



DANIELE CARVALHO ABRANTES

**REPELENTES CONTENDO MISTURAS DE ATIVOS EM ASSOCIAÇÃO COM
CARREADORES NANOESTRUTURADOS PARA COMBATE À PROPAGAÇÃO
DE ARBOVIROSES**

Sorocaba

2022

PROGRAMA DE PÓS-GRADUAÇÃO em

*ciências
ambientais*



unesp
Sorocaba

DANIELE CARVALHO ABRANTES

**REPELENTES CONTENDO MISTURAS DE ATIVOS EM ASSOCIAÇÃO COM
CARREADORES NANOESTRUTURADOS PARA COMBATE À PROPAGAÇÃO
DE ARBOVIROSES**

Tese apresentada como requisito para a obtenção do
título de Doutor em Ciências Ambientais da
Universidade Estadual Paulista "Júlio de Mesquita
Filho" na Área de Concentração Diagnóstico,
Tratamento e Recuperação Ambiental

Orientador: Prof. Dr. Leonardo Fernandes Fraceto

Co-orientadora: Prof^a. Dr^a. Daniele Ribeiro de Araújo

Sorocaba

2022

PROGRAMA DE PÓS-GRADUAÇÃO em

*ciências
ambientais*



unesp
Sorocaba

A161r	Abrantes, Daniele Carvalho Repelentes contendo misturas de ativos em associação com carreadores nanoestruturados para combate à propagação de arboviroses / Daniele Carvalho Abrantes. -- Sorocaba, 2022 122 p. : il., tabs. Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia, Sorocaba Orientador: Leonardo Fernandes Fraceto Coorientadora: Daniele Ribeiro de Araújo 1. Nanopartículas. 2. Repelentes. 3. Arboviroses. 4. Nanogéis. 5. Reologia. I. Título.
-------	---

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Ciência e Tecnologia, Sorocaba. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: REPELENTES DE LONGA DURAÇÃO CONTENDO MISTURAS DE ATIVOS EM ASSOCIAÇÃO COM CARREADORES LIPÍDICOS NANOESTRUTURADOS PARA COMBATE À PROPAGAÇÃO DE ARBOVIROSES

AUTORA: DANIELE CARVALHO ABRANTES

ORIENTADOR: LEONARDO FERNANDES FRACETO

COORIENTADORA: DANIELE RIBEIRO DE ARAUJO

Aprovada como parte das exigências para obtenção do Título de Doutora em CIÊNCIAS AMBIENTAIS, área: Diagnóstico, Tratamento e Recuperação Ambiental pela Comissão Examinadora:

Prof. Dr. LEONARDO FERNANDES FRACETO (Participação Virtual)
Engenharia Ambiental / Unesp ICT Sorocaba

Profª. Drª. PATRICIA SEVERINO (Participação Virtual)
Universidade Tiradentes

Profª. Drª. LIGIA NUNES DE MORAIS RIBEIRO (Participação Virtual)
Universidade Federal de Uberlândia

Prof. Dr. MARLUS CHORILLI (Participação Virtual)
Departamento de Fármacos e Medicamentos / Faculdade de Ciências Farmacêuticas - UNESP - Araraquara

Profª. Dra. MARCIA REGINA DE MOURA AOUADA (Participação Virtual)
Departamento de Física e Química / Faculdade de Engenharia de Ilha Solteira - Unesp

Sorocaba, 12 de agosto de 2022

Dedico este trabalho especialmente
à minha filha Helena, uma benção em
minha vida, e ao meu marido Vinícius por
todo companheirismo e incentivo ao
longo destes anos.

E por último, e não menos
importante, à mais um coração que bate
aqui em mim, que ainda não o tenho por
inteiro, e nem te conheço direito, mas já
posso te ouvir. Te amo Fernando

AGRADECIMENTO

Agradeço a Deus, pois nele encontro a força, a motivação, e o sentido em tudo que faço.

Agradeço a FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo – 2018/14734-0) pelo apoio financeiro ao longo do projeto.

Ao Prof. Dr. Leonardo Fernandes Fraceto, pela orientação impecável e por todas as oportunidades de crescimento profissional que me proporcionou ao longo destes anos.

À Prof^a. Dr^a. Daniele Ribeiro de Araújo, pela coorientação e por toda a disponibilidade nos testes e ensaios realizados no laboratório da Universidade Federal do ABC (UFABC), e por todo carinho e generosidade dedicado aos seus alunos.

À Prof^a. Dr^a. Renata de Lima pelo apoio e parceria nos ensaios de citotoxicidade realizados no Laboratório de avaliação de Biotividade e Toxicologia de Nanomateriais (LaBiTON) da UNISO.

À empresa Ipanema Indústria de Produtos Veterinário, pela parceria e apoio na avaliação das formulações repelentes. Em especial ao César Pampana, Marcelo Duarte e Geórgio Valadarez que estiveram ligados diretamente ao projeto.

A todos os colegas e amigos do Laboratório de Nanotecnologia Ambiental, onde eu pude compartilhar momentos de aprendizagem, troca de experiências e descontração. Em especial ao Jhones, Estefânia e Anderson que repassaram muitos conhecimentos de nanotecnologia, técnicas, uso de softwares e afins.

À Carol, que foi minha parceira de vários projetos ao longo da pandemia, e que me ajudou ao longo da licença maternidade. Sou eternamente grata a você!

Ao Programa de Pós-Graduação em Ciências Ambientais e a ao ICTS-UNESP (Instituto de Ciência e Tecnologia de Sorocaba) pelo apoio institucional e oportunidade de evolução profissional.

Agradeço a minha família, em especial a minha filha Helena e meu marido Vinícius que estiveram comigo, praticamente 24 horas por dia durante a pandemia, e que de alguma forma participaram e foram pacientes ao longo de todo o processo.

Agradeço também aos meus pais, Jarbas e Heloísa, que mesmo de longe sempre foram meu porto seguro e sempre me apoiam em tudo.

Ao meu irmão Rafael, que tem um coração doce e gentil, e sempre esteve ao meu lado e admirou minha dedicação aos estudos.

A todos os meus familiares que sempre se orgulharam e apoiaram a minha profissão.

Às minhas amigas de colégio, Bia, Rê, Fer, Lari, Loni e Clau pelos momentos de alegria, descontração e desabafos através de videoconferências ao longo da pandemia. Vocês são mulheres que eu tenho orgulho, e juntas nos fortalecemos.

Enfim, agradeço de coração a todos que fizeram parte deste trabalho diretamente ou indiretamente. Viva a Ciência!

RESUMO

As arboviroses são doenças causadas por vírus transmitidos através de vetores, como o *Aedes aegypti*. Nos últimos anos, o surgimento de novos arbovírus como o Zika (ZIKV) e o Chikungunya (CHIKV) serviram como alerta de possíveis epidemias devido a expansão destas arboviroses concomitantemente com os arbovírus preexistentes da dengue (DENV) e febre amarela. As pesquisas estimam que até 2050 mais da metade da população mundial esteja sob o risco de contrair uma arbovirose. Portanto, o uso de repelente é uma estratégia de proteção individual importante que auxilia no combate à transmissão dos arbovírus. Porém, algumas classes de indivíduos são negligenciadas, como as gestantes e crianças que ficam restritas ao uso dos produtos comerciais. À vista disso, o presente estudo investigou o desenvolvimento de carreadores lipídicos nanoestruturados (NLCs) carregados com a mistura dos compostos repelentes botânico e sintético (icaridina e geraniol) incorporados em hidrogéis de celulose. Também foi desenvolvido uma nanopartícula polimérica de poli-(épsilon-caprolactona) (PCL) com a mistura de repelentes IR3535 e geraniol. As formulações possuem dosagens reduzidas de ativos repelentes, tornando-se uma alternativa promissora, pois prolongam o tempo de liberação dos ativos e, conseqüentemente, reduzem a toxicidade. Os NLCs apresentaram um tamanho médio de 252 ± 5 nm e eficiência de encapsulação de 99% de ambos os ativos, enquanto o tamanho médio dos PCLs foi de 372 ± 2 nm e uma média de 60% de IR3535 encapsulado e 99% do geraniol. O perfil de estabilidade não apresentou diferença significativa ($p > 0,05$) na média do tamanho de ambos, indicando estabilidade considerável dos sistemas. Através da análise de morfologia por microscopia de força atômica (AFM) foi possível visualizar a presença de nanopartículas esféricas em ambos os sistemas. Além disso, os estudos de permeação dos NLCs e NP_PCL mostraram baixos fluxo de ativo e coeficiente de permeabilidade através da membrana Strat-M®, indicando menor permeação através da barreira epidérmica. A incorporação das nanopartículas em hidrogéis diminuiu significativamente o perfil de liberação de IR3535 das NP_PCL e de icaridina dos NLC. A NP_PCL apresentaram maior toxicidade compara aos NLC, no entanto a concentrações tóxicas dos ativos são maiores do que as permeadas em 24h. Sendo assim, os resultados demonstram que os sistemas têm potencial promissor, abrindo perspectivas para a sua utilização em gestantes e crianças.

Palavras-chave: Nanopartícula; Arboviroses; Repelente; *Aedes aegypti*; Carreadores lipídicos nanoestruturados; poli-(épsilon) caprolactona

ABSTRACT

Aedes aegypti is the main vector of diseases caused by Arbovirus. In recent years, Zika (ZIKV) and Chikungunya (CHIKV) have appeared such new arboviruses globally. It's warning of possible epidemics due to expansion concurrently with the pre-existing arboviruses of dengue (DENV) and yellow fever. Research estimates that by 2050 more than half of the world's population will be at risk of contracting an arbovirus disease. Therefore, repellent helps to act against transmission of arboviruses. However, some individuals are neglected, such as pregnant women and children, who have restricted the use of commercial products. Therefore, we investigated the development of nanostructured lipid carriers (NLCs) loaded with the mixture of botanical and synthetic repellent compounds (icaridin and geraniol) incorporated into cellulose hydrogels. Also, we developed the polymeric poly-(epsilon-caprolactone) (PCL) nanoparticle with the mixture of IR3535 and geraniol repellents. This a promising alternative because this technology extends the release time of active ingredients allowing reduced dosages and toxicity. The NLCs showed a mean diameter of 252 ± 5 nm and 99% encapsulation efficiency of both activities. Also, PCLs showed a mean diameter of 372 ± 2 nm and an average of 60% of encapsulated IR3535 and 99% of geraniol. The stability profile showed no significant difference ($p > 0.05$) in the average size of both, indicating considerable stability of the systems. Atomic force microscopy (AFM) morphology assessed the presence of spherical nanoparticles in both systems. Also, the permeation studies of NLCs showed low active flux and permeation coefficient through the Strat-M® membrane, indicating lower permeation through the epidermal barrier. The NP-gel hybrid system significantly decreased the release profile of IR3535 from NP_PCL and icaridin from NLC. NP_PCL showed greater toxicity compared to NLC, however the toxic concentrations of the actives are higher than those permeated in 24h. The results contribute to understand opening perspectives for their use in pregnant women and children.

Keywords: Nanoparticle; Arboviruses; Repellent; *Aedes aegypti*; Nanostructure lipid carrier; poly(epsilon) caprolactone.

SUMÁRIO

INTRODUÇÃO	10
OBJETIVOS	13
REVISÃO BIBLIOGRÁFICA	14
CHAPTER I	41
RECENT DEVELOPMENTS IN NANOTECHNOLOGY FOR DETECTION AND CONTROL OF AEDES AEGYPTI-BORNE DISEASES	42
CHAPTER II	59
DEVELOPMENT OF A MOSQUITO REPELLENT FORMULATION BASED ON NANOSTRUCTURED LIPID CARRIERS	60
CHAPTER III	73
REPELLENT ACTIVE INGREDIENTS ENCAPSULATED IN POLYMERIC NANOPARTICLES: POTENTIAL ALTERNATIVE FORMULATIONS TO CONTROL ARBOVIRUSES	74
CONCLUSÕES GERAIS E PERSPECTIVAS	107
REFERENCIAS	109

INTRODUÇÃO

No último século, o *Aedes aegypti* se proliferou de maneira desenfreada em áreas urbanas, principalmente em países tropicais e subtropicais (LIU et al., 2020; NARARAK et al., 2019). Ele é o principal vetor de transmissão de arboviroses como a Dengue, a febre amarela, e mais recentemente da Zika e Chikungunya (GOULD et al., 2017; MARKLEWITZ; JUNGLEN, 2019). Os estudos estimam que, num futuro próximo, mais da metade da população mundial estará sob o risco de contrair uma arbovirose. As manifestações clínicas de infecções por arbovírus pode variar de quadros febris leves a síndromes febris hemorrágicas e neurológicas.

No mundo, a dengue infecta 390 milhões de indivíduos a cada ano, sendo que 25% do total apresentam episódios sintomático da infecção (MESSINA et al., 2019; STANAWAY et al., 2016). Em 2019, o número de casos de dengue no Brasil foi cinco vezes maior quando comparado a 2018. Da mesma forma, os casos de Zika e Chikungunya também tiveram acréscimo em 53% e 30%, respectivamente (MINISTÉRIO DA SAÚDE, 2020)

O impacto das arboviroses no sistema de saúde é considerável, além disso o fator econômico é preocupante. No Brasil, um estudo analisou os gastos públicos obtidos em todo o país no período de 2016. Mais da metade dos custos foram gastos no combate ao vetor, e a outra parcela em tratamento médico ambulatorial e em custos indiretos relacionados a licenças médicas (TEICH; ARINELLI; FAHHAM, 2017).

Diante dos desafios no controle do vetor e um quadro preocupante em relação à expansão das arboviroses no mundo inteiro, torna-se imprescindível a adoção de estratégias específicas. Dentre elas, a nebulização aeroespacial, os dispositivos de liberação lenta e contínua, as telas e roupas impregnadas com inseticidas são estratégias de controle químico (ZARA et al., 2016). Além disso, os órgãos de saúde recomendam a proteção individual através do uso de repelentes em spray, loção, creme ou gel, principalmente quando o mosquito tem padrões de hábito diurno que coincidem com as atividades humanas. Nestes casos, os repelentes tópicos são essenciais, pois diminuem a chance de contrair as doenças transmitida pelo vetor (OHRT; NGO; NGUYEN, 2018; TAVARES et al., 2018a).

Os estudos demonstram que a eficácia dos repelentes é diretamente proporcional ao aumento da concentração do ativo, o que os torna menos seguros (KURI-MORALES et al., 2017a; LUPI; HATZ; SCHLAGENHAUF, 2013). Portanto, existe uma preocupação quanto a segurança destes produtos aos seres humanos e ao meio ambiente, o que motiva a busca por formulações biodegradáveis, menos tóxicas, mais seguras e um bom custo-benefício. Outra questão refere-se a busca por formulações que diminua as chances de resistência do

insetos, buscando diversificar os componentes repelentes da formulação (NORRIS; COATS, 2017; SENTHIL-NATHAN, 2020).

Ao longo das últimas décadas, muitas plantas foram selecionadas como fontes potenciais de repelentes. Os óleos essenciais extraídos dessas plantas são misturas voláteis de hidrocarbonetos com uma diversidade de grupos funcionais, e sua atividade repelente tem sido associada à presença de monoterpenos e sesquiterpenos (NERIO; OLIVERO-VERBEL; STASHENKO, 2010; SHARMA et al., 2013). Inclusive, há evidências que os compostos botânicos e sintéticos quando misturados apresentam um efeito sinérgico potencializando a eficácia repelente (LUPI; HATZ; SCHLAGENHAUF, 2013; RAGHAVENDRA; PRATHIBHA; VIJAYAN, 2013a; SHAALAN et al., 2005a). Embora os repelentes possuam o modo de ação semelhante, cada composto promove efeito repelente se ligando e interagindo com receptores específicos. (DICKENS; BOHBOT, 2013; KWON et al., 2010; PORTILLA-PULIDO et al., 2020).

