acid is commonly used in liquid crystalline system (Calixto et al., 2016; 2018; Miyashiro et al., 2020) and is known to act as stabilizer. Additionally, it is naturally present in grape seed oil (Arruda, Moreira, 2021). The new oily phase of the system was formulated with a high percentage of grape seed oil to maintain the stability of the active ingredients (Otto et al., 2009).

On the other hand, hyaluronic acid has been previously incorporated into liquid crystal systems for corneal gene therapy, as described by Abelha (2012). Additionally, De Castro (2022) reported its use in *in situ* gelling systems, combined with indomethacin, for the treatment of ocular lesions. Thus, hyaluronic acid stands out not only for its moisturizing properties but also for its fundamental role in cell biology, justifying its inclusion in the formulation developed in the present study.

Another important component of the formulation developed in the present study is lidocaine, which has been widely used in mouthwashes and as a standalone anesthetic, is effective in relieving pain in patients with oral mucositis (Chaveli-López et al., 2016; Sant Ana

et al., 2020; Elad et al., 2020). We have opted to include it at 5% as it was previously demonstrated to promote pain relief in patients with oral mucositis in concentration range between 2% and 5% (Brasil et al., 2012; Sant'Ana et al., 2020).

The obtained formulation was able to maintain its structure with the incorporation of lidocaine hydrochloride in the aqueous phase of the system. Additionally, the formulation exhibeted desirable physicochemical characteristics, including improved mucoadhesion and slow permeation in porcine buccal mucosa.

The ratio of 40% oil phase (grape seed oil and oleic acid – 4:1), 40% surfactant (Procetyl®), and 20% aqueous phase (hyaluronic acid dispersion) resulted in a viscous formulation, characterized with the presence of streaks visualized by PLM throughout the sample. According to Chorilli et al. (2009), this characteristic indicates system stability. In fact, the resulting formulation showed no conformational changes in its internal structure 24 hours after preparation and resting. Moreover, it did not have its structure modified when combined with saliva, as demonstrated in other similar systems that used the same surfactant and oleic acid (Calixto et al., 2018; Miyashiro et al., 2020; Victorelli et al., 2020; Calixto et al., 2021).

The increase in water content from saliva allowed greater mobility of the polar groups of Procetyl®, causing disorder in the lipid chain and increasing the volume of the hydrophobic chain. Despite this, the strong hydrogen interactions kept the area of the polar groups constant as previously demonstrated (Borgheti-Cardoso et al., 2015; Marena et al., 2020; Victorelli et al., 2020).

Nevertheless, a transition behavior from a liquid-crystalline structure to a cubic structure was observed due to water absorption, as demonstrated in the 30% and 100% saliva dilutions (FL30 and FL100), with a tendency to occur *in situ*. This behavior can be explained by the critical packing parameter (CPP) of the combined lipids used (grape seed oil and oleic acid), which relates to the properties of the surfactant, such as the area of the polar group, volume, and length of the apolar chain. These factors influence the curvature of the polar-apolar interface and, consequently, the type of liquid crystal formed (Souza et al., 2014 Marena et al., 2020).

The addition of substances to the system, such as drugs, solvents, co-solvents, and other solutes, can alter the critical packing parameter (CPP), modifying the formed mesophase and/or promoting phase transitions (Guo et al., 2010). Highly hydrophobic molecules, like the grape seed oil used in the study, favor the formation of cubic and hexagonal phases (Shah et al., 2001). In the study, it was observed that FL spontaneously formed a liquid crystalline system

(LCS) with a hexagonal mesophase, and after water absorption (FL30 e FL100), transitioned to a cubic mesophase, corroborating the results of Shah et al. (2001). This occurs because the presence of lipophilic molecules increases the volume of the lipid's hydrophobic chain and, consequently, the CPP, favoring cubic and hexagonal phases (Souza et al., 2014). The transition to the cubic phase generally occurs with 20-25% water absorption, which, being viscous, limits additional water uptake and promotes rapid swelling of the formulation (Souza et al., 2014).

From a pharmaceutical perspective, the mesophase behavior, showing a transition from hexagonal to cubic in all dilution variations, with and without drug incorporation, is very promising for the topical administration of drugs in the oral cavity. The changes in CPP values induced by excess water also imply rheological changes in the mesophases, as already described in the literature (Engström & Engström L, 1992) and confirmed in the present study. Thus, the developed formulation remained viscous in *in situ* conditions, i.e. in the presence of artificial saliva, with improved *in vitro* mucoadhesion, corroborating previous studies, such as Calixto et al. (2023), which used mucoadhesive liquid crystal precursor systems for the development of a formulation intended for oral cancer topical treatment. This aspect is clinically essential to overcome the limitations of the oral cavity and ensure retention at the site of action, which is crucial for the efficacy of topical formulations intended for oral cavity use.

This study reveals that in liquid crystalline systems (LCS), the spacing between lamellar layers or hexagonal micelles allows greater penetration and retention of water, as seen in the case of lidocaine containing liquid crystalline formulation, which originally exists in a hexagonal mesophase. This phase is more flexible for structural reorganization upon contact with water, facilitating absorption. The cubic structure formed by water absorption is more compact, with interconnected channels offering less space for water incorporation. This was demonstrated by Kumar et al. (2004), who observed that the cubic mesophase, with its dense three-dimensional structure, has a lower capacity for water incorporation. This aspect is important because water absorption implies the swelling of the system, which likely likely occurs at the application site, influencing its release profile as demonstrated by other authors (Burrows et al., 1994; Chang & Bodmeier, 1997b; Lara et al., 2005; Shah & Paradkar, 2007; Souza et al., 2014).

When evaluating the obtained texture profile results, the lidocaine-containing liquid crystalline formulation, with or without saliva showed a decrease in the mechanical properties such as viscosity, adhesiveness, hardness, compressibility, and cohesion. This decrease is consistent with findings from previous studies investigating the impact of drugs on the physical

properties of LCS and lipid formulations, as reported by Calixto et al. (2018, 2021). Furthermore, studies such as those by Müller and Müller-Goymann (1986) demonstrated that lidocaine can act as a plasticizing agent, increasing the mobility of molecules within the liquid crystal matrix, resulting in reduced hardness and cohesiveness. Additionally, the introduction of lidocaine can interfere with intermolecular interactions of surfactants, as observed in the research by Siqueira Leite et al. (2018), which discussed the disruption of molecular packing due to the presence of hydrophilic drugs. These interactions can lead to a reorganization of the structure, making the matrix less resistant to external forces. Consequently, the decrease in the mechanical properties of the formulations with lidocaine observed in this study reflects an expected trend and is consistent with the literature, reinforcing the need to optimize the composition of formulations to balance drug delivery efficacy and maintain desirable mechanical properties for topical application.

Regarding rheology measures, the developed lidocaine-containing liquid crystalline formulation exhibited Newtonian fluid behavior. This characteristic is important to ensure uniform application on the oral mucosa, as it indicates that the formulation's viscosity remains constant regardless of the shear rate. These findings corroborate previous studies that highlighted the importance of viscosity in the stability and efficacy of lipid formulations (Calixto et al., 2018; Victorelli et al., 2020). This behavior was maintained despite the presence of 30% saliva, indicating that the formulation maintains its rheological stability even when exposed to a simulated oral mucosa environment. This result is consistent with the cited literature and suggests that the formulation is robust enough to withstand variations in the application environment. This characteristic is especially relevant since patients undergoing cancer treatment (chemotherapy and/or radiotherapy) often experience reduced salivary flow (Silva et al., 2022).

However, when diluted with 100% saliva, we observed a change in rheological behavior, indicating pseudoplastic fluid characteristics. This means that the formulation's viscosity decreased with increased shear rate, but tends to become more viscous again after the shear force ceases, which can be beneficial for more effective application and spreading on the oral mucosa of patients with normal salivary flow, with the viscosity returning upon cessation of topical application tension. This observation is supported by previous studies that emphasized the importance of adapting the rheological behavior of formulations to oral mucosa conditions (Chorilli et al., 2016). In the present study, formulations containing lidocaine presented ideal rheological characteristics in different saliva proportions, indicating suitible

properties for topical application in the oral cavity with different salivary flows, as previously addressed (Kim et al., 2015).