Neste cenário, a utilização de sistemas nanoparticulados é visto como uma ferramenta tecnológica promissora, uma vez que aumenta a estabilidade e biodisponibilidade dos ativos, bem como reduz a dosagem e a necessidade de reaplicação, proporcionando uma menor toxicidade e maior sustentabilidade (LANG et al., 2021; LOMBARDO; KISELEV; CACCAMO, 2019). Atualmente, muitos estudos visam desenvolver sistemas nanocarreadores para aplicações farmacêuticas focando nas principais propriedades e aplicabilidade de carreadores orgânicos (lipídicos, proteicos ou poliméricos) e inorgânicos (nanotubos de carbono, nanopartículas de ouro ou dots) (LANE, 2011; LOMBARDO; KISELEV; CACCAMO, 2019; SOUTO et al., 2020). Os sistemas lipídicos ganharam atenção nas últimas décadas devido a sua biocompatibilidade, facilidade de preparação, custo-efetividade e a viabilidade da produção em larga escala (FANG et al., 2008; KHOSA; REDDI; SAHA, 2018b; SOUTO et al., 2020). Os carreadores poliméricos também são sistemas adequados para uso tópico. Similarmente aos NLC são considerados ideais para ativos que tenham como alvo a epiderme e folículos capilares.

Portanto, o objetivo deste projeto consistiu em desenvolver, sintetizar e caracterizar formulações nanoencapsuladas com a mistura de compostos repelentes botânicos e sintéticos, visando a otimização da eficácia repelente contra o *Aedes aegypti* com menores doses, menor toxicidade e mais segurança. No **primeiro capítulo**, é apresentado uma revisão que discute o uso da nanotecnologia na detecção das arbovirose e no controle do principal vetor, o *Aedes aegypti*. Assim, a pesquisa e desenvolvimento busca por novas tecnologias na produção de repelentes, larvicidas e inseticidas menos tóxicos com base em nanotecnologia. Por outro lado, a mesma tecnologia apresenta um papel importante no desenvolvimento de

sensores para o diagnóstico rápido das arboviroses. No **segundo capítulo**, é apresentado o desenvolvimento de carreadores lipídicos nanoestruturados (NLC) carregados com a mistura de icaridina e geraniol. O artigo descreve a síntese dos NLCs em suspensão e gel, bem como a estabilidade, o perfil de liberação e permeação *in vitro* e características reológicas do sistema. As formulações apresentaram-se estáveis ao longo do tempo e a taxa de permeação *in vitro* foi baixa, indicando menor penetração na pele. Além disso, a formulação em gel apresentou características reológicas que garantem melhor espalhabilidade e estabilidade físico-química. No **terceiro capítulo**, é apresentado o desenvolvimento e caracterização da formulação repelente baseada em nanopartículas poliméricas, de poli (épsilon) policaprolactona (NP_PCL) carregados com a mistura de IR3535 e geraniol. O artigo também apresenta análise reológica das formulações líquidas e em gel, a liberação e permeação dos ativos em membranas de celulose e Strat-M. Avalia também a citotoxicidade das NP_PCL através da atividade mitocondrial em células HaCaT e NIH-3T3. Além disso, faz uma comparação das formulações desenvolvidas e uma análise crítica de qual apresenta um potencial mais promissor para o mercado dos repelentes.

OBJETIVOS

1. Gerais

O presente projeto visou o preparo de carreadores nanoestruturados contendo geraniol e icaridina encapsulados em sistemas a base de lipídeos (CLN), e também geraniol e IR3535 encapsulados em sistemas a base de polímeros (PCL), e a incorporação destes sistemas em matriz de hidrogéis com a finalidade de liberação prolongada dos ativos visando o controle na transmissão de arboviroses pelos mosquitos, bem como, avaliou os possíveis efeitos tóxicos destes sistemas através de estudos de citotoxicidade e permeação visando a diminuição de efeitos tóxicos ao Homem e ao meio ambiente.

2. Específicos

- Caracterização das formulações nanoestruturadas a partir da medida de tamanho e potencial zeta, interação entre composto bioativo/nanopartícula por calorimetria, tamanho e morfologia das partículas por microscopia de força atômica, eficiência de encapsulação e estabilidade físico-química das formulações;
- Preparo do dispositivo de hidrogel, formulado a partir de hidroxipropilmetilcelulose e poloxamer e incorporação dos sistemas no hidrogel;
- Avaliar a permeabilidade *in vitro* a partir do ensaio de cinética de liberação e permeação em um sistema de difusão de células vertical.

REVISÃO BIBLIOGRÁFICA

1. Evidências epidemiológicas das arboviroses

Arboviroses ou *Arthropod-borne viruses* são um conjunto de 9 famílias de vírus diferentes, transmitidos por artrópodes vetores e espalhados/amplificados por animais hospedeiros. Essas famílias são de vírus de RNA, sendo elas: alphaviruses (*Togaviridae*); flaviviruses (*Flaviviridae*); bunyaviruses, nairoviruses e phleboviruses (*Bunyaviridae*); orbiviruses (*Reoviridae*); vesiculoviruses (*Rhabdoviridae*) e thogotoviruses (*Orthomyxoviridae*). Apenas um grupo é de vírus de DNA, o *African swine fever virus* (*Asfarviridae*). Além disso, existem mais de 500 tipos de arboviroses capazes de trazer doenças aos seres humanos e outros animais domésticos (HUBÁLEK; RUDOLF; NOWOTNY, 2014; LIANG et al., 2017; WEAVER; REISEN, 2010).

Esses vírus já foram responsáveis pela morte de um número considerável de pessoas e animais mundialmente ao longo dos anos. E, até hoje a população mundial e economia são prejudicadas, devido às doenças como, dengue, Zika, Chikungunya, encefalite japonesa e microcefalia. Nos últimos 50 anos essas doenças têm recebido mais atenção devido aos prejuízos que têm causado na economia e saúde pública. A dengue, por exemplo, atinge anualmente cerca de 390 milhões de pessoas, enquanto houve registros de mais de um milhão de casos por vírus da Zika em 2015-2018 nas Américas. O vírus Chikungunya causou diversos malefícios em 2013/2014 e o vírus do Nilo Ocidental atacou os EUA em 1999 e se espalhou para América do Norte e Sul. Além disso, esses vírus estão distribuídos mundialmente, têm alto potencial de se espalharem e não podem ser totalmente eliminadas do ambiente (LIANG et al., 2017; WILDER-SMITH et al., 2017; WILKE et al., 2019).

Essa rápida transmissão ocorre quando há cruzamento constante de população, vetores e hospedeiros em um ambiente facilitador. Em relação ao vetor deve-se analisar sua reprodução e multiplicação, mobilidade, preferência de espécies de alimentação (antropofílicos e zoofílicos) e longevidade (dependência de temperatura e umidade) (WEAVER; REISEN, 2010; WILKE et al., 2019; YOUNG et al., 2014).

Em relação a população deve-se considerar sua exposição aos vetores, anticorpos, uso de proteções contra o vetor. Além de se analisar a relação entre população e hospedeiro. Além disso, outros fatores importantes são mudanças no clima, mutações genéticas, ação antropogênica, viagens, mudança na temperatura terrestre e urbanização (concentração da população), como facilitadores da difusão das doenças. Algumas regiões são mais propícias para o desenvolvimento de mosquitos vetores e transmissão, que são de clima tropical, com

intensa urbanização e grande fluxo populacional (SHOPE; MEEGAN, 1997; WEAVER; REISEN, 2010; WILKE et al., 2019).

A temperatura, umidade e chuva têm forte influência na transmissão dessas doenças, pois muitas arboviroses são sazonais, já as larvas de mosquitos são aquáticas e demandam água para sua reprodução. As chuvas podem manter corpos d'água ou criar novos, o que também facilita a reprodução de mosquitos, além da umidade que possibilita sua sobrevivência. Por fim, altas temperaturas podem tornar o mosquito infeccioso mais rapidamente (YOUNG et al., 2014).

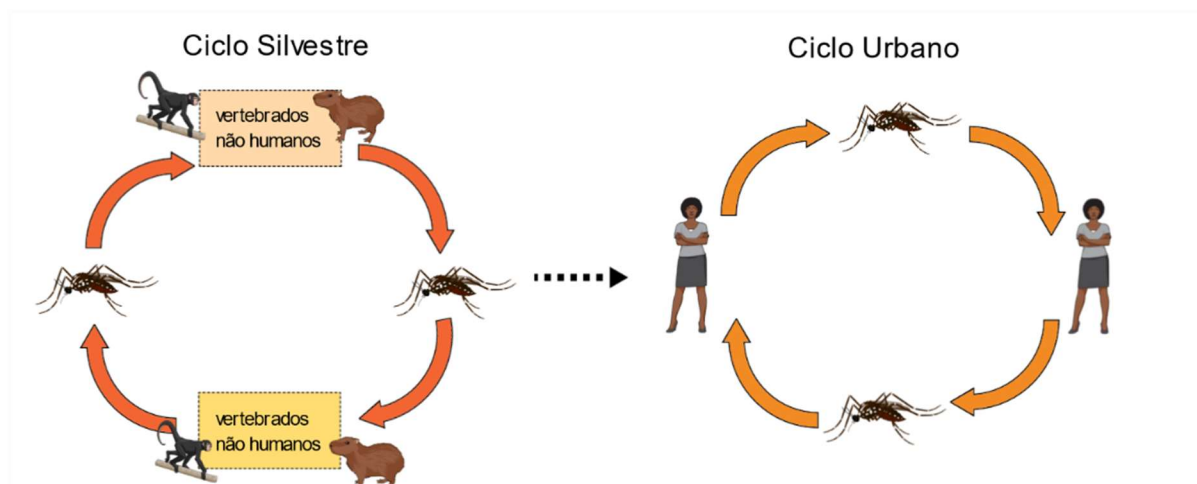
O ser humano tem forte influência em facilitar o processo de transmissão, como a construção de barragens, agricultura, como plantação de arroz, corte de vegetação antiga, criação de gado e entrada da população em regiões florestais, que facilitam o aumento do número de mosquitos, e, portanto, a propagação de doenças (YOUNG et al., 2014).

1.1. Ciclo de transmissão de arboviroses

Para que ocorra a transmissão necessariamente precisa-se de três fatores: o vetor mosquito, o hospedeiro vertebrado e condições ambientais. O ciclo de transmissão (Figura 1) ocorre em dois ciclos evolutivos: o silvestre e o urbano, em que o primeiro ocorre entre os vetores e hospedeiros não humanos (vertebrados artrópodes hematófagos e animais selvagens hospedeiros) e o segundo ocorre entre os vetores e o ser humano ou animais domésticos (MAYER; TESH; VASILAKIS, 2017; YOUNG et al., 2014).

Assim, para que a doença continue sendo transmitida, necessariamente, precisa-se de 3 fatores: um hospedeiro vertebrado suscetível a contaminação, que deve ser ave, réptil ou anfíbio, por exemplo; a fase virêmica e a fase de replicação do vírus, que ocorrem nos artrópodes “competentes”. Essa competência está associada a captura do vírus no processo de alimentação, via ingestão de sangue e fluidos corporais, entrada do vírus no tecido intestinal dos artrópodes, seguida de sua replicação no tecido intestinal e liberação nas glândulas salivares, podendo, assim, contaminar futuros hospedeiros (ARTSOB; LINDSAY, 2008; RÜCKERT; EBEL, 2018; YOUNG et al., 2014).

Figura 1 – Ciclo das arbovirose. No ciclo silvestre vê-se o mosquito vetor e os possíveis artrópodes hospedeiros, e a seta preta indica a interligação entre os dois ciclos, onde no ciclo urbano o ser humano e em laranja/amarelo uma representação dos possíveis artrópodes hospedeiros, divididos em ciclo silvestre e o urbano.



Fonte: Autoria própria.

2. Incidências arbovirose no Brasil e no Mundo

Existem mais 500 tipos de arbovirose espalhados mundialmente, porém, alguns destes podem destacar-se considerando sua importância veterinária e de saúde pública, que serão comentados neste tópico (Tabela 1) (WEAVER; REISEN, 2010).

As doenças que mais se destacam devido ao ressurgimento são transmitidas pelo mosquito *Aedes* spp, sendo elas: Zika, dengue, febre amarela (*Flavivirus*) e Chikungunya (*alfavírus*). O ciclo de transmissão envolve primatas (não humanos), mosquitos arbóreos e humanos (transmissão urbana/periurbana), ocorrendo principalmente em regiões tropicais e subtropicais. Essas doenças já atingem todos os continentes e mais de 3 bilhões de pessoas vivem em regiões com risco devido à presença de mosquitos *Aedes*. Esses vetores alimentam-se preferencialmente de seres humanos, são ativos no período diurno e próspero em regiões peridomésticas. Além disso, a urbanização desenfreada, superpopulação e a globalização são fortes aliados da maior incidência da doença (RÜCKERT; EBEL, 2018; WILDER-SMITH et al., 2017).

No Brasil, segundo boletim epidemiológico de maio de 2022, ocorreram mais de 757 mil casos de dengue até o momento, um aumento de mais de 150% dos casos quando comparado com o mesmo período do ano anterior. Os casos de chikungunya aumentaram

75% em relação ao ano anterior, contabilizando mais de 70 mil casos até abril de 2022 (MINISTÉRIO DA SAÚDE, 2022).

No mundo, a febre amarela em 2015-2016 na Angola também se espalhou para Quênia, República Democrática do Congo e China, e, em 2016 foi considerada uma ameaça mundial pela OMS. Outro exemplo disso foi o surto de Chikungunya na Itália em 2007, influenciada por viagens internacionais. Essa doença originou-se na África subsaariana, mas em 2004 houve registro de Chikungunya no Quênia, com expansão global para Ásia, Oceano Índico e Europa. Em seguida, foram registrados casos nas Américas, como Caribe, Flórida e Brasil. O vírus Zika foi identificado primeiramente em 1977 na Indonésia, em 2013 houve registros da doença na Polinésia Francesa, em 2014 houve registros de casos na Nova Caledônia, Nas Ilhas Cook e de Páscoa e em 2015 chegou nas Américas. Dessa forma, a dengue se tornou a arbovirose mais comum em seres humanos, principalmente na Ásia e América Latina (MAYER; TESH; VASILAKIS, 2017; WILDER-SMITH et al., 2017).

O vírus Mayaro, um *alfavírus* transmitido pelo mosquito *Haemagogus*, foi inicialmente detectado em Trinidad e já é encontrado, também, na América Central, do Norte e do Sul e na Bacia Amazônica. É um vírus clinicamente semelhante a Chikungunya (YOUNG et al., 2014).

O Nilo Ocidental, um tipo de *Flavivirus*, é transmitido por pássaros passeriformes e mosquito *Culex*, e surgiu em 1999 no Oriente Médio. Possui 5 tipos de linhagem: a 1 que é amplamente distribuída e invasiva, a 2, que permaneceu na África, a 3 e 4 localizadas na Europa Central e a 5, que está isolada na Índia. A dispersão da doença pode ser associada a variedade de mosquitos *Culex*, à extensa urbanização e ao excelente e invasivo hospedeiro. Atualmente essa doença é amplamente distribuída em todos os continentes exceto a Antártida (RÜCKERT; EBEL, 2018; WEAVER; REISEN, 2010).

Já o vírus da encefalite japonesa, *Flavivirus*, foi documentado pela primeira vez em 1800 e atualmente existem cerca de 3 bilhões de pessoas em regiões de risco, com registros de casos anuais variando entre 30 e 50 mil. Sua transmissão (ciclo *Culex*) ocorre através de um pássaro ardeídeo, mosquito *Culex tritaeniorhynchus* e é amplificado por um suíno doméstico, atingindo equídeos e humanos. Sua transmissão ocorre em regiões da China, Coreia, Filipinas, sudeste e sul da Ásia, Sri Lanka, Índia, Paquistão, Nepal, Indonésia e Papua Nova Guiné, abrangente em regiões com culturas de regadio (WEAVER; REISEN, 2010; YOUNG et al., 2014).