Analyzing the viscoelastic response of the formulations, we observed that the developed formulation demonstrated viscoelastic liquid fluid behavior (G”>G’), indicating the presence of both liquid and solid properties. This characteristic is advantageous as it allows the formulation to deform and recover its initial structure, facilitating its application on the oral mucosa, as previously addressed (Lee et al., 2019; Wang et al., 2016). These characteristics were maintained even when diluted with 30% and 100% saliva (FL30 and FL100), suggesting that the formulation was able to preserve its viscoelastic properties even in a simulated oral mucosa environment. This observation is consistent with previous studies that emphasized the stability of formulations under similar conditions (Calixto et al., 2018; Souza et al., 2014). However, with the higher saliva proportion (100%), the formulation exhibited viscoelastic solid behavior (G’>G”). This suggests a transition in the formulation structure, making it more resistant to deformations, findings that corroborate with studies by Lee (Lee et al., 2019) and Wang (Wang et al., 2016). This fact may be particularly relevant for patients with normal saliva flow, as the formulation's increased resistance means saliva would not easily remove the formulation, making adhesion more effective and stable at the application site. Thus, it would better overcome the barrier imposed by saliva flow in such patients.

These findings align with mucoadhesion results. Despite the presence of lidocaine have decreasing the maximum mucoadhesive force, the formulation showed higher mucoadhesion work. Together, the obtained mucoadhesion properties are above the adequate mucoadhesive values (peak force > 0.587 N and mucoadhesion work > 0.468 N.s) as determined in the same experimental conditions for a well-known mucoadhesive commercial formulation (Pestana et al., 2024). These results indicate that the developed formulation presents satisfactory *in vitro* mucoadhesiveness and may also have clinical adherence to the application site.

Moreover, previous studies have shown that formulations with a high degree of internal rigidity can impair the interaction between the formulation and biological membrane proteins, as the interpenetration between polymers and mucosa proteins is a key factor in mucoadhesion (Carvalho et al., 2014; Salmazi et al., 2015). This characteristic corroborates the results found in this research, as formulations without lidocaine incorporation exhibit greater hardness, as demonstrated in the texture profile test, showing lower average mucoadhesion work values when compared to formulations containing drug incorporation.

Regarding *in vitro* release kinetics, lidocaine release was described by the Weibull model, as already demonstrated in studies using this drug in LCS and/or precursor systems

(Calixto et al., 2018). The transport of lidocaine from the lidocaine solution (S-LDC) occurred predominantly by Fickian diffusion, considering a b-value less than 0.75, as described by Rodero et al. (2018). In contrast, the developed liquid crystalline formulation presented a b-value greater than 1, suggesting that lidocaine release is due to a complex mechanism involving multiple processes such as diffusion, swelling, and erosion. This behavior can be attributed to the liquid crystal structure, which, besides allowing drug diffusion, also undergoes swelling and possible matrix erosion, as previously evidenced (Kim et al. 2015). This characteristic can contribute to a sustained therapeutic effect, which is aligned with the objectives of the present study.

Similarly, lidocaine permeation across porcine buccal mucosa revealed both immediate and slow permeation, as seen in the *in vitro* release experiment using artificial membrane. This rapid onset of permeation can be attributed to the presence of oleic acid in the formulation, which acts as a permeation enhancer, as previously verified in studies (Calixto et al., 2016; Calixto et al., 2018). Additionally, this characteristic is advantageous when immediate drug action is needed (Wang et al., 2022). The low flux observed for lidocaine is desirable in sustained release systems, allowing the anesthetic effect to be maintained for a prolonged period, enabling longer intervals between applications. Specifically, in the treatment of oral mucositis, Yarom et al. (2020) emphasize the importance of formulations providing quick and lasting symptomatic relief, contributing to improved quality of life for cancer patients.

On another note, evaluating the toxicity of the test formulation is necessary to verify possible toxicity caused by both the local anesthetic and other components of the new proposed formulation, such as the surfactant. The model using the chorioallantoic membrane (CAM) of the chicken embryo is a promising toxicity evaluation model for this purpose since the CAM is composed of easily accessible veins, arteries, and capillaries (Victorelli et al., 2020), allowing *in vivo* evaluation of substance toxicity at low cost. Moreover, it is an alternative method that does not cause pain to the animals involved, as the tests occur before the fourteenth day of embryonation, and the neuronal structures for pain perception are not yet formed (Tavakkoli et al., 2020). In this perspective, Bresciani et al. (2016) corroborate, mentioning that the use of CAM offers a robust approach to investigate the toxic effects of compounds without the need for experimentation on more advanced mammalian animals, contributing to reducing the use of animals in scientific research. This method has already been used to study the toxicity of other topical formulations for oral cavity use, such as commercial toothpaste (Corrado et al., 2012) and to evaluate a liquid crystal formulation containing an innovative peptide for oral cancer treatment (Calixto et al., 2021).

The results of the present study revealed that the liquid crystalline system (LCS) formulation composed of grape seed oil and hyaluronic acid with or without incorporated lidocaine received a classification of "moderately irritating," similar to that obtained with the corresponding commercial formulation widely used in dentistry (Boyce et al., 2016). Moreover, the commercial Lidocaine® formulation has been clinically used for over seven decades (Jeske, 1998; Donaldson & Goodchild, 2018) and since then, it has been considered the most widely used local anesthetic in medicine and dentistry in most countries where it is marketed, representing the gold standard for comparison (Malamed, 2021).

It is worth mentioning that there are reports in the literature that the CAM method may generate overestimated responses regarding the toxicity of a substance compared to those found in human oral mucosa (Corrado et al., 2012). This can be justified due to the morphological differences between these tissues. Histologically, the CAM is composed of two epithelial layers surrounding a thin stroma layer, where blood vessels are located (Nowak-Sliwinska, 2014). The human oral mucosa, on the other hand, has a lining epithelium (keratinized or non-keratinized) and underlying connective tissue, separated by a basement membrane (Li et al., 2018). Thus, the CAM is more susceptible to the possible effects of test substances, having a simpler morphology with fewer protective layers, making it a more sensitive model to the irritating effects of topical formulations. Therefore, the "moderately irritating" effect exerted by the formulations developed in this study may be potentially safe clinically.

5. CONCLUSION

In conclusion, the study developed an innovative formulation for the treatment of oral mucositis, composed of a liquid crystalline system with grape seed oil, oleic acid, PPG-5-CETETH-20 (Procetyl®) as a surfactant, hyaluronic acid, and lidocaine. The formulation presented ideal and promising characteristics for the treatment and symptomatic relief of the condition. It demonstrated appropriate viscosity, high mucoadhesive capacity, immediate and sustained permeation of the anesthetic, and toxicity similar to the commercial product. These results represent a significant contribution to health and science, offering a promising perspective for future *in vivo* evaluation using an oral mucositis animal model.

ACKNOWLEGEMENTS

This study was supported by São Paulo Research Foundation (FAPESP) (Grant # 2020/03786-9). The author MBPF acknowledges the scholarship provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

The authors would like to thank Prof. Dr. Marlus Chorilli and Dr. Aline Martins dos Santos (School of Pharmaceutical Sciences – São Paulo State University "Júlio de Mesquita Filho") for their assistance in performing the rheology analyses, as well as to express their gratitude to laboratory technician Fábio Padilha, from the Piracicaba Dental School, for his invaluable help with the porcine samples, which was fundamental for the development of this research.

We also are very thankfull to Cristália Laboratory, for donating the local anesthetic used in the study.

REFERENCES

1. Arruda LV, Moreira CGB. Uso do óleo de semente de uva em formulações cosméticas. *Rev Intellectus.* 2021;63(1):219.
2. Bezerra AAC. Toxicidade de anestésicos tópicos de uso odontológico a partir do modelo da membrana corioalantoica de embrião de galinha [dissertação de mestrado]. Piracicaba, São Paulo: Universidade Estadual de Campinas; 2023.
3. Borgheti-Cardoso LN, Silva AR, Ibanes SO, Visentainer JV, Carvalho IC, Bruschi ML. Mucoadhesive properties and stability of hydrogels containing glycolic extract of *Phyllanthus niruri. Eur J Pharm Sci.* 2015;70:93-101.
4. Boyce JM, Havill NL, Maria B. Use of oral antiseptics and mouth rinses in dental practice. *Am J Infect Control.* 2016;44(4).
5. Bortolazzo FO. Efeito combinado da aplicação de células tronco e microcorrente na cicatrização do tendão calcanear de ratos após transecção parcial [tese de doutorado]. [s.l.]: [s.n.]; 2018.
6. Brasil. Ministério da Saúde. Instituto Nacional de Câncer (INCA). Estimativa de Câncer no Brasil. 2023. Disponível em: https://www.inca.gov.br/estimativa
7. Calixto GMF, Garcia CM, Cury BSF, Chiavacci LA, Chorilli M. Design and Characterization of a Novel p1025 Peptide-Loaded Liquid Crystalline System for the Treatment of Dental Caries. *Molecules.* 2016;21(2):158.
8. Calixto G, Yoshii R, dos Santos M, Kaneko T, Pereira M, Baby A, et al. Development and characterization of lyotropic liquid crystalline systems for topical delivery of lidocaine hydrochloride. *J Pharm Sci.* 2018;107(10):2678-89.
9. Calixto G, Fonseca-Santos B, Chorilli M, Leonardi GR. Nanotechnology-based drug delivery systems for treatment of oral mucositis. *J Control Release.* 2021;330:714-30.