Vírus da febre do Vale do Rift, um tipo de *Phlebovirus*, causa epidemias esporádicas no leste da África, podendo afetar ovinos, bovinos, o ser humano e animais domésticos. O ciclo de transmissão ocorre entre búfalos africanos e mosquitos, em que são mais afetados

por períodos chuvosos, que permitem a eclosão de ovos de mosquitos e maior geração de adultos capazes de transmitir a doença. Inicialmente a doença afetou regiões da África Ocidental subsaariana, com surtos registrados na África do Sul, Vale do Nilo, do Sudão ao delta do Egito e na Arábia Saudita (WEAVER; REISEN, 2010).

Já o vírus O'nyong-Nyong está concentrado na região da África Oriental e em 1959 atingiu mais de 2 milhões de pessoas em Uganda, Quênia, Tanzânia, Zaire, Malawi, Moçambique, Senegal, Zâmbia, Sul do Sudão, República Centro-Africana e Camarões. Em 1994 outra epidemia atingiu o Quênia. E Uma variante do vírus já foi encontrada na África Ocidental e o principal vetor é o *Anopheles funestus* (YOUNG et al., 2014).

O vírus da Floresta Barmah é um *alfavírus* exclusivo do continente australiano. Sua epidemiologia é muito semelhante ao vírus do Rio Ross, que é encontrado na Austrália, Fiji, Samoa Americana, Ilhas Cook e Nova Caledônia. Esse vírus é transmitido pelo *Aedes vigilax*, *Ae. camptorhynchus*, *Cx. annulirostris*, *Ae. notoscriptus*, *Ae. sagax* (na Austrália), *Ae. polynesiensis* (nas Ilhas Cook). Espera-se que os hospedeiros desse vírus sejam cangurus e ocorrem anualmente entre verão e outono (YOUNG et al., 2014).

Tabela 1 – Dados de nome de vírus, vetores, hospedeiros (humanos e não-humanos) e localização de algumas arboviroses.

Vírus	Vetor	Hospedeiros	Distribuição
<i>Alfavírus</i>			
Mayaro	<i>Haemagogus</i> spp.	Humanos, primatas e aves silvestres	América do Sul/Central
UNA	<i>Psorophora</i> spp.	Humanos, pássaros e equinos	América do Sul, Trinidad e Tobago
Pixuna	Não identificado	Humanos, roedores e equinos	América do Sul
Everglades	<i>Cx. cedecei</i>	Humanos, cães e roedores	Flórida
O'nyong nyong	<i>Anopheles</i> ssp.	Humanos e primatas	África subsaariana

Rio Ross	<i>Aedes</i> spp. e <i>Culex</i> spp.	Humanos e cangurus	Austrália, Ilhas do Pacífico
Flavivírus			
Spondweni	<i>Ae. circumluteolus</i>	Humanos e roedores	África subsaariana
Dengue	<i>Ae. aegypti</i> e <i>Ae. Albopictus</i>	Humanos e primatas	Em todo mundo nos trópicos
Rocio	<i>Aedes</i> spp. e <i>Psorophora</i> spp.	Humanos e pássaros	América do sul
Encefalite japonesa	<i>Culex</i> spp.	Humanos, porcos e aves	Ásia, Ilhas do Pacífico
Encefalite de Murray Valley	<i>Culex</i> spp.	Humanos e aves	Austrália
Nilo do Oeste	<i>Culex</i> spp.	Humanos e aves	África, Ásia, Europa e Américas
Febre amarela	<i>Aedes</i> spp., <i>Hemagogus</i> spp., <i>Sabethes</i> spp.	Humanos e primatas	África e América do Sul
Orthobunyavírus			
Oropouche	<i>Aedes</i> spp. e <i>Coquillettidia</i> spp.	Humanos e primatas	América do Sul
La Crosse	<i>Ae. triseriatus</i>	Humanos e esquilos	América do Norte
Flebovírus			
Febre do Vale do Rift	40 espécies de mosquitos	Humanos, ovinos, bovinos e animais domésticos	África subsaariana

Fonte: Adaptado de (MILLER, 2008; RÜCKERT; EBEL, 2018).

O vírus da encefalite de Murray Valley é um *Flavivirus* encontrado na Austrália e Nova Guiné e transmitido principalmente pelo mosquito *Cx. annulirostris*. O ciclo envolve aves aquáticas e mosquitos. O vírus do Rocio foi identificado no Brasil em 1975-1976, reaparecendo esporadicamente, mas sem causar epidemias. Seus principais vetores são *Psorophora ferox* e *Ae. scapularis*. Há também o vírus da Encefalite de São Luís que é um arbovírus identificado nos EUA, também é encontrado na América do Norte, Trindidad, América Central, Brasil e Argentina. O ciclo de transmissão ocorre entre pássaros e mosquitos (*Cx. tarsalis*, *Cx. nigripalpus* e *Cx. quinquefasciatus*), com maior incidência no verão (YOUNG et al., 2014).

Outro vírus é o da Encefalite da Califórnia, um *Bunyavirus*, foi identificado na Califórnia em 1940, que raramente causa doenças em humanos. Seu ciclo de transmissão passa por coelhos e roedores, e mosquitos *Aedes*. O vírus Oropouche, um *Bunyavirus*, é encontrado na região Amazônica (Brasil) e Peru, transmitido principalmente pelo mosquito *Colicoides paraenses* (YOUNG et al., 2014).

3. Mosquitos vetores

A distribuição geográfica dos mosquitos vetores depende de fatores como, clima, temperatura e chuva, visto que determinadas espécies precisam de temperatura mínimas para sobreviverem certos locais. Além disso, a temperatura influencia também na replicação e disseminação do vírus (HUANG; HIGGS; VANLANDINGHAM, 2019).

De modo geral as arboviroses são transmitidas por mosquitos vetores dos gêneros *Culex* e *Aedes* (HUANG; HIGGS; VANLANDINGHAM, 2017). O *Ae. aegypti* e o *Ae. albopictus* são responsáveis pela transmissão de diversas doenças emergentes/reemergente, como dengue, Zika, Chikungunya e febre amarela, são invasores e muito bem adaptados aos ambientes urbanos. No entanto, existem mais de 30 mosquitos vetores de febre amarela, como o *Ae. aegypti*, *Ae. africanus*, *Ae. luteocephalus*, *Ae. furcifer*, *Ae. simpsoni* e *Ae. opok* (FONTENILLE; POWELL, 2020; HUANG; HIGGS; VANLANDINGHAM, 2017).

Essas espécies de *Aedes* depositam ovos próximos a residências, onde tenha água acumulada, como pneus (VONTAS et al., 2012). Sabe-se que o *Ae. aegypti* é um mosquito tropical, que evoluiu para se reproduzir e se alimentar em torno de áreas urbanas, possui hábitos diurnos e as fêmeas conseguem picar várias pessoas em um único período de alimentação. Este mosquito é endofílico e endofágico, encontrado em todo o mundo, devido à globalização e aquecimento global (CONWAY; COLPITTS; FIKRIG, 2014; REINHOLD; LAZZARI; LAHONDÈRE, 2018; TAMBWE; SWAI; MOORE, 2022).

O *Ae. albopictus* (mosquito tigre asiático), de clima temperado, foi encontrado em diversas regiões além do sudeste asiático, de onde é nativo (CONWAY; COLPITTS; FIKRIG, 2014; LI et al., 2021). Nessa região pode-se encontrar 5 subgrupos de mosquitos dessa espécie, que são: *Ae. novalbopictus*, *Ae. patriciae*, *Ae. seatoi*, *Ae. subalbopictus* e *Ae. pseudalbopictus* e existem 40 tipos de vírus que podem ser transmitidos por esses mosquitos (FONTENILLE; POWELL, 2020; HUANG; HIGGS; VANLANDINGHAM, 2017). Além disso, eles são endofílicos, exofágicos e vivem mais que o *Ae. aegypti*. Nos EUA e Europa o *Ae. albopictus* é o novo vetor de dengue e Chikungunya e se provou tão competente quanto o *Ae. aegypti*, embora provoquem epidemias geralmente mais brandas que o *Ae. aegypti* (CONWAY; COLPITTS; FIKRIG, 2014; DIAZ, 2016; REINHOLD; LAZZARI; LAHONDÈRE, 2018).

Já os mosquitos do gênero *Culex* podem transmitir vírus da encefalite japonesa e do Nilo Ocidental (HUANG; HIGGS; VANLANDINGHAM, 2017). Esses mosquitos são vetores durante o verão e reservatórios durante o inverno (hibernação). Essas espécies têm preferência por aves, mas podem contaminar humanos e cavalos na ausência de um hospedeiro aviário (CONWAY; COLPITTS; FIKRIG, 2014; NELMS et al., 2013).

Embora esses mosquitos (*Aedes* e *Culex*) sejam mais responsáveis pela transmissão de arboviroses, o *Anopheles* também tem sido citado como transmissor de doenças, como o vírus O'nyong-nyong e a febre do Vale do Rift (CARISSIMO et al., 2014). Os dados resumidos de algumas arboviroses e seus principais mosquitos vetores estão listados abaixo na Tabela 2.

Tabela 2 – Dados de nome de vírus, vetores, hospedeiros (humanos e não-humanos) e localização de algumas arboviroses.

Arbovirose	Mosquito vetor	Referência
Chikungunya	<i>Ae. aegypti</i>	(MARTINET et al., 2019)
	<i>Ae. albopictus</i>	
	<i>Ae. japonicus</i>	
	<i>Ae. destritus</i>	
	<i>Ae. koreicus</i>	
Dengue	<i>Ae. aegypti</i>	(MARTINET et al., 2019)
	<i>Ae. albopictus</i>	
	<i>Ae. japonicus</i>	
Febre amarela	<i>Ae. aegypti</i>	(FONTENILLE; POWELL, 2020)
	<i>Ae. africanus</i>	

	<i>Ae. luteocephalus</i> <i>Ae. furcifer</i> <i>Ae. simpsoni</i> <i>Ae. opok</i>	
Zika	<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. japonicus</i>	(MARTINET et al., 2019; THANGAMANI et al., 2016)
La Crosse	<i>Ae. triseriatus</i>	(LEQUIME; LAMBRECHTS, 2014)
Vírus Usutu	<i>Ae. japonicus</i> <i>Cx. pipiens</i> <i>Cx. antennatus</i> <i>Cx. neavei</i>	(MARTINET et al., 2019; NDIAYE et al., 2018)
Vírus do Nilo Ocidental	<i>Ae. detritus</i> <i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. dalzieli</i> <i>An. rufipes</i> <i>Cx. modestus</i> <i>Cx. pipiens</i> <i>Cx. torrentium</i> <i>Cx. neavei</i> <i>Cx. perfuscus</i> <i>Cx. poicilipes</i> <i>Cx. quinquefasciatus</i> <i>Cx. tritaeniorhynchus</i>	(MARTINET et al., 2019; NDIAYE et al., 2018; WILKE et al., 2019)
Vírus Bagaza	<i>Cx. antennatus</i> <i>Cx. neavei</i>	(NDIAYE et al., 2018)
Vírus Ndumu	<i>Ae. dalzieli</i> <i>Ae. vexans</i> <i>An. rufipes</i> <i>An. ziemanni</i> <i>Cx. antennatus</i> <i>Cx. Poicilipes</i>	(NDIAYE et al., 2018)

Vírus da encefalite japonesa	<i>Ae. albopictus</i> <i>Cx. quinquefasciatus</i>	(CONWAY; COLPITTS; FIKRIG, 2014; PRIDGEON; BERNIER; BECNEL, 2009)
O'nyong-nyong	<i>An. gambiae</i>	(CARISSIMO et al., 2014)

Fonte: Autoria própria.

4. Medidas de proteção contra mosquitos vetores

A expansão de doenças e vetores faz necessária a busca por diferentes estratégias de controle de vetores, e, assim, redução da infestação da doença. Esse método é amplamente aceito visto que o tratamento dessas doenças é bastante limitado (WILKE et al., 2019), existindo poucas vacinas para a alta demanda de arboviroses existentes (ARTSOB; LINDSAY, 2008). Apesar de a erradicação dos vetores ser improvável, algumas medidas protetivas podem ser evidenciadas buscando-se a redução da população de vetores (HUANG; HIGGS; VANLANDINGHAM, 2017; SHOPE; MEEGAN, 1997).

Primeiramente é importante ter consciência de quais vírus estão circulando e ter a população em alerta para poder se prevenir corretamente. Essas informações são essenciais para avaliar os riscos associados, prevenir a transmissão de doenças e controlar vetores. Informações coletadas na América do Norte sobre o vírus do Nilo Ocidental, por exemplo, têm sido importantes para uma análise primária de risco, como por exemplo, observações sobre aves mortas e circulação de mosquitos. Essas informações alimentam o site US Geological Survey dos EUA e monitoram o acesso ao público e sua educação sanitária. De modo semelhante, a dengue também é monitorada pela população, considerando-se a análise de febre, reconhecimento de febre hemorrágica e vigilância virológica (ARTSOB; LINDSAY, 2008).

Além disso, as medidas preventivas podem estar relacionadas ao controle do vetor ou medidas protetivas à população (SHOPE; MEEGAN, 1997). Em relação as medidas protetivas podem-se considerar o uso de roupas, como camisas de manga comprida e calças, minimizando a pele exposta; evitar ambientes ao ar livre em horários com maior atividade de mosquitos vetores; usar telas de proteção em residências (ARTSOB; LINDSAY, 2008).

E para o controle de vetores, de modo geral essas medidas podem ser classificadas em 3 abordagens: controle químico, biológico e manejo ambiental. A abordagem química refere-se ao uso de inseticidas e repelentes. Já a abordagem biológica refere-se à inserção de predadores e parasitas de vetores e o manejo ambiental refere-se a remoção/alteração de habitats de reprodução de vetores, como exemplo, água parada (ARTSOB; LINDSAY, 2008; HUANG; HIGGS; VANLANDINGHAM, 2017; SHOPE; MEEGAN, 1997).

Programas de controle de mosquitos vetores costumam ser complexos e caros, implementados em âmbito municipal, de modo a remover água parada, utilizando controle biológico e aplicando larvicidas e inseticidas. Esses programas são mais eficazes quando integrados, ou seja, quando há o monitoramento dos mosquitos e larvas, análise de temperatura e chuva e educação da comunidade (ARTSOB; LINDSAY, 2008). Porém deve-se considerar que eliminar habitats de proliferação de mosquitos é difícil e pouco eficaz, pois depende de toda a comunidade e muitas vezes esses espaços não são percebidos. Outro fator são os mosquitos que se localizam em matas próximos as florestas, que não serão eliminados. Assim, o uso de proteções individuais, como repelentes e proteção da população é fortemente indicado (TAVARES et al., 2018).

5. Repelentes tópicos e tipos de compostos repelentes

Repelentes são substâncias naturais ou sintéticas que inviabilizam a picada de insetos, por tornar a pele um ambiente prejudicial a esses animais e impedir seu contato com os insetos. Além disso, repelentes devem ser eficazes, seguros para os seres humanos (mesmo durante a gravidez), não devem causar danos a roupas, sem odor ou com odor agradável, persistente contra água e suor, estabilidade química, baixa toxicidade e com preço acessível (DIAZ, 2016; TAVARES et al., 2018).

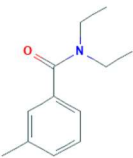
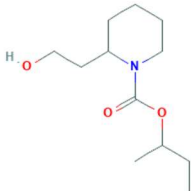
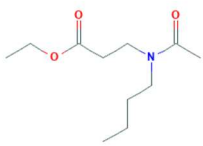
Esses produtos podem ser divididos em repelente espacial, pois sua ação é através do vapor liberado pelo repelente; ou repelente de contato, pois o mosquito entra em contato com a pele quimicamente tratada (XU et al., 2019).

A picada do mosquito além de trazer desconforto, pode apresentar um risco de transmissão de doenças. Assim, o uso de repelentes é uma forma segura e eficaz contra arboviroses (DA SILVA; RICCI-JÚNIOR, 2020). Abaixo são citados alguns repelentes tópicos sintéticos e botânicos.

5.1. Repelentes sintéticos

O uso de repelentes tópicos contra picada de mosquitos vetores de arboviroses é uma importante linha de defesa e proteção contra essas doenças. Alguns produtos utilizados na formulação repelente são o N,N-dietil-3-metilbenzamida (DEET), o éster 1-metilpropil do ácido 2-(2-hidroetil)-1-piperidina-carboxílico (picaridina/icaridina) e etil butilacetilaminopropionato (IR3535) (DIAZ, 2016; FRANCES; DEBBOUN, 2022). Suas estruturas químicas podem ser observadas na Tabela 3.

Tabela 3 – Estruturas químicas dos principais tipos de ativos sintéticos utilizados como repelentes de insetos em produtos comerciais.

Compostos	DEET	Icaridina	IR3535
Fórmula estrutural			
Fórmula molecular	C ₁₂ H ₁₇ NO	C ₁₂ H ₂₃ NO ₃	C ₁₁ H ₂₁ NO ₃
Solubilidade em água (25 °C)	0,9 g/L	8,2 g/L	70g/L
pKa	-0,95	15,9/ -2,4	-0,57
logP (20 °C)	2,1	2,2 (pH 4-9)	1,7
Pressão de vapor mmHg (25°C)	2x10 ⁻³	4,4x10 ⁻⁴	1.1x10 ⁻³

Fonte: Autoria própria.

5.1.1. DEET

O DEET foi sintetizado e distribuído em 1956/1957 nos EUA pelo Departamento de Agricultura, sendo amplamente utilizado como repelente contra moscas, mosquitos carrapatos e pulgas. Esse repelente é utilizado em todo mundo em diversas formulações, como exemplo, aerossóis, cremes, géis, sprays e protege contra *Culex* spp., *Aedes* spp., *Mansonia* spp.,

Verrallina spp. e com menor eficácia, o *Anopheles* spp., principalmente contra o vírus do Nilo Ocidental e Zika vírus (DIAZ, 2016; FRANCES; DEBBOUN, 2022)

O DEET é utilizado como repelente padrão ouro em análises com outros possíveis repelentes, ou seja, a eficácia dos novos compostos é comparada à eficácia do DEET. Porém, ele possui algumas desvantagens, como odor desagradável, sensação gordurosa na pele e o alto nível de penetração causando reações alérgicas ou tóxicas. A concentração comercializada varia entre 5 e 25%. (BRIASSOULIS; NARLIOGLOU; HATZIS, 2001; KURI-MORALES et al., 2017a). Ele atua sensorialmente nos mosquitos, sensibilizando os seus receptores olfativos, sendo que seu efeito é projetado para repelir os insetos e não os matar. Ele também pode atuar como um reversor do odor atrativo para mosquitos e impedir a alimentação dos mosquitos através de receptores gustativos, indicando que seu mecanismo de ação como repelente é bastante complexo (DIAZ, 2016; FRANCES; DEBBOUN, 2022; LEGEAY et al., 2018).

Estudos da Agência de Proteção Ambiental dos EUA determinaram em 2014 que não há evidências suficientes que o DEET seja um risco à população humana, meio ambiente e espécies não-alvo (EPA - UNITED STATES ENVIRONMENTAL PROTECTION, 2021; HALEEM et al., 2020).

No entanto, seu uso constante e generalizado (> 200 milhões de pessoas), faz necessário seu estudo ecotoxicológico. Além disso, deve-se considerar que estudos mostraram atividade inseticida do DEET, cuja LD50 varia conforme espécie e condições experimentais. O DEET também pode causar coceira e inflamação na pele, e convulsões em crianças (ALZOGARAY, 2016; LEGEAY et al., 2018; SWALE et al., 2014).

Pridgeon; Bernier; Becnel (2009), por exemplo, estudou a toxicidade do DEET, IR3535 e picaridina, dentre outros repelentes, para *Ae. aegypti*, *Cx. quinquefasciatus*, *An. quadrimaculatus* e *An. albabimanus*. Os valores de LC50 foram de 0,94, 1,09 e 1,88 mg/mosquito, respectivamente para DEET, picaridina e IR3535, considerando o mosquito *Ae. aegypti*. Já os valores de LC50 para DEET, picaridina e IR3535, respectivamente, foram de 0,40, 0,63 e 2,35 mg/mosquito para *An. quadrimaculatus*. A LC50 para DEET, picaridina e IR3535 foram, respectivamente, de 0,22, 0,50 e 1,67 considerando o mosquito *An. albabimanus*. E, considerando o mosquito *Cx. quinquefasciatus*, os valores de LC50 obtidos para DEET, picaridina e IR3535 foram, respectivamente, de 0,64, 1,62 e 3,61 mg/mosquito.

Já os autores Swale et al. (2014) estudaram o efeito do DEET para os mosquitos *Ae. aegypti* e *An. gambiae*, e para a mosca *Mosca doméstica*. Os resultados de LC50 foram de 1102 e 1175 ng/mg de mosquito, respectivamente para *Ae. aegypti* e *An. gambiae* e de 8104

ng/mg de mosca. Além disso, o DEET causou uma hiperexcitação, seguido de letargia em mosquitos *Ae. aegypti*.

Campos et al. (2016) por sua vez, estudaram os efeitos do DEET no mosquito *Chironomus riparius*. A menor concentração de efeito observada (LOEC) foi de 13,9 mg/L e nas concentrações de 21,7 e 34,3 mg/L de DEET, o crescimento de larvas reduziu em 16,23 e 39%, respectivamente. Além disso, também se observou menor atividade antioxidante nas enzimas catalase e glutathione-S-transferase quando as moscas foram tratadas com DEET.

Alzogaray (2016) estudou os efeitos do DEET e IR3535 em ninfas de *Rhodnius prolixus*. Para ambos os repelentes a LOEC foi de 74 µg/cm², mas quando aplicados topicamente, 750 µg/cm² foram capazes de causar efeito em 40% das ninfas (7 dias), mas não foi observada intoxicação das ninfas com IR3535 na mesma concentração. Além disso, apenas o DEET foi capaz de causar hiperatividade locomotora das ninfas. Outro fator é que a repelência foi mais eficaz em ninfas com antenas.

Dessa forma, apesar de muito utilizado o DEET pode causar alguns efeitos tóxicos em diversos organismos teste, como alteração na atividade locomotora de insetos, reações na derme de mamíferos, degeneração neural, hipotensão e neuro excitação. Além disso, também pode causar problemas à saúde humana, como convulsão em crianças, reações na pele, manifestações neurológicas, respiratórias e cardíacas, letargia, confusão, desorientação e dores de cabeça. Alguns casos mais graves envolveram 16 casos de encefalopatia, sendo que 3 morreram. Porém, o EPA determinou que não há ligação direta com o uso de DEET (AFIFY et al., 2019; ALFHILI et al., 2019; ALZOGARAY, 2016; FRADIN, 2019; KATZ; MILLER; HEBERT, 2008; LEGEAY et al., 2018).

5.1.2. Icaridina

A picaridina ou icaridina ou KBR3023 é um repelente novo e promissor derivado da pimenta, também se mostra eficaz na proteção de picadas de insetos. Ela foi desenvolvida na década de 90 e começou a ser usada em 1998 (NAUCKE et al., 2007). É um ativo incolor, não oleoso e não tem odor acentuado como o DEET e podem ser usadas em sprays, loções e lenços umedecidos. Inclusive, a Organização Mundial da Saúde (OMS) indica o uso em crianças acima de dois anos de idade. As formulações registradas variam em concentração de 7% - 25% (KURI-MORALES et al., 2017a; TAVARES et al., 2018a). Ela foi desenvolvida pela Bayer AG e posteriormente comercializada por 50 países, sendo bastante usado na Europa e Austrália. Sendo comercializado nos EUA pelo nome de Cutter Advanced (FRANCES; DEBBOUN, 2022; KATZ; MILLER; HEBERT, 2008).

Este produto oferece proteção contra moscas, mosquitos, abelhas, pulgas e carrapatos. Contra os mosquitos *Ae. albopictus* e *Cx. quinquefasciatus*, a picaridina é tão eficiente quanto o DEET, evapora mais lentamente que o DEET, podendo indicar um efeito prolongado e é menos tóxico que o mesmo. Pode ser usado em crianças com mais de 6 meses de idade em concentrações de 5 a 10% (DIAZ, 2016; FRANCES; DEBBOUN, 2022; NGUYEN; VU; HEBERT, 2018; TAVARES et al., 2018).

Além disso, a picaridina (5-20%) tem efeito diversas espécies de *Aedes*, *Culex* e *Anopheles*. Em concentrações de até 20% podem ter efeito protetor de até 10 h. Esse composto tem boa estabilidade luminosa, contra água e suor, é compatível com pele e mucosa e seu mecanismo pode estar associado ao bloqueio dos receptores de ácido láctico do mosquito. Espera-se que se forme uma barreira de vapor na pele e que a picaridina afete os sensores olfativos dos mosquitos (KATZ; MILLER; HEBERT, 2008; NGUYEN; VU; HEBERT, 2018; SANGHA, 2010).

Assim, o extenso uso desse repelente torna necessário estudos de toxicidade para avaliar seu risco aos seres humanos e ao ambiente. Embora a Agência de Proteção Ambiental dos EUA (EPA) tenha informado que a picaridina não apresenta toxicidade significativa em animais. Além disso, não há relatos na Europa ou Austrália de efeitos adversos graves nos seres humanos. No entanto, a picaridina pode causar irritações na pele e olhos e é considerada menos tóxica que o DEET (ANTWI; SHAMA; PETERSON, 2008; KATZ; MILLER; HEBERT, 2008; NGUYEN; VU; HEBERT, 2018; TAVARES et al., 2018).

5.1.3. IR3535

O IR3535 (Figura 2c) foi desenvolvido pela Merck e inicialmente comercializado nos EUA, na década de 1970. Atualmente está presente em cerca de 170 produtos e é muito comercializado na Europa. Ele é inodoro e transparente, podendo ser usado em aerossóis, loções e sprays. Esse composto é eficaz contra moscas, mosquitos, carrapatos, piolhos, vespas e abelhas, sendo que repele os mosquitos vetores: *An. gambiae*, *An. funestus*, *Ae. aegypti* e *Ae. albopictus*. (FRADIN, 2019; TAVARES et al., 2018).

Seu efeito repelente se dá em concentrações acima de 10%, mas só é permitido seu uso em formulações com até 30% de IR3535. Seu mecanismo de ação está ligado à sua capacidade de se volatilizar, que forma uma barreira de vapor ao redor da pele impedindo seu contato com mosquitos. (DIAZ, 2016; FRANCES; DEBBOUN, 2022; KURI-MORALES et al., 2017a; LUPI; HATZ; SCHLAGENHAUF, 2013; MERCK, 2001; NAUCKE et al.) Esse repelente é indicado para crianças acima de 6 meses de idade, menores de 2 anos quando usado em

concentrações de 12,5% e gestantes, visto que é considerado de baixa toxicidade. Além disso, quando comparado ao DEET apresenta menor toxicidade à mamíferos, causa menor irritabilidade na pele e tem melhores propriedades físicas (ALZOGARAY, 2016; TAVARES et al., 2018). Apesar do alerta de baixa toxicidade é importante realizar alguns testes toxicológicos para garantir a segurança dos seres humanos e ambiente.

Broschard et al. (2013) estudaram absorção e excreção de IR3535 em humanos (5 homens e 5 mulheres) após exposição dérmica, cuja concentração utilizada variou de 418-731 mg/pessoa. Os indivíduos não relataram irritação na pele, no plasma obteve-se uma concentração de 8 $\mu\text{mol/L}$ de IR3535 e de 4,2 $\mu\text{mol/L}$ de seu metabólito. Os autores perceberam que o repelente é rapidamente excretado pelo corpo humano, com intensa biotransformação.

5.2. Repelentes botânicos

Os repelentes botânicos são produtos extraídos de plantas, de baixa toxicidade, eficazes, de fácil degradação e formados por misturas de compostos orgânicos voláteis, baseados em monoterpenos, sesquiterpenos e fenóis. Os monoterpenos e sesquiterpenos são cadeias ligadas de isopreno, e os monoterpenos são o principal grupo de repelentes de insetos. Os fenóis são anéis aromáticos ligados a grupos hidroxilas e podem ser divididos em: flavonas, isoflavonóides e taninos (GOU et al., 2020; TAVARES et al., 2018). Alguns componentes de óleos essenciais de monoterpenos são α -pineno, limoneno, terpinoleno, citrionelol, citrionelal, cânfora e timol. Já o β -cariofileno é um importante sesquiterpeno contra *Anopheles* spp. (REHMAN; ALI; KHAN, 2014; UNİYAL et al., 2016).

As principais plantas envolvidas na repelência de insetos são das famílias: Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae e Piperaceae (REHMAN; ALI; KHAN, 2014).

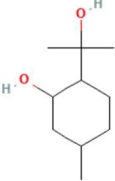
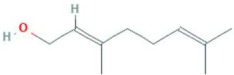
Os óleos essenciais mais conhecidos são óleo de eucalipto-limão e óleo de citronela (DIAZ, 2016; TAVARES et al., 2018). O principal componente do extrato de óleo de eucalipto-limão é o 1,6,4-p-metano-3,8-diol (Tabela 4), já o óleo de citronela é formado por citrionelal, citrionelol, geraniol (Tabela 4), citral, α -pineno e limoneno (CARROLL; LOYE, 2006; DA SILVA; RICCI-JÚNIOR, 2020; TAVARES et al., 2018). Outros óleos essenciais conhecidos são: Neem, Catnip, planta de tomate selvagem, *Hyptis suaveolens*, *Thymus vulgaris*, *Lantana camara* e *Lippia javanica* (TAVARES et al., 2018).

O PMD é um extrato da *Corymbia citriodora*, conhecido como capim-limão. Este composto é gerado por um processo de hidrodestilação, que foi investigado pelos EUA na

década de 1990 e reconhecido como repelente em 2000 pelo EPA. Este composto está disponível em sprays em concentrações de 10 a 40%, sendo que 30% de PMD em formulações tem duração comparável com formulações de DEET (5-10%) (FRANCES; DEBBOUN, 2022; NGUYEN; VU; HEBERT, 2018; WEBB; HESS, 2016).

Na Califórnia, o repelente se mostrou eficaz contra *Ae. melanimon* e *Ae. vexans* (CARROLL; LOYE, 2006). Mas também é eficaz contra o *Aedes*, *Anopheles* e *Culex*, além de carrapatos quando usados em concentrações acima de 30%. No entanto, esse repelente pode causar irritação ocular e pode ser usado somente em crianças acima de 3 anos. Este é o único repelente botânico reconhecido pelo Centro de Controle de Doenças (CDC) (FRADIN, 2019; ISLAM et al., 2017; MAIA; MOORE, 2011).

Tabela 4 – Estruturas químicas dos principais tipos de ativos botânicos utilizado como repelentes de insetos em produtos comerciais.

Compostos	PMD	Geraniol
Fórmula estrutural		
Fórmula molecular	C ₁₀ H ₂₀ O ₂	C ₁₀ H ₁₈ O
Solubilidade em água (25 °C)	0,29 g/L	0,1g/L
pKa	14,6/-2,8	16,33/ -2,2
logP (20 °C)	1,88	3,56
Pressão de vapor mmHg (25°C)	1,4 x 10 ⁻³	3.0 x10 ⁻²

Fonte: Autoria própria.