10. Calixto G, Luiz M, Di Filippo L, Chorilli M. Mucoadhesive liquid crystal precursor system for photodynamic therapy of oral cancer mediated by methylene blue. *Photodiagnosis Photodyn Ther.* 2023;44:103739. doi:10.1016/j.pdpdt.2023.103739.
11. Carvalho FC, Chorilli M, Gremião MPD. Plataformas bio (muco) adesivas poliméricas baseadas em nanotecnologia para liberação controlada de fármacos-propriedades, metodologias e aplicações. *Polímeros.* 2014;24:203-213.
12. Chaveli-López B, Bagán-Sebastián JV. Treatment of oral mucositis due to chemotherapy. *J Clin Exp Dent.* 2016;8(2).
13. Chorilli M, Prestes PS, Leonardi GR, Scarpa MV, Scarpa CH. Mucoadhesive thermoresponsive in situ gelling liquid crystalline systems: characterization and biocompatibility. *J Mater Sci Mater Med.* 2009;20(7):1523-31.
14. Chorilli M, Silva GV, Ferrari PC, et al. Rheological Characterization and Safety Evaluation of Non-Ionic Lamellar Liquid Crystalline Systems Containing Retinyl Palmitate. *J Biomed Nanotechnol.* 2016;12(2):394-403.
15. Contri RV, Frank LA, Kaiser M, Pohlmann AR, Guterres SS. Antioxidant properties of grape seed oil and its potential application in food systems: a review. *J Pharm Pharmacol.* 2014;66(7):1081-1095.
16. Corrado AD, de Andrade JB, dos Santos MG, Pinto GR, Gomes IP. Safety evaluation of toothpaste and oral care formulations using the chorioallantoic membrane model. *Toxicol In Vitro.* 2012;26(2):239-44.
17. De Araújo JSM, De Oliveira CP, De Souza JB, et al. Resistivity technique for the evaluation of the integrity of buccal and esophageal epithelium mucosa for in vitro permeation studies: Swine buccal and esophageal mucosa barrier models. *Pharmaceutics.* 2021;13(5):643.
18. Dinh AT, Pham TL, Lee NT, Phan TN, Pham TT, et al. Cubic liquid crystals as innovative carriers for drug delivery. *J Control Release.* 2024;338:26-41.
19. Donaldson M, Goodchild JH. Pharmacology of local anesthetics used in dental practice. *Anesth Prog.* 2018;65(2):55-65.
20. Dos Santos Ramos M, Crepaldi AL, Silva AR, et al. Syngonanthus nitens Bong. (Rhul.) -sistema nanoestruturado carregado para o tratamento da candidíase vulvovaginal. *Int J Mol Sci.* 2016;17(8):1368.
21. Elad S, Cheng KKF, Lalla RV, Yarom N, Hong C, Logan RM, et al. MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer.* 2020;126(19):4423-31.

22. Engström S, Engström L. Phase behaviour of the lidocaine-monoolein-water system. *Int J Pharm.* 1992;79(1-3):113-22.
23. Engström S, Engström L. Preparation of liquid crystalline phases in the presence of saliva. *J Colloid Interface Sci.* 1992;149(1):44-50.
24. Franz-Montan M, Silva ALR, Bonferoni MC, Sandri G, Ferrari F, Silva LM, et al. Nanostructured systems for transbuccal drug delivery. In: Andronescu E, Grumezescu AM, editors. *Nanostructures for Oral Medicine.* 1st ed. Elsevier; 2017. p. 87-121.
25. Franz-Montan M, Ribeiro L, Santana MH. Recent advances and perspectives in topical oral anesthesia. *Expert Opin Drug Deliv.* 2017;14(5):673-684.
26. Griesser J, Hetényi G, Nguyen HyD, et al. Thiolated Hyaluronic Acid as Versatile Mucoadhesive Polymer: From the Chemistry Behind to Product Developments-What Are the Capabilities? *Polymers (Basel).* 2018;10(7):748-763.
27. Guo C, Wang J, Cao F, Lee RJ, Zhai G. Lyotropic liquid crystal systems in drug delivery. *Drug Discov Today.* 2010;15(23-24):1032-1040.
28. Isopencu G, Constantin M, Fundueanu G, Ascenzi P, Marin LM, Dorobantu D, et al. Hyaluronic acid-based mucoadhesive drug delivery systems for improved therapeutic efficacy. *Int J Biol Macromol.* 2023;166:890-903.
29. Jambi HAS, Saleh H, Abdallah MA, et al. Potential antioxidant anti-inflammatory and gastroprotective effect of grape seed extract in indomethacin-induced gastric ulcer in rats. *Int J Farmacol.* 2019;15(2):209-18.
30. Jeske AH. Local anesthetics in dentistry: then and now. *Gen Dent.* 1998;46(2):178-80.
31. Kim DH, Jahn A, Cho SJ, et al. Lyotropic liquid crystal systems in drug delivery: a review. *J Pharm Investig.* 2015;45:1-11.
32. Kumar P, Goyal A, Kumar N. Cubic liquid crystalline phases in drug delivery systems. *Pharm Res.* 2004;21(7):1279-83.
33. Kunieda H, Ozawa K, Huang KL. Effect of oil on the surfactant molecular curvatures in liquid crystals. *J Phys Chem B.* 1998;102(5):831-838.
34. Lee CM, Otte KS, Anderson S, Williams RO. Characterization of liquid crystalline drug formulations for improved solubility and bioavailability. *J Drug Deliv Sci Technol.* 2019;53:101151.
35. Leal AM, Souza MG, Gomes RM, et al. Potencial terapêutico da camomila (Matricaria chamomilla L. Asteraceae) no manejo da mucosite oral: revisão integrativa. *Rev Bras Planta Med.* 2024;24(1):123-30.

36. Li W, Liu Z, Chen W, Jiang Y, Feng X, Chen X. Histological comparison of the chorioallantoic membrane and oral mucosa for evaluation of mucoadhesive properties. *J Pharm Sci.* 2018;107(4):1065-71.
37. Liu S, Pan J, Liu H, et al. Status of Treatment and Prophylaxis for Radiation-Induced Oral Mucositis in Patients With Head and Neck Cancer. *Front Oncol.* 2021;11:642575.
38. Liu Q, Zheng C, Yang B, Sun H. Codelivery of doxorubicin and MDR1-siRNA by mesoporous silica nanoparticles polymer-polyethylenimine to improve oral squamous carcinoma treatment. *Int J Nanomedicine.* 2018;13:187-198.
39. Logan RM, Ahmed SM, Adkins D, et al. Serum levels of NFkappaB and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer Biol Ther.* 2018;7(7):1139-45.
40. Malamed SF. Manual de Anestesia Local. 7ª ed. Rio de Janeiro: Grupo GEN; 2021.
41. Marques M, et al. Simulated biological fluids with possible application in dissolution testing. *Dissolution Technologies.* 2011;18(3):15-28.
42. Marena GD, Cervini P, Silva ER, Ribeiro MS, Avila RE, Araujo GL. Structural transitions in liquid crystals induced by saliva interaction. *J Mol Liq.* 2020;314:113618.
43. Miyashiro C, Taira MC, Penedo LA, Rossi AG, Yamashita F, Barros SB. Evaluation of grape seed oil in the development of lipid-based formulations for skin treatment. *Pharm Dev Technol.* 2020;25(1):25-35.
44. Mohammed M, Devnarain N, Elhassan E, Govender T. Exploring the applications of hyaluronic acid-based nanoparticles for diagnosis and treatment of bacterial infections. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2022;14(4)
45. Müller RH, Müller-Goymann CC. Interaction of lidocaine and lidocaine-HCl with the liquid crystal structure of topical preparations. *Int J Pharm.* 1986;32(2-3):229-238.
46. Mueller-Goymann CC. Transdermal delivery of lidocaine: evaluation of different penetration enhancers in liquid crystal systems. *Pharm Res.* 1986;3(6):300-4.
47. Nowak-Sliwinska P, Seguin J, Pache JC, van den Bergh H. The chorioallantoic membrane model in tumor biology. *Exp Cell Res.* 2014;328(2):318-28.
48. Oliveira RM, Gonçalves RF, Machado TD, Costa AM, Barros BS, Rezende KR, et al. Grape seed oil: physicochemical characterization and benefits in dermatology. *J Appl Pharm Sci.* 2024;14(1):52-64.
49. Otto A, du Plessis J, Wiechers JW. Formulation effects of topical emulsions on transdermal and dermal delivery. *Int J Cosmet Sci.* 2009;31(1):1-19.

50. Pestana AM, De Lima FS, Santos CR, et al. Analysis of key factors for evaluating mucosal adhesion using swine buccal tissue. *J Pharm Sci.* 2024.
51. Pestana AM, Bezerra AAC, Barbin T, Franz-Montan M. Tissue-based in-vitro models for buccal permeability studies. In: *Concepts and Models for Drug Permeability Studies.* Woodhead Publishing; 2024. p. 275-308.
52. Price Z, Robinson R, Adeyemi A, Sikiti L, Snyder M. Multifunctional nanostructured systems for drug delivery and therapy. *Adv Drug Deliv Rev.* 2017;117:63-88.
53. Rodero A, González-Rodríguez ML, Rabasco AM. Release and diffusion of drugs in liquid crystal systems. *Int J Pharm.* 2018;544(1):101-10.
54. Rodriguez-Lopez JM, Pereyra AM, Chattah AK, et al. Antimicrobial properties of grape seed extract and its applications in oral health care. *J Appl Microbiol.* 2022;133(2).
55. Salmazi R, Lima AC, Saczko J, Spolnik P, Zielinska-Pisklak M, Cabeça LF, et al. A curcumin-loaded liquid crystal precursor mucoadhesive system for the treatment of vaginal candidiasis. *Int J Nanomedicine.* 2015;10:4815-23.
56. Sant Ana G, Normando AGC, De Toledo I, Dos Reis PED, Guerra ENS. Topical Treatment of Oral Mucositis in Cancer Patients: A Systematic Review of Randomized Clinical Trials. *Asian Pac J Cancer Prev.* 2020;21(7):1851-1866.
57. Santos MA, Franco FN, Caldeira CA, de Araújo GR, Vieira A, Chaves MM, Lara RC. Antioxidant effect of Resveratrol: Change in MAPK cell signaling pathway during the aging process. *Arch Gerontol Geriatr.* 2021;92:104266.
58. Santos MA, Franco FN, Soares DP, et al. O resveratrol tem seus mecanismos protetores antioxidantes e anti-inflamatórios diminuídos no envelhecimento. *Arco Gerontol Geriatr.* 2023;107:104895.
59. Shah JC, Sadhale Y, Chilukuri DM. Cubic phase as drug delivery systems. *Adv Drug Deliv Rev.* 2001;47:229-250.
60. Silva IA, Lanza AO, Rodrigues Filho AB, et al. Impacto do tratamento antineoplásico na microbiota da cavidade oral e orofaríngea de pacientes acometidos pelo câncer de cabeça e pescoço: revisão sistemática. *Rev Bras Cancerol.* 2022;68(1)
61. Siqueira Leite C, Coelho J, Muehlmann L, Azevedo R, Sousa M. Microemulsions as Platforms for Transdermal Delivery of Hydrophilic Drugs – A Review. *Curr Nanosci.* 2018;14.

62. Singh P, Kumar A, Khan S, Arif M, Sharma S, Jain SK. Resveratrol enhances the anti-inflammatory effects of nonsteroidal anti-inflammatory drugs in animal models. *J Inflamm Res.* 2019;12:195-203.
63. Souza C, Watanabe E, Borgheti-Cardoso LN, Fantini MCA, Lara MG. Mucoadhesive System Formed by Liquid Crystals for Buccal Administration of Poly (Hexamethylene Biguanide) Hydrochloride. *J Pharm Sci.* 2014;103(12):3914-3923.
64. Souza AG, Mota JR, Medeiros VP, Gomes AL, Braga MB. Mucoadhesive properties of thermoresponsive polymers for drug delivery applications. *Eur J Pharm Sci.* 2014;59:111-20.
65. Tavakkoli M, Shabani N, Kalhori MR, Tavakoli-Yaraki M. Alternative methods for toxicity evaluation using the chorioallantoic membrane assay. *Adv Pharm Bull.* 2020;10(1):1-8.
66. Terescenco D, Picard C, Clemenceau F, et al. Influence of emollient structure on the properties of cosmetic emulsions containing lamellar liquid crystals. *Colloids Surf A Physicochem Eng Asp.* 2018;536:10-19.
67. Victorelli FD, Lopes PS, Mercurio DG, Oliveira RF, Leonardi GR. Potential of grape seed oil in topical formulations for dermal delivery of therapeutic agents. *Int J Cosmet Sci.* 2020;42(5):510-20.
68. Victorelli FD, Rodero CF, Lutz-Bueno V, Chorilli M, Mezzenga R. Amyloid fibrils enhance the topical bio-adhesivity of liquid crystalline mesophase-based drug formulations. *Adv Healthc Mater.* 2023;12(12).
69. Vilas Boas de Almeida B, Gomes dos Santos M, Tescarollo IL. Nanotecnologia: revisão integrativa sobre uso de nanoemulgéis em cosmecêuticos. In: *Revista Ensaios Pioneiros.* 2023;7(1).
70. Wang CY, Lee I, Chen M, et al. Recent advances in discovery and biosynthesis of natural products from myxobacteria: an overview from 2017 to 2023. *Nat Prod Rep.* 2024.
71. Wang M, Wang L, Han Z, Guo Z. Recent advances in drug delivery using thermoresponsive and mucoadhesive materials. *Int J Pharm.* 2016;502(1-2):189-98.
72. Wang Y, Fang Z, Zhang Y, Dong X. Thermoresponsive and mucoadhesive drug delivery systems for targeted therapy. *J Drug Target.* 2022;30(4):421-35.
73. Yadav P, Kalonia DS, Narang AS. Enhanced permeability of hydrophilic drugs in microemulsion systems. *Int J Pharm.* 2023;627:122230.

74. Yarom N, Hovan AJ, Bossi P, Brennan MT, Dupuis LL, Gibson RJ, et al. Systematic review of natural and miscellaneous agents for the management of oral mucositis in cancer patients. *Support Care Cancer.* 2020;28(5):2281-94.

SUPPLEMENTARY MATERIAL

1. DEVELOPMENT OF A LIQUID CRYSTAL SYSTEM

Initially, a pilot test of the formulation composition was evaluated using the surfactant polyoxypropylene (5) polyoxyethylene (20) cetyl ether, that is, Procetyl®, grape seed oil, and water. However, the mixture of these three components in different 54 proportions combining visual and PML classification did not result in the formation of a liquid crystal system, as the microscope images were of a dark field, and liquid systems with phase separation were basically formed. The same behavior was observed when hyaluronic acid, characterized as the dispersion component chosen for the aqueous phase, was added.

Upon examining both the structure of grape seed oil and its solubility characteristics as well as the surfactant and critical packing parameter, oleic acid was added to the oily phase.

Tests conducted with the study of the oily phase proportions defined an 80% grape seed oil and 20% oleic acid ratio (4:1) as shown in Table 1, ensuring a high percentage of the first and sufficient quantity of the second to guarantee the formation of the structure, and additionally presented a more viscous texture based on visual and PML classification analysis.

Proportion of grape seed oil – oleic acid (%)	Microscopic Image	Microscopic Classification	Visual Classification
50 – 50	Maltese cross	Cubic LC	SVTr
75 – 25	Maltese cross	Cubic LC	SVTr
80-20	Stretch marks	Hexagonal LC	SVT
85-15	Stretch marks	Hexagonal LC	SLT
90 - 10	Dark Field	-	SLT
95 – 5	Dark Field	-	SLT

Table 1. Proportion of the oily phase composition (%), microscopic and visual classification of the formed systems.

The non-immediate formation of liquid crystal can be explained by the critical packing parameter (CPP) (Otto Plessis & Wiechers, 2009). The high polarity of the grape seed oil components as previously reported (Miyashiro et al., 2020) and its molecular structure possibly influenced the CPP, preventing the formation of a liquid crystal system, necessitating the adjustments made in the present study. Oliveira et al. (2024) demonstrated that the combination of oleic acid can promote stability to grape seed oil.

2. CONTROL FORMULATION OF CONVENTIONAL LIPID

2.1. Material and Methods

2.1.1. Development of the formulation

In the present study, for comparison purposes, a conventional lipid formulation was developed to evaluate the results of the LC system against a formulation without the spontaneous formation of the system. The conventional formulation consisted solely of oleic acid, a commonly used component in pharmaceutical formulations as a vehicle or excipient. This formulation was included in the study as a control group to assess the relative efficacy of the test formulation. The formulation had the same proportion of the phases of the test formulation, in this case, 40% oily phase, 40% surfactant, and 20% aqueous phase.