O limoneno possui atividade repelente contra *Ae. aegypti* e *Culex* spp e o citronelal tem efeito contra *Ae. aegypti* e *Cx. quinquefasciatus* (DA SILVA; RICCI-JÚNIOR, 2020). Já o geraniol é um álcool monoterpênóide, amarelo claro, insolúvel em água, com odor de rosa, sendo um dos compostos mais encontrados em diferentes óleos essenciais, que possui maior

atividade repelente contra *Ae. aegypti* e *Ae. albopictus* (CHEN; VILJOEN, 2010; DA SILVA; RICCI-JÚNIOR, 2020).

Diversos produtos à base de geraniol têm sido comercializados como repelentes naturais, como exemplo, velas e difusores. Sendo que o geraniol tem repelência maior que o linanol e citronela em ambientes internos e externos (CHEN; VILJOEN, 2010). Ele é praticamente insolúvel em água, mas solúvel em solventes orgânicos. É um importante constituinte do óleo de rosas, citronela e da palmarosa (SHARMA et al., 2013).

Assim, o óleo de citronela pode ser extraído de *Cymbopogon nardus* e foi registrado em 1948 nos EUA, podendo ser encontrado como óleos de banho, velas e loções em concentrações de 0,5-20% (NGUYEN; VU; HEBERT, 2018). O repelente é muito comercializado na Europa e na América do Norte. Seu efeito repelente é próximo ao DEET, possui baixa toxicidade, boa eficácia em concentrações variando de 0,05 e 15% (DA SILVA; RICCI-JÚNIOR, 2020; MAIA; MOORE, 2011; TAVARES et al., 2018). Ele é mais eficiente contra Anopheles e mosquitos culicines, como o Aedes e ineficaz contra carrapatos, pulgas e moscas (FRADIN, 2019; NGUYEN; VU; HEBERT, 2018).

No entanto, o uso dos repelentes botânicos é limitado, devido à rápida volatilização da formulação aplicada, o que reduz o seu tempo de proteção e torna necessário um maior número de aplicações, por isso poucos produtos estão disponíveis no mercado (LUPU; HATZ; SCHLAGENHAUF, 2013; SENTHIL-NATHAN, 2020). Portanto, diversos estudos têm sido feitos para analisar a capacidade repelente de diversos compostos, sendo que as misturas entre óleos essenciais e/ou sintéticos têm auxiliado na melhoria da capacidade de repelência destes compostos.

Hazarika et al. (2022), por exemplo, desenvolveram um creme repelente utilizando os óleos essenciais contra o mosquito *Ae. albopictus*. A melhor repelência se deu com a mistura de óleo de cravo, citronela e capim-limão, com uma capacidade de 96% na concentração de 0,04 mg/cm²; seguido da mistura do óleo de lavanda, capim-limão e citronela, com repelência de 88% na mesma concentração. Também foram feitos testes de citotoxicidade em células epiteliais de pulmão humano (L-132) e apenas concentrações acima de 0,25 g/L. Não houve reações dérmicas em coelhos (*Oryctolagus cuniculus*) e o creme foi considerado não irritante no teste de irritação ocular, para a mesma espécie.

Das et al. (2015) estudaram óleos essenciais à base de *Crucuma longa* (CL), *Pogostemon heyneanus* (PH) e *Zanthoxylum lomonella* (ZL) contra *Ae. albopictus*, sendo que a mistura deles em uma proporção de 1:1:2 para CL:ZL:PH ocasionou em um tempo de proteção de 329,4 e 391,0 min, em laboratório e campo, respectivamente. Isoladamente, o máximo de tempo atingido de proteção foi de 123,4 min para o óleo baseado em PH. Esse

efeito sinérgico tem grande importância para o desenvolvimento de novos repelentes eficientes contra picada de mosquito.

Odalo et al. (2005) extraíram óleos essenciais de 6 plantas do Quênia: *Croton pseudopulchellus*, *Mkilua fragrans*, *Endostemon tereticaulis*, *Ocimum forkolei*, *Ocimum fischeri* e *Plectranthus longipes* para avaliar seu efeito repelente, como ou sem presença de DEET em *An. gambia*. Sendo que as misturas com *C. pseudopulchellus* e *P. longipes* foram superiores em repelência aos óleos originais, indicando efeitos aditivos ou sinérgicos com a presença de um repelente sintético.

6. Formulações de uso tópico

Muitos destes óleos essenciais são facilmente volatilizados e instáveis, e a busca por novas formulações capazes de garantir uma ação prolongada e mais resistentes se fazem necessária, como o uso de nanoestruturas carreadoras que possibilitem o uso destes materiais de forma tópica (DA SILVA; RICCI-JÚNIOR, 2020; TAVARES et al., 2018).

A nanotecnologia manipula materiais e estruturas em escala nanométrica e tem se buscado estratégias associadas à redução da poluição ambiental, mais sustentáveis (menor quantidade de ativos) e com materiais biocompatíveis e biodegradáveis. Esses produtos obtidos da nanotecnologia são vantajosos considerando a perda e disponibilidade do óleo essencial e sua proteção (DUARTE et al., 2020), devido a geração de sistemas de liberação controlada, em que os extratos vegetais podem estar adsorvidos na superfície do carreador ou aprisionados pelas estruturas. Além disso, podem minimizar os efeitos em organismos não-alvos, serem mais bem solubilizados e ter melhor atividade devido ao tamanho diminuto (ORTIZ ZAMORA et al., 2021).

Para a produção destes materiais deve-se considerar qual princípio ativo deve ser escolhido, o tipo de formulação usado, o tipo de tecnologia de liberação controlada, o público-alvo desse produto (crianças, adultos, gestantes), tempo de ação e ambiente propício para o uso (DA SILVA; RICCI-JÚNIOR, 2020).

Alguns materiais desenvolvidos são a ciclodextrina, que aumenta a solubilidade de substâncias lipofílicas e disfarça o odor; as nanopartículas, que reduzem a volatilização de moléculas; nanoemulsão, que possuem boa estabilidade cinética; nanopartícula lipídica sólida, que possui efeito de filme na pele e biocompatibilidade e nanofibras, que possuem excelente eficiência de encapsulação e alta área superficial (DA SILVA; RICCI-JÚNIOR, 2020).

Há uma tendência crescente de uso da nanotecnologia no setor industrial de cosméticos, em que os líderes mundiais em fabricação estão usando esta inovação em muitos de seus produtos. Atualmente, já são mais de 918 produtos cosméticos produzidos com base nanotecnológica. Por isso, o desenvolvimento de uma nova geração de repelentes utilizando como ferramenta a nanotecnologia é essencial para um avanço nessa linha de cosméticos que é tão importante na proteção contra as arboviroses (StatNano, 2022).

6.1. Carreadores lipídicos nanoestruturados (NLC¹)

Os carreadores nanoparticulados possuem inúmeras vantagens como proteção do ativo, liberação controlada, reduzindo, assim, a quantidade de produto utilizado, circulação prolongada e entrega direcionada (GAO et al., 2016; KHOSA; REDDI; SAHA, 2018).

Dentre esses sistemas nanoestruturados, os produzidos a partir de lipídios (carreadores lipídicos nanoestruturados – NLC) tem a vantagem de serem biocompatíveis, estabilidade físico-química, biocompatibilidade cutânea, fácil produção e maior eficiência de encapsulamento. Além de encapsular mais facilmente compostos ativos que sejam lipofílicos (DA SILVA; RICCI-JÚNIOR, 2020; KHOSA; REDDI; SAHA, 2018; TAMJIDI et al., 2013).

Os NLCs são uma modificação das nanopartículas lipídicas sólidas, que possuem mais vantagens e melhores propriedades para armazenamento de ativos em sua estrutura. Esses carreadores são formados por uma matriz lipídica sólida e uma porção de óleo, resultando em uma matriz menos ordenada, em uma proporção variando de 70:30 a 99,9:0,1 de sólidos:líquidos (BELOQUI et al., 2016; KHOSA; REDDI; SAHA, 2018; TAMJIDI et al., 2013).

Além disso, lipídios líquidos podem solubilizar melhor alguns ativos do que os lipídios sólidos, enquanto a matriz sólida oferece maior estabilidade ao sistema e protege o ativo (BELOQUI et al., 2016; GARCÊS et al., 2018). Assim, os NLCs têm maior espaçamento entre os ácidos graxos e mais imperfeições, o que permite melhor acomodação do ativo em sua estrutura (MÜLLER; RADTKE; WISSING, 2002).

Esses carreadores podem ser divididos em três subgrupos, que são do tipo imperfeito, do tipo amorfo e do tipo múltiplo de óleo em gordura sólida em água (O/F/W) (Figura 2). O tipo imperfeito é composto por uma mistura lipídica de diferentes comprimentos de cadeia. Já o tipo amorfo é uma mistura de lipídios especiais, por exemplo, isopropilmorostato, com lipídio sólido, ou seja, é uma NLC sólida, mas não cristalina. E, o O/F/W é composto por

¹ NLC é uma abreviação do termo referente aos carreadores lipídicos nanopartículas, que em inglês é nanostructured lipid carriers.

compartimentos de óleo líquido distribuído em uma matriz sólida (KHOSA; REDDI; SAHA, 2018; TAMJIDI et al., 2013).

Figura 2 – Tipos de NLCs: tipo imperfeito, amorfo e múltiplo (O/F/W).



Fonte: Autoria própria.

Os principais reagentes para formação de NLCs são lipídios, tensoativos e água, sendo que os lipídios líquidos podem ser óleo de soja, triglicerídeos, ácido oleico, vitamina E, óleo de milho, entre outros. E, para se produzir um NLC eficiente deve-se considerar a solubilidade do ativo antes de incorporá-lo na matriz carreadora; os lipídios líquidos e sólidos devem ser espacialmente incompatíveis e o lipídio líquido deve ser mais estável (TAMJIDI et al., 2013).

Os NLCs podem ter diversas aplicações terapêuticas, sendo que em administrações dérmicas têm as vantagens de biocompatibilidade, segurança, e proteção do ativo encapsulado (GARCÊS et al., 2018; SALVI; PAWAR, 2019).

A absorção pela pele pode ocorrer via transepidérmica e/ou transapendageal (via glândulas sebáceas). Além disso, o tamanho das nanopartículas pode influenciar na sua absorção pela pele, bem como as propriedades dos compostos encapsulados, do surfactante utilizado na preparação do NLC e o método de produção (BELOQUI et al., 2016).

Os NLCs já têm sido reportados na literatura como encapsuladores de óleos essenciais, embora poucos trabalhos demonstrem seu uso como repelentes para mosquitos e pragas. Tortorici et al. (2022) desenvolveram NLC pelo método de temperatura de inversão de fase, para encapsular separadamente três óleos essenciais, a lavanda, o alecrim e a hortelã-pimenta. As partículas obtidas tinham tamanhos menores de 200 nm, PDI inferior a 0,3 e potencial zeta negativo. A eficiência de encapsulação foi de ~70% para os três óleos e

a formulação com alecrim foi a mais estável por um mês. Para a praga *Aphis gossypii*, todas as formulações (com ou sem óleos essenciais) causaram mortalidade significativa para o inseto. Já para o inseto *Spodoptera littoralis*, apenas sua atividade alimentar foi afetada pelas formulações com óleo de alecrim e lavanda. Já a *Tuta absoluta* não foi afetada em termos de sobrevivência e alimentação pelas formulações.

Radwan et al. (2022) utilizaram NLCs encapsuladoras de erva-doce e chá verde contra o mosquito *Cx. pipiens*. As partículas desenvolvidas tinham tamanho inferior a 300 nm, com formato esférico, homogêneas e potencial zeta negativo. Para o efeito larvicida (em laboratório), a porcentagem de mortalidade (24 h) das formulações com óleo de erva-doce e chá verde foi de 100%, com LC_{50} de 251,71 ppm e 278,63 ppm, respectivamente. Como efeito adulticida, as formulações com óleo de erva-doce e chá verde causaram a mortalidade de 100% dos organismos.

Ghodrati; Farahpours. Hamishehkar (2019) propuseram um NLC, por fusão a quente, como agente encapsulante de óleo essencial hortelã-pimenta para atividade antibacteriana e cicatrização de feridas em camundongos. O tamanho das partículas variou de 40 a 250 nm, o PDI foi de aproximadamente 0,4 e potencial zeta negativo. A atividade antibacteriana foi semelhante para *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Listetia monocytogenes*, *Escherichia coli* e *Pseudomonas aeruginosa*. Já o ferimento foi causado nos camundongos e inoculado em *P. aeruginosa* e *S. aureus*, cujos resultados foram positivos como tratamento de região infectada, pelos dados de infiltração de fibroblastos e deposição de colágeno, por exemplo.

Saporito et al. (2018) prepararam NLCs baseadas em manteiga de cacau como sistema carreador de óleo de alecrim ou eucalipto pelo método de homogeneização de alto cisalhamento como agente de cicatrização e em atividade antimicrobiana de *S. aureus* e *Streptococcus puogenes*. O tamanho das partículas variou entre 220 e 300 nm, PDI obtido foi de 0,5 e a estabilidade medida foi de 3 meses. Além disso, as partículas (independentes do óleo essencial) apresentaram boa citocompatibilidade e o encapsulamento dos óleos também melhorou a atividade antimicrobiana do óleo de eucalipto para ambas as bactérias. As nanopartículas à base de azeite carreando óleo de eucalipto apresentaram melhor proliferação *in vitro* de fibroblastos dérmicos humanos normais, indicando boa cicatrização. E, os NLCs de óleos essenciais também auxiliaram na redução da lesão tecidual (*in vivo*) causada por queimadura em ratos.

6.2. Nanopartículas poliméricas

As nanopartículas poliméricas também têm recebido atenção por apresentarem vantagens como, tamanho, potencial para sistemas de liberação controlada, proteção do ativo encapsulado e biodisponibilidade. Esses materiais podem ser nanoesferas ou nanocápsulas. As nanoesferas são formadas por uma matriz densa com ativos dispersos nessa matriz ou aderido à sua superfície, geralmente esférica. Já as nanocápsulas são um reservatório, ou seja, uma cavidade líquida (óleo/água) revestida por um polímero, cujos ativos estão em seu interior ou adsorvidos na membrana (Figura 3). Sendo que seu tamanho varia entre 100 e 1000 nm (MADKOUR, 2019; ORTIZ ZAMORA et al., 2021; ZIELÍ NSKA et al., 2020).

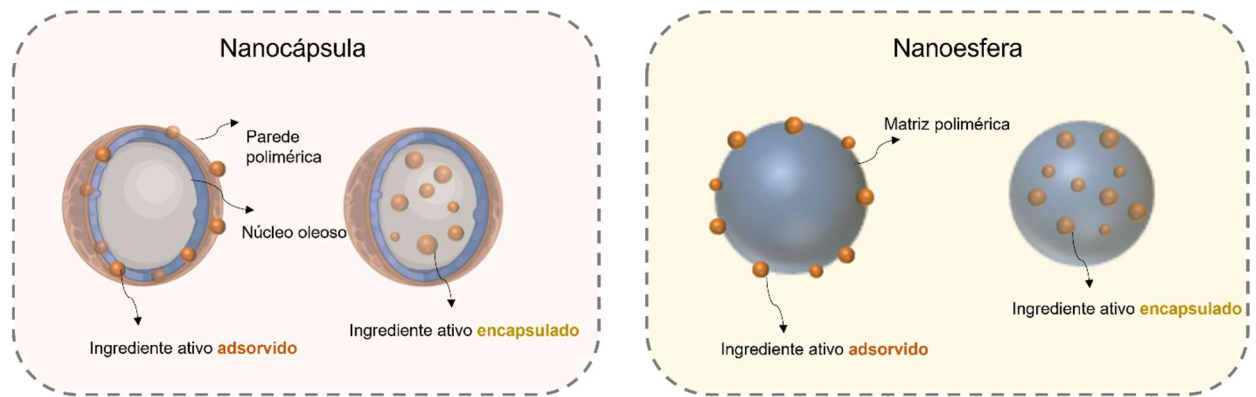
Essas nanopartículas possuem características interessantes para o uso em aplicações tópicas, pois facilita a estabilização de suspensões. Já sua área superficial auxilia na maior área de contato do ativo encapsulado e o alvo. Além disso, o método de produção e os reagentes (polímeros) utilizados podem controlar as propriedades físico-químicas das nanopartículas (MADKOUR, 2019).