The formulations were obtained following the method carried out by Calixto et al. (2018). The diagram was constructed using oleic acid as the oily phase, water as the aqueous phase, and the same surfactant, Procetyl®, resulting in the formation of 54 points. Similarly, the control formulation was characterized using techniques such as polarized light microscopy, swelling tests in artificial saliva, rheological studies, release kinetics, permeation capacity, and *in vitro* mucoadhesion.

2.2. Results

2.2.1. Development of liquid crystal systems and characterization of the formulations

The diagram was constructed following the same protocol established for the test formulation, selecting the phase diagram point corresponding to the same proportion of the test formulation.

The conventional lipid formulations composed of Procetyl®, oleic acid, and water selected the formulation – Fao – a control formulation with the same phase proportion as the test formulation, i.e., 40% oily phase (oleic acid), 40% of the same surfactant, and 20% aqueous phase (water), characterized as located in a translucent liquid system area and near a transition region from liquid system to viscous system with increasing aqueous phase.

The control formulation Fao increased viscosity from dilution with 30% and 100% artificial saliva. The same behavior was observed with the incorporation of lidocaine. Formulations with lidocaine were less viscous compared to those without lidocaine.

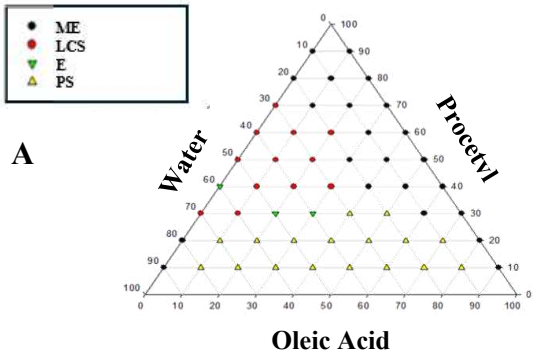
Based on microscopic and visual characterization, Fao was classified as a microemulsion due to the isotropic dark field image characteristics visualized by polarized light microscopy, as illustrated in Figure 1 along with visual classification as translucent liquid. This characteristic was altered upon dilution with 30% and 100% saliva (Fao30 and Fao100),

becoming a viscous system and still showing dark field in microscopy, indicating the formation of a liquid crystal system in the cubic mesophase.

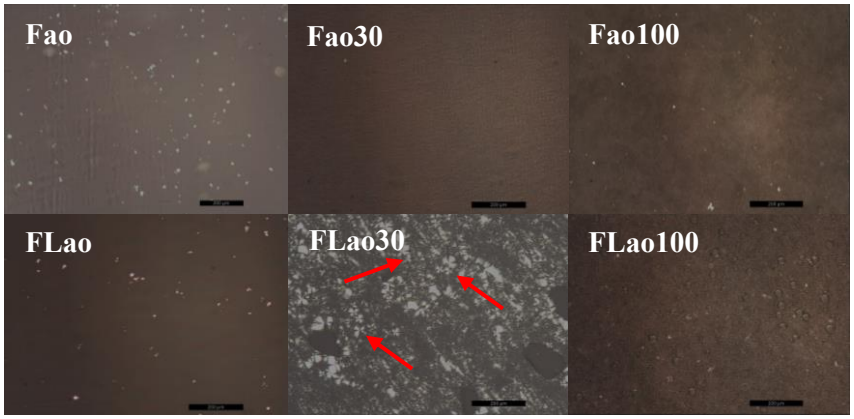
With the incorporation of lidocaine (FLao), the behavior as a precursor of the liquid crystal system was maintained; however, with 30% saliva dilution (FLao30), there were signs of lamellar mesophase formation, as indicated by the Maltese crosses in PML, with rearrangement to cubic mesophase upon dilution with 100% saliva (FLao100).

The behavior observed in the microscopy can be seen in the images and is arranged in figure 1.

A



B



C

Formulation	Surfactant	Fase oleosa	Aqueous Phase	Dilution Medium	Drug	Visual Classification	Microscopic Classification
	Procetyl®	Oleic acid	Water	Artificial saliva	Lidocaine		
Fao	40	40	20	-	-	SLTr	ME
Fao30	40	40	20	30	-	SVO	Cubic
Fao100	40	40	20	100	-	SVO	Cubic
FLao	40	40	20	-	5	SLTr	ME
FLao30	40	40	20	30	5	SVO	Lamelar
FLao100	40	40	20	100	5	SVO	Cubic

Figure 1. A) Phase diagram with 54 different proportions of the control formulation composition with classification by PML. B) Photomicrographs of the control formulations (conventional lipid): Fao; Fao30; Fao100; FLao; FLao30; FLao100. Magnification of 20x. The red arrows point to the formation of Maltese crosses. C) Composition (%, w/w), visual and microscopic classification of the test formulations. Fao is the control formulation of conventional lipid, a precursor system of liquid crystal without the incorporation of lidocaine. FLao is the control formulation of conventional lipid, a precursor system of liquid crystal with 5% lidocaine hydrochloride. Fao30 and FLao30 are Fao and FLao with 30% artificial saliva, and Fao100 and FLao100 are Fao and FLao with 100% saliva, respectively.

2.2.2. Texture profile analysis and determination of rheological behavior

The texture profile analysis charts are shown in sequence. The control formulation is characterized as a liquid formulation, with viscosity increasing with 30% and 100% saliva dilution (Fao30 and Fao100), with Fao100 being the most viscous among the three conditions ($p<0.05$), resulting in higher hardness and compressibility compared to Fao30 and even more so than the original Fao formulation.

The most cohesive formulation among the control variations studied was the base formulation without saliva dilution and with lidocaine incorporation (FLao) ($p<0.05$).

Fao and FLao are the formulations with the lowest viscosity ($p>0.05$) among the ones studied, and thus they do not have significant adhesiveness values. However, these formulations diluted with 30% saliva began to present values for the mechanical parameters, which generally increased with the concentration of artificial saliva.

The addition of lidocaine decreased the mean values ($p>0.05$) of the mechanical properties compared to the drug-free formulation, making the formulation more cohesive.

The qualitative analysis of the rheograms (rheological continuous) illustrated in Figure 2 showed that the control formulation with and without lidocaine incorporation (Fao, FLao, and FLao100) presented as Newtonian fluids. However, the other formulations showed non-Newtonian fluid behavior.

Fao30, Fao100, and FLao30 demonstrated non-Newtonian pseudoplastic behavior. Furthermore, it was clearly demonstrated that the addition of saliva gradually reduced the flow behavior index values, increasing the pseudoplasticity of the formulations. On the other hand, the difference between the polymers did not interfere with this behavior.

Data showed that both the formulation and artificial saliva, as well as the interaction of the two variables, have a significant effect on all continuous rheological parameters ($p<0.0001$, two-factor ANOVA).

Overall, dilution with 30% saliva increased ($p<0.0001$) the consistency index, yield stress, and thixotropy of the formulations, followed by dilution with 100%. Thus, the continuous rheological analysis of the formulations showed that both the formulation and the saliva concentration have a significant impact on continuous rheological parameters ($p<0.0001$).

About oscillatory rheology, the control formulation of conventional lipid, the Fao and FLao formulations, demonstrated viscoelastic behavior and were classified as viscoelastic liquids with gel characteristics in their dilutions. This behavior is interesting for its application

in the oral cavity as it allows good adhesion to the mucosa and controlled drug release. The viscoelastic nature of the formulations is advantageous as it provides a suitable consistency to keep the formulation in the treatment area, while saliva dilutions provide a transition to the gel state, further improving adhesion and contact time with the mucosa.

Thus, the oscillatory rheological analysis of the formulations showed that both the formulation and the saliva concentration significantly affect the storage and loss moduli ($p<0.0001$).

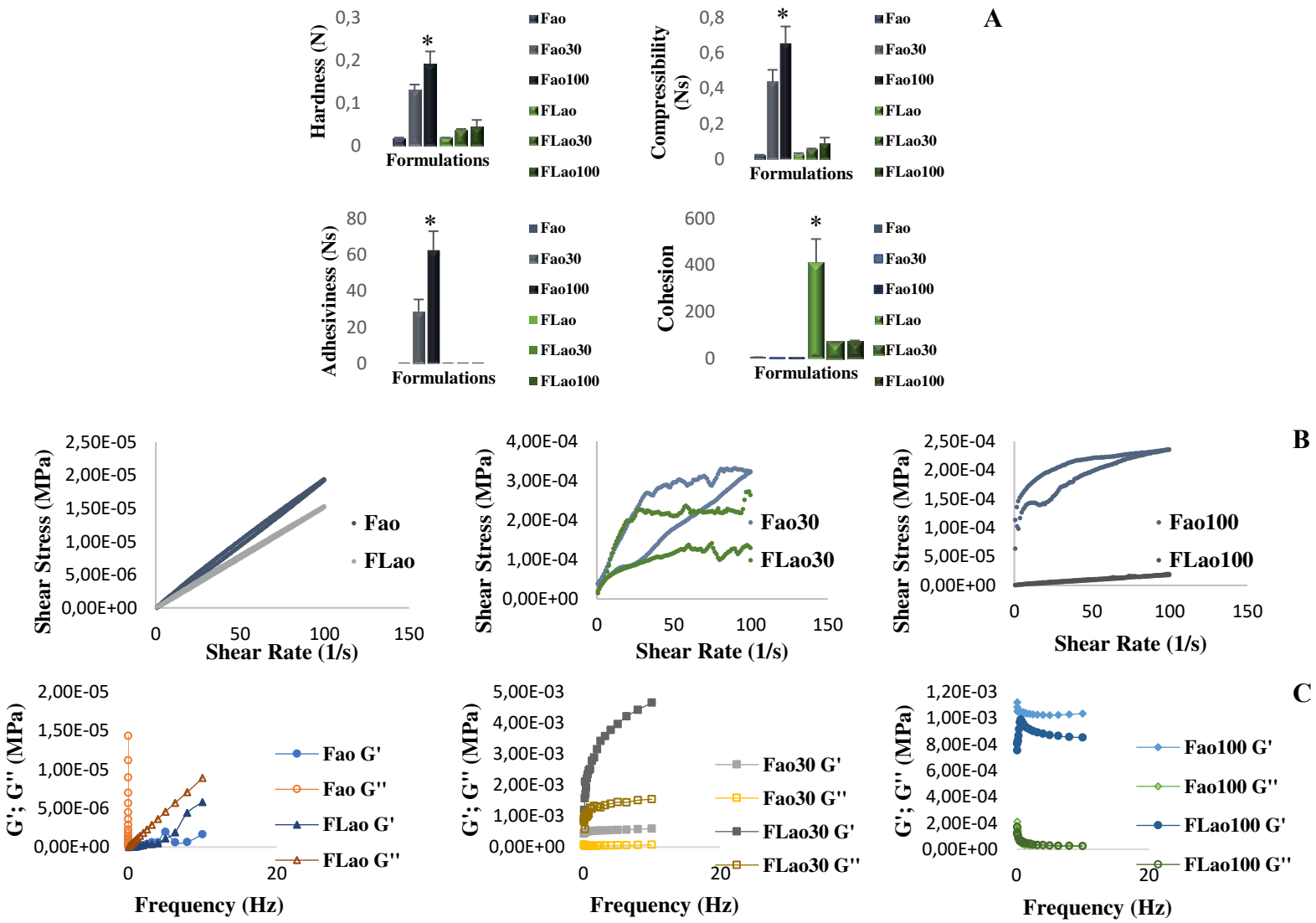


Figure 2. A) Texture Profile. Mechanical properties: Hardness; Compressibility; Adhesiveness; Cohesion. Control Formulation (Fao, Fao30, Fao100 - FLao, FLao30, FLao100). *($p<0.05$) (One-way ANOVA test with Tukey post-test at a significance level of 0.01%, n=10. B) Flow curves (shear stress versus shear rate) of the formulations. Standard deviations were omitted for clarity. Data were collected at 37 ± 0.5 ºC. The coefficient of variation of the triplicate analyses was below 10%. (ANOVA with Games Howell post-test. Significance level adopted was 0.05). C) Variation of storage modulus G' and loss modulus G'' as a function of frequency for all formulations. Standard deviations were omitted for clarity. Data were collected at 37 ± 0.5 ºC. The coefficient of

variation of the triplicate analyses was below 10%. (ANOVA with Games Howell post-test. Significance level adopted was 0.05). Fao is the control formulation of conventional lipid, a precursor system of liquid crystal without lidocaine incorporation. FLao is the control formulation of conventional lipid, a precursor system of liquid crystal with 5% lidocaine hydrochloride. Fao30 and FLao30 are Fao and FLao with 30% artificial saliva, and Fao and FLao100 are Fao and FLao with 100% saliva, respectively.

2.2.3. In Vitro release kinetics, permeation and mucoadhesion assays (LDC)

The release profiles illustrated in Figure 3. The FLao sample, the control formulation of conventional lipid, reaches an almost complete release, but the release profile is more gradual compared to S-LDC. Despite a slower initial release in the first few hours, it accelerates and reaches near 100% saturation within 24 hours. This kinetics offers a balance between onset of action and duration, which may be suitable for applications requiring moderately rapid release and prolonged coverage.

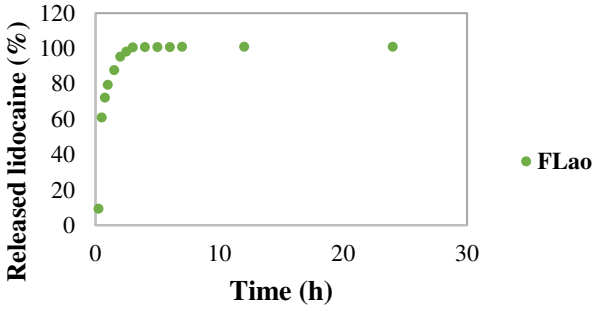
Mathematical modeling was employed to fit the kinetics of the formulations using different models (zero-order, first-order, Korsmeyer-Peppas, Higuchi, and Weibull) as described in figure 3. In this case, the Weibull model best fit with the highest R^2.

For the control formulation considering the higher determination coefficient value (R^2), FLao (R^2=0.9926) was best described by the Weibull model with the shape parameter (b) less than 1, indicating that FLao releases lidocaine through Fickian diffusion, which posits that the drug flux goes from regions of high concentration to regions of low concentration, with a magnitude proportional to the concentration gradient. In this context, the control formulation (FLao), with characteristics of a liquid crystal precursor but originally a microemulsion, showed a faster release of lidocaine, probably due to its water absorption capacity and its composition with oleic acid. The permeation profile of FLao through porcine buccal mucosa and as well as the permeation parameter value for lidocaine is shown in figure 3.

The control formulation FLao had an intermediate flux among those tested, with a longer latency time indicating slower and delayed permeation. Based on these data, it was found that the control formulation also presents a slow and sustained permeation, with a rapid yet not immediate onset, as it spontaneously does not form a liquid crystal but is characterized as a precursor system and contains oleic acid, which is a permeation enhancer.

The mucoadhesive properties results can be seen in Figure 3. The control formulation had a marginal impact on the properties when considering the original formulation with incorporated lidocaine for the formulation diluted in 30% saliva, i.e., it marginally decreased the average values of mucoadhesive peak force and work of all formulations ($p>0.05$). The formulation diluted in 100% saliva showed similar results, yet with lower average values compared to the test formulation.

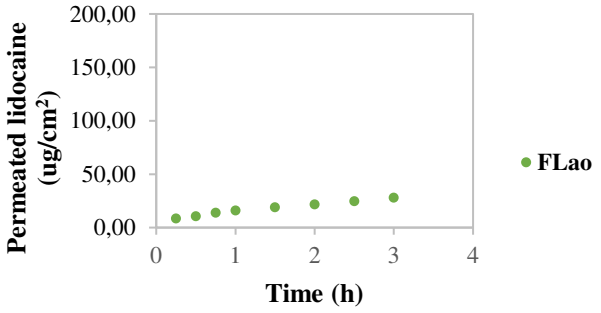
A



B

Mathematical Models	Formulation FLao
Korsmeyer-Peppas	
R^2	0,8145
N	0,2689
Higuchi	
R^2	0,8864
K	6,9574
1ª ordem	
R^2	0,9057
K	0,0322
Weibull	
R^2	**0,9926**
B	**0,6396**

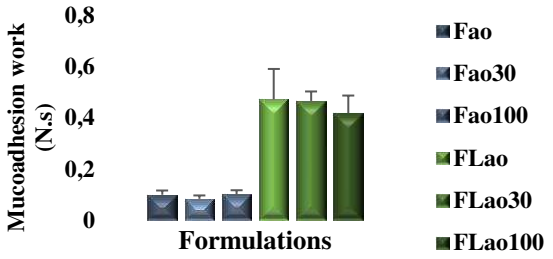
C



D

	Formulation FLao
Flux (µg.cm^{-2}.h^{-1})	56,53 ± 13,63
Lag time (h)	1,82 ± 1,06

E



F

Figure 3: A) Release profile of lidocaine incorporated in the control formulation of conventional lipid (FLao). B) Adjusted parameters of the kinetic models used in the lidocaine release. C) Permeation profile of lidocaine through porcine buccal mucosa epithelium incorporated in FLao. D) Kinetic parameters calculated from the lidocaine permeation profiles. E) Mucoadhesive Work. F) Mucoadhesive Peak Force (Fao, Fao30, Fao100, FLao, FLao30, and FLao100) (two-factor ANOVA). Fao is the control formulation of conventional lipid, a precursor system of liquid crystal without lidocaine incorporation. FLao is the control formulation of conventional lipid, a precursor system of liquid crystal with 5% lidocaine hydrochloride. Fao30 and FLao30 are Fao and FLao with 30% artificial saliva, and Fao100 and FLao100 are Fao and FLao with 100% saliva, respectively.