Os sistemas baseados em nanopartículas poliméricas também podem auxiliar na solubilização dos ativos e o controle da liberação maximiza o efeito dos ativos com menor efeito colateral, reduz a administração dos ativos e protege os produtos encapsulados (PELTONEN; SINGHAL; HIRVONEN, 2020).

Bem como os NLCs, as nanopartículas poliméricas têm sido estudadas no aprisionamento de substâncias lipofílicas para uso tópico. Da Silva et al. (2020) produziram nanocápsulas do tipo core-shell baseados em quitosana e poli- ϵ -policaprolactone como sistema carreador de óleo de tea tree para tratamento de acne. O tamanho das nanopartículas desenvolvidas foi de 268 nm, PDI menor 0,3 e o potencial zeta foi de + 31 mV, pH 5 e eficiência de encapsulação de 95%. Além disso, as nanopartículas apresentaram melhores resultados na atividade anti-Cutibacterium acnes se comparado ao óleo livre.

González et al. (2017) produziram nanopartículas poliméricas baseadas em quitosana e polietilenoglicol carreadoras de dois óleos essenciais, o gerânio e a bergamota como larvicida de *Cx. pipiens*. O tamanho das nanopartículas foi inferior a 255 nm, PDI menor que 0,3 e eficiência de encapsulação variando de 68 a 77%, para o polietilenoglicol. Já para as nanopartículas de quitosana o tamanho foi inferior a 535 nm, o PDI menor que 0,4 e a eficiência de encapsulação variou de 22 a 38%. Para a atividade larvicida notou-se que as nanopartículas foram mais tóxicas que os óleos livres (causada pela redução da LC₅₀) e as nanopartículas de quitosana foram mais tóxicas que o polietilenoglicol para ambos os óleos.

Figura 3 – Representação esquemática das nanopartículas poliméricas, que podem ser nanocápsulas ou nanoesferas: a) fármaco adsorvido na superfície; b) fármaco encapsulado; c) fármaco adsorvido na superfície e d) fármaco disperso na matriz.



Fonte: Autoria própria.

Froio et al. (2019) produziram nanopartículas baseadas em Eudragit® RS 100 para encapsular óleo de bergamota e óleo de laranja doce como antibactericidas para *E. coli* (in vitro). O diâmetro médio das nanopartículas variou de 57 a 208 nm e o potencial zeta variou de + 39 a + 74 mV. A atividade antimicrobiana das nanopartículas-óleos foi de 90% e o óleo essencial de laranja parece mais eficiente que o de bergamota. Além disso, as nanopartículas com óleo de laranja doce foram usadas para conservação de suco de laranja, que mostraram resultados promissores.

Yeguerman et al. (2020) produziram nanopartículas baseadas em polietilenoglicol como carreadores de óleo de hortelã-pimenta e óleo de palmarosa como inseticidas contra a barata alemã (*Blattella germanica* L.). As nanopartículas com óleo de hortelã-pimenta tinham tamanhos de 380 nm, PDI maior que 0,4 e eficiência de encapsulação de 72,25%, e as nanopartículas com óleo de palmarosa tinham tamanho de 191 nm, PDI menor que 0,25 e eficiência de encapsulação de 89,75%. Além disso, as nanopartículas potencializaram o efeito repelente dos óleos essenciais de 12 h para 36 h do óleo de hortelã-pimenta e de 36 h para 72 h para o óleo de palmarosa.

6.3. Hidrogéis baseados em nanopartículas (NP-gels)

A incorporação de nanopartículas com outros materiais para formar novos sistemas híbridos também tem sido estudada. Como exemplo há nanopartículas incorporadas em hidrogel (NP-gels), que tem função de proteger as nanopartículas e diminuir riscos ambientais e à saúde humana, bem como modificar suas propriedades iniciais, como resistência mecânica e propriedades adesivas (GAO et al., 2016; THONIYOT et al., 2015).

Os hidrogéis são redes 3D de polímeros hidrofílicos reticulados, que podem preservar a integridade das nanopartículas e auxiliar em sua eficácia terapêutica. São materiais macios, úmidos, com capacidade de absorver água (de 10% a milhares de vezes em seu peso seco) e com características sólidas e líquidas. Além disso, esses materiais são muito utilizados na biomedicina, pois suas características se assemelham a matriz extracelular, são mais semelhantes às propriedades dos tecidos se comparados a outros biomateriais e são biocompatíveis (DANNERT; STOKKE; DIAS, 2019; GAO et al., 2016; HOFFMAN, 2012; LIU et al., 2015).

Os hidrogéis podem ser do tipo físico (reversível) ou químico (permanentes). O primeiro é formado de ligações de hidrogênio, iônicas e/ou hidrofóbicas, o que acarreta um gel heterogêneo, que pode ser reversível por mudanças de pH, temperatura e estresse, por exemplo. Já o segundo tipo são redes formadas através de ligações covalentes, são géis heterogêneos e possuem *clusters*, que são regiões de baixo inchamento inseridas em regiões de alto inchamento (HOFFMAN, 2012).

Para a formação do sistema híbrido NP-gels, o hidrogel pode ser formado diretamente na suspensão de nanopartículas, ou as nanopartículas podem ser formadas dentro do gel formado previamente, as nanopartículas podem ser incorporadas ao gel, seguido do processo de gelificação (hidrogel nanocompósito), a reticulação do gel pode ser feita pelas nanopartículas ou há uma mistura de nanopartículas, polímeros (para formação do gel) e agentes reticulantes. A ligação das nanopartículas pode ser por ligações hidrofóbicas ou covalentes (GAO et al., 2016; PALMESE et al., 2019).

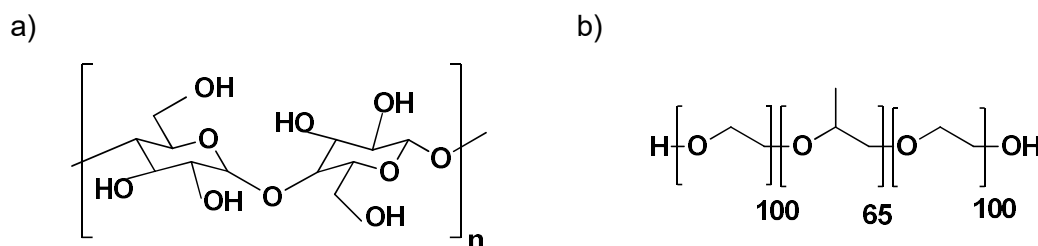
Diversos polímeros têm sido tradicionalmente usados na produção de hidrogéis, como o polidimetilsiloxano, polietilenoglicol e poli(ácido láctico-co-glicólico), que podem ser divididos em naturais ou sintéticos (LIU et al., 2015). Um polímero que tem recebido destaque é a hidroxipropilmetilcelulose (HPMC), um polímero natural baseado em celulose e um polímero sintético que é o Pluronic F-127, um poloxamer.

Dentre os polímeros utilizados, o composto HPMC (Figura 4, a) tem sido reportado no uso de formação de hidrogéis para sistemas de liberação controlada. O HPMC é um polímero

derivado de éter de celulose, inodoro, branco, com características antimicrobianas, que tem seu volume expandido quando em contato com a água, devido aos grupos polares de sua estrutura (hidroxipropil). Mas também pode fazer interações hidrofóbicas devido aos grupos apolares de sua estrutura (metil) (DHARMALINGAM; ANANDALAKSHMI, 2019; KHANUM et al., 2018).

Além disso, também tem sido reportado o uso de poloxamer (Pluronic F-127) (Figura 4, b) para a geração de hidrogéis. Esse polímero é feito pela automontagem, em água, de copolímeros de óxido de etileno e polopropileno. É um material tribloco, não iônico, atóxico, biocompatível, biodegradável, termossensível que favorece a adesão dérmica e seu gel possui alta viscosidade (CHATTERJEE et al., 2019; DINIZ et al., 2015; NASCIMENTO et al., 2018).

Figura 4 – Estruturas químicas de polímeros utilizados na formação de hidrogéis: a) Hidroxipropilmetilcelulose e b) Poloxamer (Pluronic F-127).



Fonte: Autoria própria.

Pivetta et al. (2018), por exemplo, produziram um gel baseado em NLC carreadora de timol, cujas partículas tinham características de: tamanho de 107,7 nm, potencial zeta de -11,6 mV e eficiência de encapsulação de 89,1%. As nanopartículas foram incorporadas no gel, e a formulação final de NP-gels obtida tinha características necessárias para aplicação tópica, como pH variando entre 5,4 e 5,6, fluido não newtoniano e comportamento reológico ajustado no modelo de Herschel-Bulkley. Além disso, o gel de timol encapsulado foi testado em camundongos (*in vivo*) e apresentou atividade anti-inflamatória em edema de orelha e psoríase, mostrando melhores resultados do que o timol livre.

Buntum et al. (2021) produziram nanopartículas poliméricas de quitosana/tripolifosfato de sódio como sistema carreador de óleo essencial de cúrcuma e cravo, que foram incorporadas no hidrogel produzido a partir de poli(álcool vinílico), para aplicação de curativos. O tamanho das nanopartículas carreadoras de óleo essencial de cravo foi inferior a 227 nm,

enquanto para o óleo essencial de cúrcuma o tamanho variou de 375 a 888 nm, dependendo da concentração de quitosana. Já a eficiência de encapsulação das nanopartículas carreadoras de óleo essencial de cravo variou de 13 a 50 %, enquanto para o óleo essencial de cúrcuma a eficiência de encapsulação variou de 9 a 37 %. Em relação ao hidrogel, aqueles com óleo de cúrcuma mostraram melhores resultados em termos de adesividade. A liberação máxima dos sistemas de hidrogéis contendo nanopartículas de quitosana com óleo essencial de cravo e cúrcuma foram de 25,9 e 19,8%, respectivamente. Além disso, as NPs-gel para ambos os óleos não se mostraram tóxicas para as células NCTC-929 e fibroblastos dérmicos humanos normais (NHDF) (viabilidade celular > 70%).

Ngampunwetchakul et al. (2019) também produziram nanopartículas de quitosana reticuladas com tripolifosfato de sódio para encapsular óleo essencial de gengibre em diferentes concentrações. Essas nanopartículas foram imobilizadas em hidrogéis de poli(álcool vinílico) para tratamento tópico de feridas. O tamanho das partículas foi inferior a 259 nm e a eficiência de encapsulação variou de 21 a 44 %, aumentando em maiores concentrações de óleo essencial. Já os hidrógeis contendo nanopartículas mostraram que os dados de dureza e compressibilidade foram menores conforme maiores concentrações de óleo essencial. Já a adesividade não variou conforme a concentração de óleo essencial, mostrando que os hidrógeis podem ser facilmente aplicados em uso tópico. Já os estudos de liberação do óleo essencial variaram de 30 a 72%, conforme aumento da concentração do ativo na formulação de quitosana. Além disso, essas formulações foram testadas em NCTC-929 e NHDF para uma avaliação de citotoxicidade indireta, e não se mostraram tóxicas nas concentrações testadas já que a viabilidade celular foi superior a 70%.

CHAPTER I
RECENT DEVELOPMENTS IN NANOTECHNOLOGY FOR DETECTION AND CONTROL
OF AEDES AEGYPTI-BORNE DISEASES

Frontiers in Bioengineering and Biotechnology, V. 8, 2020

doi: 10.3389/fbioe.2020.00102



Recent Developments in Nanotechnology for Detection and Control of *Aedes aegypti*-Borne Diseases

Estefânia Vangelie Ramos Campos¹, Jhones Luiz de Oliveira²,
Daniele Carvalho Abrantes², Carolina Barbára Rogério², Carolina Bueno²,
Vanessa Regina Miranda², Renata Aparecida Monteiro² and
Leonardo Fernandes Fraceto^{2*}

¹ Human and Natural Sciences Center, Federal University of ABC, Santo André, Brazil, ² São Paulo State University—UNESP, Institute of Science and Technology, Sorocaba, Brazil

OPEN ACCESS

Edited by:

Eden Morales-Narváez,
Centro de Investigaciones en
Optica, Mexico

Reviewed by:

Abdelrahim H. A. Hassan,
Beni-Suef University, Egypt
Jie Liu,
South China Agricultural
University, China

*Correspondence:

Leonardo Fernandes Fraceto
leonardo.fraceto@unesp.br

Specialty section:

This article was submitted to
Nanobiotechnology,
a section of the journal
Frontiers in Bioengineering and
Biotechnology

Received: 27 November 2019

Accepted: 03 February 2020

Published: 20 February 2020

Citation:

Campos EVR, de Oliveira JL,
Abrantes DC, Rogério CB, Bueno C,
Miranda VR, Monteiro RA and
Fraceto LF (2020) Recent
Developments in Nanotechnology for
Detection and Control of *Aedes*
aegypti-Borne Diseases.
Front. Bioeng. Biotechnol. 8:102.
doi: 10.3389/fbioe.2020.00102

Arboviruses such as yellow fever, dengue, chikungunya and zika are transmitted mainly by the mosquito vector *Aedes aegypti*. Especially in the tropics, inefficacy of mosquito control causes arboviruses outbreaks every year, affecting the general population with debilitating effects in infected individuals. Several strategies have been tried to control the proliferation of *A. aegypti* using physical, biological, and chemical control measures. Other methods are currently under research and development, amongst which the use of nanotechnology has attracted a lot of attention of the researchers in relation to the production of more effective repellents and larvicides with less toxicity, and development of rapid sensors for the detection of virus infections. In this review, the utilization of nano-based formulations on control and diagnosis of mosquito-borne diseases were discussed. We also emphasizes the need for future research for broad commercialization of nano-based formulations in world market aiming a positive impact on public health.

Keywords: *Aedes aegypti*, vector control, biosensors, larvicides, arboviruses, nanobiotechnology

INTRODUCTION

Rampant growth of human population has led to major challenges of sustainable food production and disease control in the twenty-first century (Roni et al., 2015). Arthropods are vectors of some deadly diseases, which can lead to epidemics or pandemics (Murugan et al., 2015). On top of the list are mosquitoes (Diptera: Culicidae) that are a cause of a major concern around the world because they can act as vectors of a variety of harmful pathogens and parasites (Benelli, 2016; Benelli and Mehlhorn, 2016). *Aedes aegypti* and *Aedes albopictus* are the most important global vectors of arboviruses, such as dengue, yellow fever, chikungunya, and zika viruses (Durán et al., 2016). Arboviruses have long been treated as neglected diseases around the world. However, in recent years there have been a number of epidemics caused by arboviruses - such as dengue, chikungunya, yellow fever and unprecedented zika (Wilder-Smith et al., 2017). The main factors contributing to these outbreaks have been considered to be urbanization, modernization and increased international mobility of the general population (Tavares et al., 2018).

Control of arboviruses is difficult due to many factors, such as lack of effective vaccines for most of the arboviruses, lack of antiviral drugs, insecticide resistance in the vectors such as *Aedes* species

and failure of vector control strategies that would decrease human-vector contact (Batool et al., 2018). In this scenario, development of new approaches to rapidly detect, and control dissemination of arboviruses are a priority and a public health imperative. In this regard, the importance of nanobiotechnology has been gradually realized as an emerging technology of the future due to exceptional new benefits (Suganya et al., 2017). In the vector control applications, nanoparticles could be applied for: (a) the development of new drugs, with higher activity, decreased toxicity and sustained release; (b) development of new repellent formulations based on natural or synthetic compounds; (c) control of vectors by the use of nanoparticles with repellent, insecticidal or larvicidal activities (Magro et al., 2019); and (d) development of biosensors that can rapidly detect and diagnose the mosquito transmitted viral diseases (Durán et al., 2016; Benelli et al., 2017; Nicolini et al., 2017a). Due to the lack of specific drugs for viral diseases, nanobiotechnology has appeared as an important new breakthrough, which could be potentially used for treatment of patients infected with arboviruses. VivaGel® is a poly-L-lysine dendrimer-based formulation, which has shown efficient antiviral activity against zika virus (ZIKV) (Starpharma, 2016). Recently, a number of reviews have been published on the contribution of nanotechnology to control arboviruses epidemics.

The developments in the area of nanomedicines is also promising new treatments for different diseases, improving the efficacy and bioavailability of drugs, with controlled release formulations that require optimal doses and consequently lesser adverse effects. This review discusses the current status of nanobiotechnology relevant to the control of arbovirus mosquito vectors, and highlights how it provides key tools for exploring new perspectives in the treatment of arboviruses.

NANOTECHNOLOGY FOR ARBOVIRUS DETECTION AND CONTROL

Several strategies have been applied to prevent proliferation of *Aedes* species using physical, biological, and chemical control approaches. Other methods under research and development, are also being studied, including the use of nanotechnology to produce repellent and larvicidal formulations that are more efficacious and less toxic. The development of nanotechnology-based sensors for rapid viral detection has also attracted the attention of scientific community (Figure 1).

Biosensors

Rapid diagnosis of important arboviruses-borne diseases such as dengue, chikungunya, zika, and yellow fever is essential in order to reduce and avoid further dissemination of the infections within the general population (Patterson et al., 2016). The WHO has emphasized the importance of developing point-of-care (POC) tests that are ASSURED (Affordable, Sensitive, Specific, User-friendly, Robust and rapid, Equipment-free, and Deliverable) (Pashchenko et al., 2018). An ideal technique for the on-site detection of arboviruses should have these characteristics and enable early detection of the disease. Fast and timely diagnosis

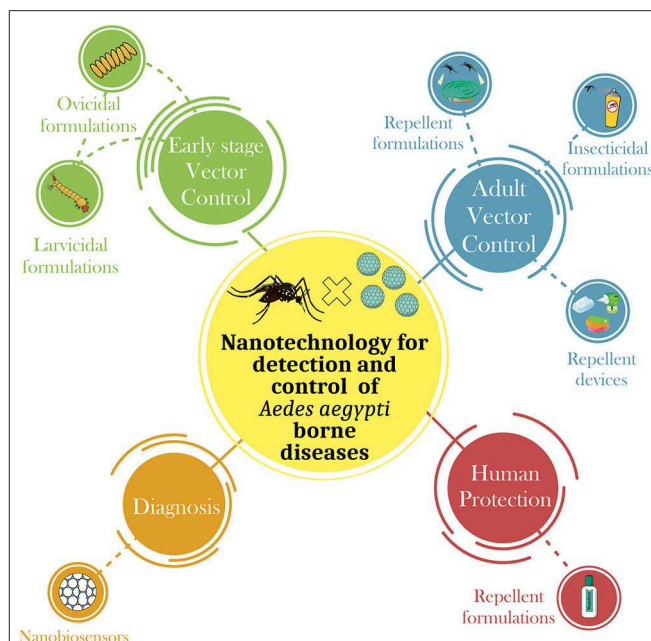


FIGURE 1 | Summary of the main applications of nanotechnology in the control of *Aedes aegypti*-borne diseases. There are four main areas where nanotechnology can be connected: (i) *Early stage vector control*—The nanodevices for the control of *Aedes aegypti* can be developed in order to control this pest in its different stages of life. Many studies have developed nanodevices to control the early stages of mosquitoes, i.e., products that exhibit ovicidal and/or larvicidal activity; (ii) *Adult vector control*—adult insect control is also highly studied form of control through the development of nanoformulations that exhibit larvicidal and/or repellent activity; (iii) *Human protection*—following the recent epidemic of arboviruses transmitted by *Aedes aegypti*, many studies have focused on the development of forms of immunization of humans through the development of nanovaccines, and (iv) *Diagnosis*—the development of nanobiosensors that quickly detect the presence of arboviruses in the host, thus expediting the decision for the most effective treatment.

is crucial for the confirmation of viral infection so that it can be followed by clinical treatment, and necessary measures can be put in place for monitoring and protection of public health (Rashid and Yusof, 2018). Currently, diagnosis of the infections caused by arboviruses in the genus *Flavivirus* (family *Flaviviridae*) is often late, ineffective, and dependent on the clinical symptoms. The final decision on the infection generally requires a long waiting time, collection of samples from suspected patients (blood, urine, or saliva), transportation and preservation, and laboratory procedures by trained health staff.

Given such difficulties in the early detection of an impending epidemic of a viral infection, such as dengue, chikungunya, zika, and yellow fever (Nakata and Röst, 2015), there has been an urgent need for the improvement of existing tools and development of new biosensing technologies that are rapid, effective, and applicable in terms of real-time diagnosis. Biosensors are biologically-selective analytical devices that are able to recognize analytes in a complex sample matrix without the need for lengthy sample treatments. The biologically-selective part of biosensors enables them to produce highly specific

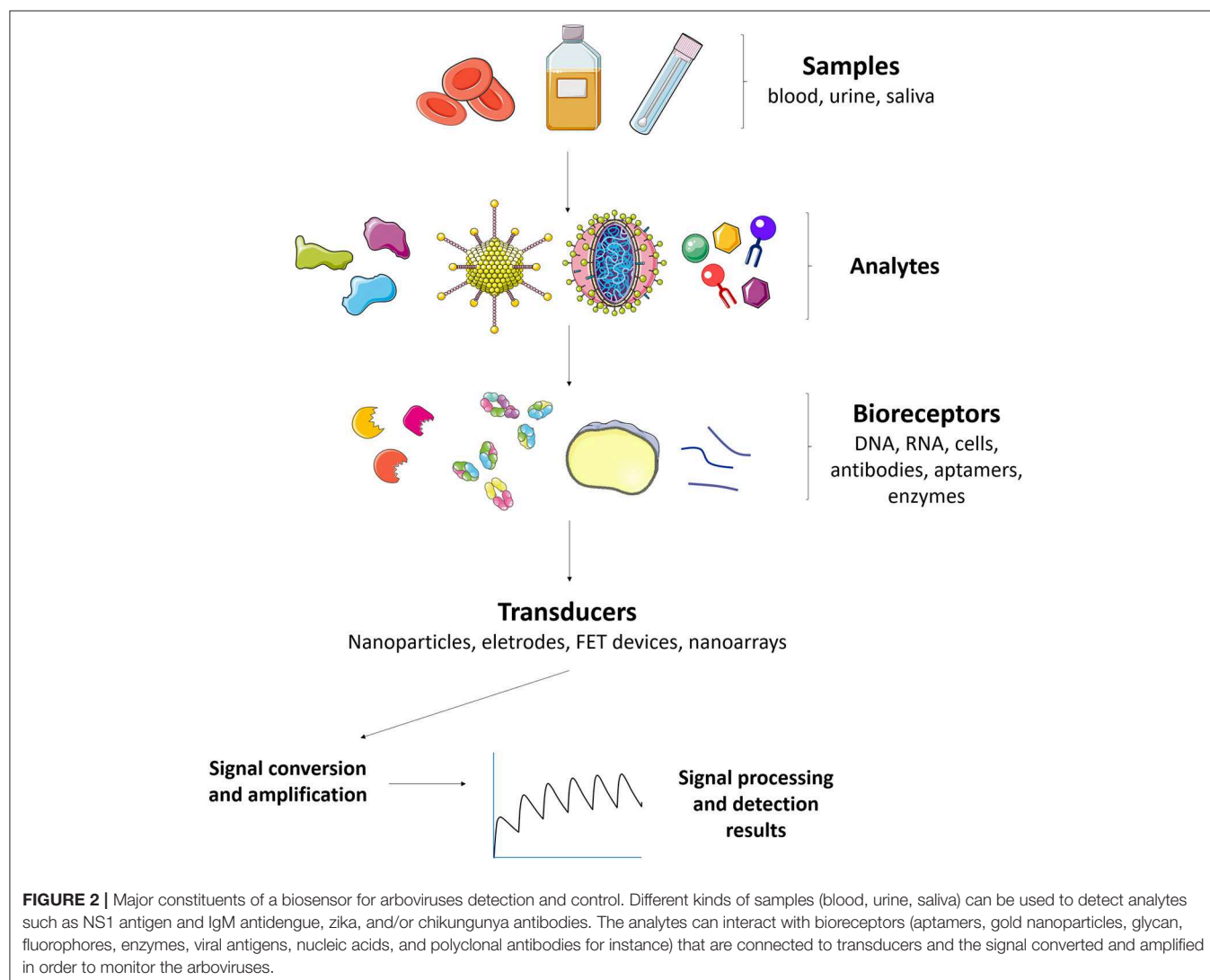
responses by means of a transduction system that acts to convert the biological recognition into a quantifiable electrical signal (**Figure 2**). For the detection of arboviruses, the system must be able to identify low concentrations in complex sample matrices, such as blood, saliva, urine, and serum, without pretreatment or with minimal sample preparation.

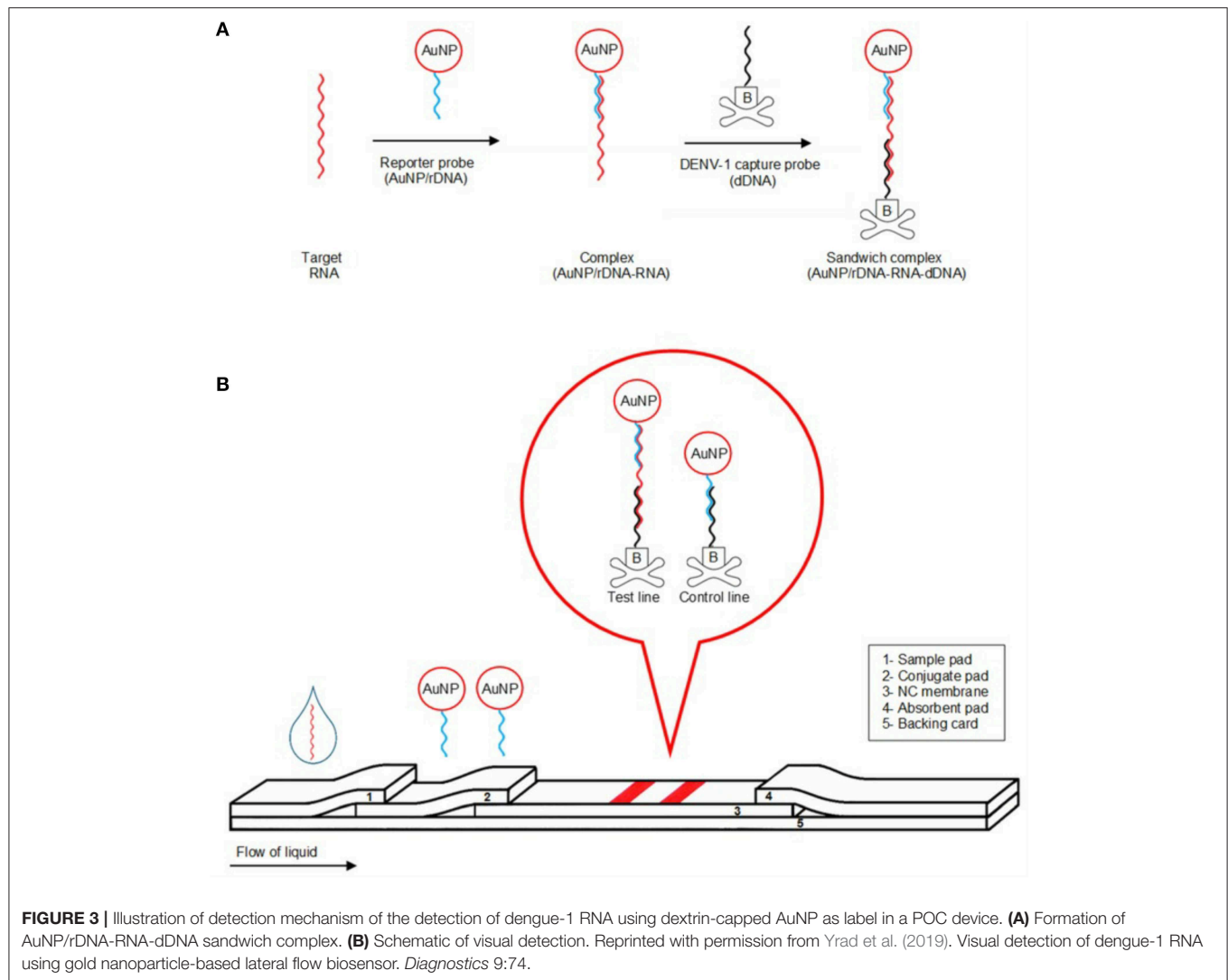
The great advantage of a biosensor is that the bioreceptor interacts specifically with an analyte molecule. The specific interaction causes one or more physicochemical changes (production of ions, colored moieties, electrons, gases, heat, mass, or light) (Sethi, 1994). These responses can then be amplified and transformed into easily interpretable results. For the control and diagnosis of an endemic disease, an ideal biosensor device should be able to detect the arbovirus during all stages of infection, so the device must be designed to carry more than just one bioreceptor (multiplex sensing). Such features can be achieved using lateral-flow assays (LFAs) and lateral flow immunoassay (LFIA).

Several recent studies have used mainly LFAs as the basis for the construction of ASSURED biosensing devices. These assays

can be performed using microfluidic technologies such as paper-based miniaturized devices that combine several recognition steps in a small area for naked-eye detection and quantification of compounds in complex mixtures, with the sample being collected on a test device and the results being displayed in real time (Koczula and Gallotta, 2016). Glucose, urine, and pregnancy test strips are examples of LFA devices, where the fluid containing the sample (blood, saliva, serum, etc.) moves by capillary action through various stages where antibodies and conjugated labels (nanoparticles, for instance) can interact and react to the fluid and, finally, show (sandwich assays) or not (competitive assays) a colored line at the test line position. More detailed information about LFAs can be found at (Sajid et al., 2015; Koczula and Gallotta, 2016; Carrell et al., 2019). **Figure 3** provides a scheme of biosensor based on LFA where a colorimetric lateral flow biosensor (LFB) for the visual detection of dengue-1 RNA using dextrin-capped gold nanoparticle (AuNP) as label can be seen.