3. CONCLUSÃO

O presente estudo evidenciou a viabilidade da incorporação da lidocaína em um sistema líquido cristalino composto por óleo de semente de uva e ácido oleico como fase oleosa, ácido hialurônico e da lidocaína na fase aquosa, juntamente com PPG-5-CETETH-20 (Procetyl®) atuando como tensoativo. Esse sistema apresentou aumento na viscosidade e a formação de sistemas líquidos cristalinos cúbicos com a progressiva incorporação de água presente na saliva. Avaliações reológicas, mecânicas e mucoadesivas revelaram uma formulação estruturada e mucoadesiva quando a saliva e a lidocaína foram incorporadas. Em estudos *in vitro*, a formulação apresentou liberação e permeação através de mucosa bucal suína controlada e lenta do anestésico local. E finalmente, apresentou toxicidade semelhante à formulação comercial em modelo de membrana corioalantóica do embrião de galinha (CAM). Em conjunto, estes resultados sugerem que a formulação desenvolvida possui potencial para ser avaliado quanto à sua capacidade de proporcionar analgesia e cicatrização de lesões de mucosite oral em modelo *in vivo*. Tais achados são promissores, pois as características identificadas para a formulação desenvolvida são clinicamente desejáveis com potencial para promover eficácia clínica.

REFERÊNCIAS*

1. Apolinário AC, Pessoa A, Moraes AM. Abrindo a caixa de pandora dos nanomedicamentos: há realmente muito mais 'espaço lá embaixo'. *Quim Nova.* 2020;43(2):212-225.
2. Benergossi J, Silva FA, Baptista MS. Highlights in peptide nanoparticle carriers intended to oral diseases. *Curr Top Med Chem.* 2015;15(4):345-355.
3. Berto C, Hermes DM. Bases da resistência antifúngica: uma revisão. In: Oliveira TRS, Neto OB, editores. *Ciências biológicas e da saúde: integrando saberes em diferentes contextos.* 3ª ed. São Paulo: Científica digital; 2023. p. 52-72.
4. Brasil. Ministério da Saúde. Instituto Nacional de Câncer (INCA). Estimativa de Câncer no Brasil, 2023. Disponível em: https://www.inca.gov.br/estimativa
5. Bortolazzo FO. Efeito combinado da aplicação de células tronco e microcorrente na cicatrização do tendão calcanear de ratos após transecção parcial [tese de doutorado]. [s.l.]: [s.n.]; 2018.
6. Calixto GMF, Garcia CM, Cury BSF, Chiavacci LA, Chorilli M. Design and characterization of a novel p1025 peptide-loaded liquid crystalline system for the treatment of dental caries. *Molecules.* 2016;21(2):158.
7. Calixto GMF, Victorelli FD, Dovigo LN, et al. Polyethyleneimine and chitosan polymer-based mucoadhesive liquid crystalline systems intended for buccal drug delivery. *AAPS PharmSciTech.* 2018;19(2):820-836.
8. Calixto GMF, Victorelli FD, Franz-Montan M, Baltazar F, Chorilli M. Innovative mucoadhesive precursor of liquid crystalline system loading anti-gellatinolytic peptide for topical treatment of oral cancer. *J Biomed Nanotechnol.* 2021;17(2):253-262.
9. Chorilli M, Calixto GMF, Rigon RB, Scudeller MA, Leonardi GR. Rheological characterization and safety evaluation of non-ionic lamellar liquid crystalline systems containing retinyl palmitate. *J Biomed Nanotechnol.* 2016;12(2):394-403.
10. Cintra G, Ferreira DC, Fialho SL, Sato E, Rossi RC, Barros TC, et al. Bioadhesive surfactant systems for methotrexate skin delivery. *Molecules.* 2016;21(2):231.
11. Colella G, Boschetti CE, Vitagliano R, Colella C, Jiao L, King-Smith N, et al. Interventions for the prevention of oral mucositis in patients receiving cancer treatment: evidence from randomised controlled trials. *Curr Oncol.* 2023;30(1):967-980.

* De acordo com as normas da UNICAMP/FOP, baseadas na padronização do International Committee of Medical Journal Editors - Vancouver Group. Abreviatura dos periódicos em conformidade com o PubMed.

12. Costa FPD, Silva MR, Ramos VH, Cyrino JSAE. Enxaguantes bucais utilizados em pacientes com mucosite oral - revisão de literatura [dissertação de mestrado]. Belo Horizonte: Universidade Federal de Minas Gerais; 2023.
13. Costa HM, Ramos VD, Cyrino JSAE. Influência do óleo de semente de uva na degradação termo-oxidativa do polipropileno (PP) reciclado. *Matéria.* 2021;26.
14. Dos Santos Ramos M, Araujo LQ, Silva AM, Egito ES, Araújo I, Mansur CR, et al. *Syngonanthus nitens* Bong. (Rhul.)-loaded nanostructured system for vulvovaginal candidiasis treatment. *Int J Mol Sci.* 2016;17(8):1368.
15. Fernandes E, Fraga CPT. A importância do cirurgião-dentista nos efeitos adversos na cavidade bucal do tratamento oncológico de cabeça e pescoço. *Rev Científica UMC.* 2019;4(1).
16. Mirza MA, Mahmood S, Hilles AR, Ali A, Khan MZ, Zaidi SAA, et al. Quercetin as a therapeutic product: evaluation of its pharmacological action and clinical applications-a review. *Pharmaceuticals (Basel).* 2023 Nov 20;16(11):1631.
17. Fonseca-Santos B, Satake CY, Calixto GMF, Dos Santos AM, Chorilli M. Trans-resveratrol-loaded nonionic lamellar liquid-crystalline systems: structural, rheological, mechanical, textural, and bioadhesive characterization and evaluation of in vivo anti-inflammatory activity. *Int J Nanomedicine.* 2017 Sep 14;12:6883-6893.
18. Franco FN, Silva MC, Rezende KC, Lima GC. Envelhecimento: papel da via de sinalização NRF2 sobre os mecanismos antioxidantes e anti-inflamatórios mediados pelo resveratrol [tese de doutorado]. Belo Horizonte: Universidade Federal de Minas Gerais; 2023.
19. Franz-Montan M, Ribeiro L, Santana MH. Nanostructured systems for transbuccal drug delivery. In: Nanostructures for Oral Medicine. 1ª ed. Elsevier; 2017. p. 87-121.
20. Franz-Montan M, Ribeiro L, Santana MH. Recent advances and perspectives in topical oral anesthesia. *Expert Opin Drug Deliv.* 2017;14(5):673-684.
21. Garavaglia J, Markoski MM, Oliveira A, Marcadenti A, Tavares do Carmo M. Grape seed oil compounds: biological and chemical actions for health. *Nutr Metab Insights.* 2016;9.
22. Glenny AM, Gibson F, Auld E, Coulson S, Clarkson JE, Craig JV, et al. The development of evidence-based guidelines on mouth care for children, teenagers and young adults treated for cancer. *Eur J Cancer.* 2010;46(8):1399-1412.
23. Hearnden V, Ligon LC, Crean SJ, Kielbassa AM, Porter SR. New developments and opportunities in oral mucosal drug delivery for local and systemic disease. *Adv Drug Deliv Rev.* 2012;64(1):16-28.

24. Huang X, Gupta AK, Poços L, Cheng YH, Long J, Wan J. Effect of hyaluronic acid on the interaction between nanoparticles and mucus. *Pharm Res.* 2014;31(12):3390-3403.
25. Idris S, Syeda K, Lone AM, AlQahtani S, Ahmed A, Zareen A, et al. Propriedades antimicrobianas e anti-inflamatórias do extrato de semente de uva: potenciais aplicações em saúde bucal. *J Med Food.* 2022;25(7):730-741.
26. Jambi HAS, Almajwal AM, Alsalhi M, Alshamrani HM, Albalawi SM, Alfheeaid H, et al. Potencial efeito antioxidante, anti-inflamatório e gastroprotetor do extrato de semente de uva em úlcera gástrica induzida por indometacina em ratos. *Int J Pharmacol.* 2019;15(2):209-218. doi:10.3923/ijp.2019.209.218.
27. Jemal F, Torre LA, Bray F, Forman D. Incidência de câncer nos cinco continentes. v. XI. Lyon: Agência Internacional de Pesquisa em Câncer; 2018.
28. Lalla RV, Sonis ST, Peterson DE. Manejo da mucosite oral em pacientes com câncer. *Dent Clin North Am.* 2021;52(1):61-77.
29. Leal AM, Lopes MM, Cassemiro MA, Corrêa RL, Soares AB. *Rev Bras Plant Med.* 2024;24(1):123-130. doi:10.1590/s0102-695x20202401200130.
30. Liu S, Zhou X, Zhou Z, Li J, Xu Y. Status do tratamento e profilaxia para mucosite oral induzida por radiação em pacientes com câncer de cabeça e pescoço. *Front Oncol.* 2021;11:642575.
31. Logan RM, Stringer AM, Bowen JM, Gibson RJ, Sonis ST, Keefe DMK. Níveis séricos de NF-κB e citocinas pró-inflamatórias após administração de drogas mucotóxicas. *Cancer Biol Ther.* 2018;7(7):1139-1145.
32. Malamed SF. *Manual de Anestesia Local.* 7ª ed. Rio de Janeiro: Grupo GEN; 2021.
33. Miyashiro C, Taira MC, Penedo LA, Rossi AG, Yamashita F, Barros SB. Evaluation of grape seed oil in the development of lipid-based formulations for skin treatment. *Pharm Dev Technol.* 2020;25(1):25-35.
34. Nascimento MHM, Lombello CB. Hidrogéis a base de ácido hialurônico e quitosana para engenharia de tecido cartilaginoso. *Polímeros.* 2016;26(4):360-370.
35. Parise G, Ferranti K, Grando C. Sais anestésicos utilizados na odontologia: revisão de literatura. *J Oral Investig.* 2017;6(1):75-84.
36. Rahman M, Vargas M, Barros ME. Compostos de óleo de semente de uva: ações biológicas e químicas para a saúde. In: Alimentação Vegana: Tecnologias e Perspectivas de Futuro. São Paulo: Editora Vegana; 2019. p. 123-145.

37. Robijns J, Bulot N, Laubach HJ, Hamblin MR, Kharkwal GB, Gupta PK, et al. Terapia de fotobiomodulação no manejo de efeitos colaterais induzidos pela terapia do câncer: artigo de posição WALT 2022. *Front Oncol.* 2022;12:927685.

38. Rodero CF, Garcia CM, Fernandes D, Moreno MB, Almeida EA, Chorilli M. Sistemas cristalinos líquidos carregados de curcumina para liberação controlada de drogas e tratamento melhorado da candidíase vulvovaginal. *Mol Pharm.* 2018;15(10):4491-4504.

39. Rodriguez-Lopez JM, Sanches JE, Vilela FM, Oliveira JS, Barros TP. *J Appl Microbiol.* 2022.

40. Salamat-Miller N, Chittchang M, Johnston TP. O uso de polímeros mucoadesivos na liberação bucal de fármacos. *Adv Drug Deliv Rev.* 2005;57(11):1666-1691. doi:10.1016/j.addr.2005.07.006.

41. Salmazi R, Calixto GMF, Bernegossi J, Sanches JER, Ishida K, de Freitas LM, et al. Um sistema mucoadesivo precursor de cristal líquido carregado de curcumina para o tratamento da candidíase vaginal. *Int J Nanomedicine.* 2015;10:4815.

42. Silva IA, Monteiro DS, Oliveira MA, Batista JM, Oliveira AE, Lira VC. Impacto do tratamento antineoplásico na microbiota da cavidade oral e orofaríngea de pacientes acometidos pelo câncer de cabeça e pescoço: revisão sistemática. *Rev Bras Cancerol.* 2023;68(1).

43. Singh P, Bastos DS, Lombardi S, Franca FM, Reis JM. O resveratrol aumenta os efeitos anti-inflamatórios de anti-inflamatórios não esteroidais em modelos animais. *J Inflamm Res.* 2019;12:43.

44. Sonis ST, Peterson RL, Edwards LJ, Lucey CA, Wang L, Mason KA, et al. Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. *Oral Oncol.* 2000;36(4):373-81.

45. Sonis ST. The biologic role for nuclear factor-kappaB in disease and its potential involvement in mucosal injury associated with antineoplastic therapy. *Crit Rev Oral Biol Med.* 2012;13:380-9.

46. Victorelli FD, Lopes PS, Mercurio DG, Oliveira RF, Leonardi GR. Potential of grape seed oil in topical formulations for dermal delivery of therapeutic agents. *Int J Cosmet Sci.* 2020;42(5):510-20.

47. Victorelli FD, Rodero CF, Lutz-Bueno V, Chorilli M, Mezzenga R. As fibrilas amiloides aumentam a bioaderência tópica de formulações de medicamentos à base de mesofase cristalina líquida. *Adv Healthc Mater.* 2023;12(12).

48. Walsh LJ. Clinical assessment and management of the oral environment in the oncology patient. *Aust Dent J.* 2010;55:66-77.

49. Wang CY, Lee I, Chen M, et al. Avanços recentes na descoberta e biossíntese de produtos naturais a partir de mixobactérias: uma visão geral de 2017 a 2023. *Nat Prod Rep.* 2024.

50. Yagiela JA, Dowd FJ, Neidle EA. *Pharmacology and Therapeutics for Dentistry.* 6ª ed. Rio de Janeiro: Elsevier; 2011.

51. Zhou DD, Li J, Xiong RG, Saimaiti A, Huang SY, Wu SX, et al. Bioactive compounds, health benefits and food applications of grape. *Foods.* 2022;11(18):2755

ANEXOS

Anexo I. Comprovante de Submissão do Artigo

Gmail — Mônica Portela <monicabpferreira@gmail.com>

Submission to Journal of Pharmaceutical Sciences - manuscript number
1 mensagem

Journal of Pharmaceutical Sciences <em@editorialmanager.com> 20 de agosto de 2024 às 19:34
Responder a: Journal of Pharmaceutical Sciences <jpharmsci@aphanet.org>
Para: Mônica Beatriz Portela Ferreira <monicabpferreira@gmail.com>

You are being carbon copied ("cc:'d") on an e-mail "To" "Michelle Franz-Montan" mfranz@unicamp.br
CC: "Mônica Beatriz Portela Ferreira" monicabpferreira@gmail.com, "Giovana Maria Fioramonti Calixto" giovana.calixto@gmail.com, "Aylla Mesquita Pestana" pestanaaylla@gmail.com, "Arthur Antunes Costa Bezerra" a220849@dac.unicamp.br

This is an automated message.

Manuscript Number: XPHS-D-24-01156

Mucoadhesive Liquid Crystalline System Based on Grape Seed Oil, Hyaluronic Acid, and Lidocaine for Topical Treatment of Oral Mucositis

Dear Prof Franz-Montan,

Your above referenced submission has been assigned a manuscript number: XPHS-D-24-01156.

To track the status of your manuscript, please log in as an author at https://www.editorialmanager.com/xphs/, and navigate to the "Submissions Being Processed" folder.

Thank you for submitting your work to this journal.

Kind regards,
Journal of Pharmaceutical Sciences

More information and support

You will find information relevant for you as an author on Elsevier's Author Hub: https://www.elsevier.com/authors

FAQ: How can I reset a forgotten password?
https://service.elsevier.com/app/answers/detail/a_id/28452/supporthub/publishing/
For further assistance, please visit our customer service site: https://service.elsevier.com/app/home/supporthub/publishing/
Here you can search for solutions on a range of topics, find answers to frequently asked questions, and learn more about Editorial Manager via interactive tutorials. You can also talk 24/7 to our customer support team by phone and 24/7 by live chat and email

---

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/xphs/login.asp?a=r). Please contact the publication office if you have any questions.

Anexo II. Relatório de verificação de originalidade e prevenção de plágio

FORMULAÇÃO DE LIDOCAÍNA INCORPORADA EM SISTEMA LÍQUIDO CRISTALINO À BASE DE ÓLEO DE SEMENTE DE UVA E ÁCIDO HIALURÔNICO PARA APLICAÇÃO TÓPICA EM LESÕES DE MUCOSITE ORAL

ORIGINALITY REPORT

16%	12%	12%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	link.springer.com Internet Source	1%
2	www.mdpi.com Internet Source	1%
3	Submitted to Universidad Estadual Paulista Student Paper	1%
4	www2.mdpi.com Internet Source	1%
5	repositorio.unicamp.br Internet Source	1%
6	Aylla Mesquita Pestana, Giovana Maria Fioramonti Calixto, Arthur Antunes Costa Bezerra, Lígia Nunes de Morais Ribeiro et al. "Analysis of key factors for evaluating mucosal adhesion using swine buccal tissue", Journal of Pharmaceutical Sciences, 2024 Publication	<1%