The detection of dengue and yellow fever has been performed with a platform for multiplexed pathogen detection employing





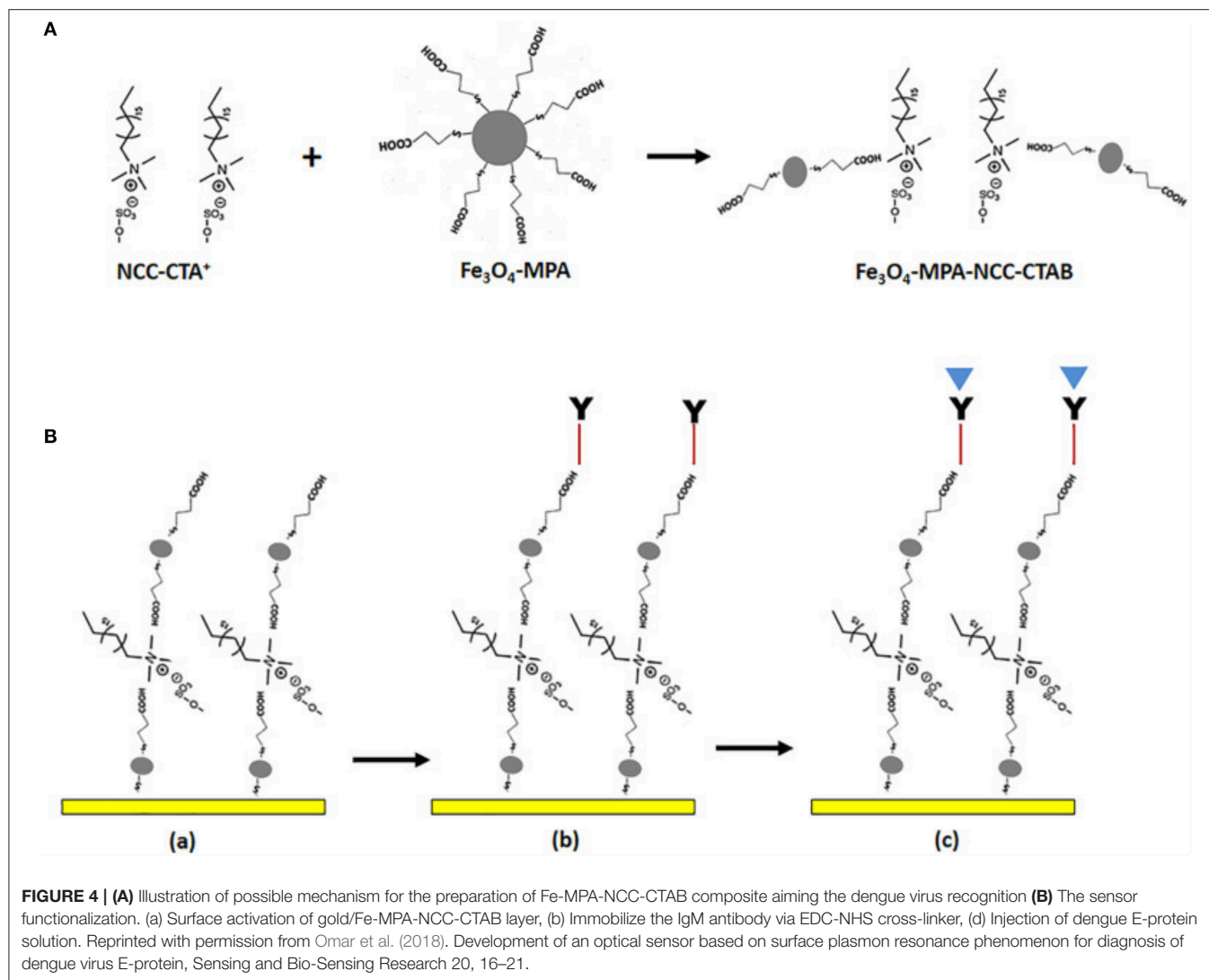
multi-colored silver nanoplates (Yen et al., 2015) as demonstrated by Yen et al. (2015). In this study, the authors showed that the color of test lines could differentiate among different bioreceptors, with the analyses being performed in various ways, including the use of mobile phone applications. Current improvements in LFA technology are associated with the use of nanotechnological tools, such as lab-on-a-chip devices and nanoparticles that change color when aggregated. These improvements have significantly enhanced diagnosis sensitivity and selectivity.

Nawaz et al. (2018) reported a novel method for the detection, classification, and antibody screening of dengue virus, based on electrochemical impedance spectroscopy (EIS), involving protein recognition by means of a self-assembly process based on polymer matrix composites. However, as mentioned previously, it is very important to be able to achieve early detection of arbovirus infection but the biosensors had generally been designed to detect NS-type proteins that are only produced from the fifth day of infection onwards (Nawaz et al., 2018). Omar et al.

(2018) overcame this limitation by designing an optical sensor based on the surface plasmon resonance phenomenon, which was applied to the diagnosis of dengue virus structural E-protein that forms the coat of the host virus itself. This protein can be detected earlier, at the start of the immune system response to the infection (Omar et al., 2018).

Figure 4 shows the schematic immobilization of IgM in gold/Fe-MPA-NCCCTAB (3-mercaptopropionic acid - nanocellulose crystalline/hexadecyltrimethylammonium bromide, respectively)/EDC-N-hydroxysuccinimide (NHS) for early detection of dengue virus E-protein using surface plasmon resonance explored by Omar et al. (2018). By introducing IgM immobilized Fe-MPA-NCC-CTAB/EDC-NHS on a gold surface, it is possible to determine the E-protein concentration in a range of 0.0001–10 nM. The sensitivity found by optical sensor in contact with DENV is $39.96^\circ \text{ nM}^{-1}$ (Omar et al., 2018).

Vinayagam et al. (2018) reported the recognition of serotype-specific DENV employing multicolor triangular silver nanoparticles (TAg), which has the potential to be a



powerful diagnosis technique that is able to differentiate between various serotypes. The color responses were established on the interaction of a TAG-DNA probe with a specific strand, resulting in the creation of a network association between the DNA probe and the dengue virus RNA, according to serotype. This was the first report of DNA conjugated to triangular silver nanoparticles, based on the pH reduction method. This biosensor has not yet been tested using real samples from infected patients, although the proposed technology appears to be very promising for use in clinical POC diagnostic testing (Vinayagam et al., 2018).

Other available methodologies and R&D developments for the improvement and development of biosensing systems for the detection of arboviruses are shown in **Table 1**.

The recent increase in the cases of epidemic infections worldwide (Nicolini et al., 2017b) has also been matched by an increase in the available commercial biosensing devices and diagnostic methodologies. An excellent example is the invention designed by Kaushik and Nair (2018). The device is based on a modeling and electrochemical immunosensing

approach for the detection of Zika virus and offers an extremely low detection limit (picomolar). The biosensor is shaped to be worn on an individual's skin for infection screening. It matches the analyte measurement to the baseline in order to determine if an infection is present. After signal interpretation, the result is transmitted as an alert message. The device can be classified as a point-of-care biosensor, since it complies with the ASSURED features as proposed by the WHO.

The Ulisse Biomed SRL product portfolio includes a biosensor for the determination of an infection, and possible associated infections, caused by several viral pathogens, including Zika, dengue, and chikungunya. The biosensor designed by Braga et al. provides a method for obtaining qualitative and quantitative data related to viral infections. This specific biosensor consists of an antigen (biologically active responsive element) bound covalently to the surface of one or more carbon nanotubes and/or metal nanoparticles present on part of a microelectrode surface, together with

TABLE 1 | Main biosensors developed for the detection of arboviruses, together with their operational principles and applications.

Biosensor type	Disease	LOD*	Detection speed	Detection medium	References
Electrochemical biosensor based on surface imprinted polymers	zika	2×10^{-4} PFU.mL ⁻¹ $2 \times 10^{-3-5} \times 10^{-2}$ PFU.mL ⁻¹	–	Buffer Serum	Tancharoen et al., 2019
Magneto-enzyme LFIA combining super-paramagnetic nanoparticles	DENV-1	0.25 ng.mL ⁻¹	90 min	Serum	Thanh et al., 2019
	DENV-2	0.1 ng.mL ⁻¹			
	DENV-3	0.25 ng.mL ⁻¹			
	DENV-4	1.0 ng.mL ⁻¹			
Nitrogen-doped porous carbon-based fluorescence sensor	zika RNA	0.23 nM	40–80 min	Saliva	Li et al., 2019
Paper-plastic microfluidic hybrid chip integrated with a lateral flow immunoassay	Dengue	84.66 ng.mL ⁻¹	<2 min	Spiked buffer	Yuzon et al., 2019
Trapezoidal SiNWs array fabricated by AFM-LAO	Dengue	2.22 fM	Real-time	DNA	Yusoh et al., 2019
Aptamer–gold nanoparticle conjugates	zika	10 ng	Real-time	<i>A. aegypti</i> salivary gland extract	Bosak et al., 2019
Acrylic-based genosensor (DNA biosensor)	Dengue (DEN-2)	1.21×10^{-16} M	30 min	Blood Urine Saliva	Mazlan et al., 2019
Gold nanoparticle-based lateral flow biosensor	Dengue	0.01 μ M	20 min	Synthetic dengue-1 target	Yrad et al., 2019
		1.2×10^4 PFU.mL ⁻¹		Pooled Human Sera	
Fluorescent lateral flow immunoassay	zika (NS1)	0.045 ng.mL ⁻¹ 0.15 ng.mL ⁻¹	20 min	Buffer Serum	Rong et al., 2019
Localized surface plasmon resonance immunosensors	Dengue (4 serotypes)	10^7 TCID50.mL ⁻¹	<5 min	–	Basso et al., 2018
Two-dimensional MoS ₂ nanosheets-based disposable biosensor (electrochemical detection)	Chikungunya	3.4 nmol.L ⁻¹	<60 min	PBS serum	Singhal et al., 2018
Paper-based DNA biosensor using gold shell-coated magnetic nanocubes	Chikungunya	0.1 nmol.L ⁻¹	–	PBS serum	Singhal et al., 2018
Laser-cut microfluidic device made of glass-fiber paper	Non-structural 1 (NS1) viral protein and specific IgM	25 ng.mL ⁻¹	<10 min	Blood and plasma	Theillet et al., 2018
Graphene-based biosensor employing precise immobilized monoclonal antibody	zika	450 pmol.L ⁻¹	5 min	–	Afsahi et al., 2018
Electrochemical immunosensor	Dengue	0.3 ng.mL ⁻¹	–	Serum	Nawaz et al., 2018
Reverse-transcription LAMP coupled with reverse dot blot	zika	$<2 \times 10^3$ (6 RNA copies per reaction)	Between 3 and 10 min	Saliva	Sabalza et al., 2018
Multiplex tools with target-specific fluorescently tagged strand displaceable probes with RT-LAMP	Dengue	~1.22 PFU equivalent viral RNAs	30 min	Urine and plasma	Yaren et al., 2017
	zika	~0.71 PFU equivalent viral RNAs			
	Chikungunya	~38 copies of viral RNA			
Electrochemical stand with electrospun semi-conducting manganese (III) oxide (Mn ₂ O ₃) nanofibers for DNA hybridization detection	Dengue	120×10^{-21} mol.L ⁻¹	–	Spiked serum	Tripathy et al., 2017
Electrochemical capacitive sensing	Dengue	0.5 ng.mL ⁻¹	–	Serum	Cecchetto et al., 2017

(Continued)

TABLE 1 | Continued

Biosensor type	Disease	LOD*	Detection speed	Detection medium	References
Coupling of reverse-transcription loop-mediated isothermal amplification (RT-LAMP) with the quenching of unincorporated amplification signal reporters (QUASR) technique	zika				
	Chikungunya				
	Dengue	$10^{3.4}$ copies. μL^{-1}	<40 min	Blood, urine, and saliva	Priye et al., 2017
Surface-enhanced Raman spectroscopy (SERS)-based sandwich immunoassays (LFA)	zika	10^5 - 10^2 PFU equivalent.mL $^{-1}$ (10^4 - 10^1 copies per rxn)	7–15 min <40 min		
	Chikungunya				
	zika (NS1)	0.72 ng.mL $^{-1}$	20 min	Serum	Sánchez-Purrà et al., 2017
Bead-based immunofluorescence assay on a microfluidic dielectrophoresis platform	Dengue	7.67 ng.mL $^{-1}$			
	Dengue	10^4 PFU.mL $^{-1}$	5 min	–	Iswardy et al., 2017
	Dengue	50 pg.mL $^{-1}$	15 min	Serum	García et al., 2017
Optical caustic plasmonic light scattering sensor	Dengue	50 pg.mL $^{-1}$	15 min	Serum	García et al., 2017
Carbon nanotube-based chemiresistor functionalized with heparin	Yellow fever	8.4×10^2 TCID 50 .mL $^{-1}$	10 min	–	Wasik et al., 2017
Multiplexed assay on a nanostructured plasmonic gold (pGOLD) platform	Dengue				
	zika (NS1)	0.33 IgG level	120 min	Serum	Zhang et al., 2017
		0.30 IgA level			
Reverse transcription strand invasion-based amplification (RT-SIBA) with fluorescence detection	zika	5,000 copies.mL $^{-1}$	<30 min	Lysis buffer	Eboigbodin et al., 2016
Detection using isothermal amplification, AC susceptometry, and magnetic nanoparticles	zika virus oligonucleotide	1 aM	27 min	Serum	Tian et al., 2016
Reverse-transcription loop-mediated isothermal amplification (RT-LAMP)	zika	50–100 PFU.mL $^{-1}$	40 min	Saliva	Song et al., 2016
Lateral flow assay using multicolored silver nanoparticles	Dengue	150 ng.mL $^{-1}$	–	Blood	Yen et al., 2015
Carbon nanotube-ink printed electrode	Dengue	12 ng.mL $^{-1}$	–	Serum	Dias et al., 2013
Optical DNA biosensor based on square-planar ethyl piperidine substituted nickel (II) salphen complex	Dengue	0.2 mol.L $^{-1}$	30–120 min	Saliva and urine	Ariffin et al., 2013
Microfluidic system combined with microvalves and micropumps for rapid DNA hybridization using shuttle flow	Dengue (4 serotypes)	100 pmol.L $^{-1}$	90 s	–	Huang et al., 2010
Microfluidic chip that accomplish DNA/RNA amplification, sample injection, and separation of nucleic acid products	Dengue	–	<5 min	–	Huang et al., 2010
Sensor-based microchip employing a magnetic bead bioassay platform	Dengue (antidengue virus IgG)	100 pg.mL $^{-1}$	–	–	Aytur et al., 2006

*Limit of detection.

an electrically conducting part where a transduction system (sensor) converts the biochemical response into an electric signal (Braga et al., 2015).

Table 2 lists some of the published patents related to the improvement of biosensing tools and emerging new technologies

for more efficient detection of analytes related to dengue, chikungunya, zika, and yellow fever.

The advancement of technology can show how to improve the ways to detect arboviruses with nano based-tools. However, just a few publications on the use of nano-tools has been

TABLE 2 | Patents related to the detection and control of arboviruses.

Country	Company	Product technology	Year	Patent number	References
United States	Mikrogen, GmbH	Method for the immunological diagnosis of a sample with a potential infection with an arbovirus and test kits suitable for this purpose	2019	US2019/0227065A1	Soutschek et al., 2019
United States	The USA, as represented by the Secretary, Dept. Of health and human services	Compositions and methods for the diagnosis and treatment of zika virus infection	2017	WO2018152496A1	Akahata and Ueno, 2017
United States	University of Central Florida Research Foundation, Inc. (UCFRF)	A payload reservoir comprising an insect attractant or insect food source; and (b) A detector conjugate comprising a gold nanoparticle conjugated to a specific detector molecule that binds specifically to a protein present in the saliva of a specific insect to be detected	2018	US20180231550A	Willenberg and Seal, 2018
United States	The Hong Kong polytechnic university	Microarray design of hybrid upconversion nanoparticles on a nanoporous anodized alumina membrane heterogeneous assay for simultaneous detection of multiple oligonucleotides	2018	US2018/0246084 A1	Hao et al., 2018
United States	Sympano, Inc.	Nano-field electrical Sensor for Biomarkers and other targets analytes by determining impedance in bodily fluid on nanoporous membrane		US2018/0067107 A1	Barrett et al., 2018
China/ United States	Ullisse Biomed SRL	Biosensors for the detection of infection and associated maladies	2017	CN106461667A US20170212116A1	Braga et al., 2015
United States	DexCom, Inc.	Transcutaneous analyte sensor for transcutaneous measurement of glucose in a host	2018	US7654956B2	Brister et al., 2012
Spain	Universidad complutense de Madrid	Biosensor for the detection of nucleic acids	2017	ES2580138B2	Cabarcos et al., 2017
United States	University of Ottawa, University of Malaya	Long-range surface plasmon-polariton biosensor	2018	WO2018090125A1	Berini and Wong, 2018
United States	Florida International University	Electrochemical sensing device based on nano-devices for fast zika Virus detection	2018	US10012645B2	Kaushik and Nair, 2018
United States	San Diego State University (SDSU) Foundation	Cell-based devices for track small molecules that restrain enzymes	2018	US10006077B2	Wolkowicz, 2018
United States	Eccrine Systems, Inc.	Biosensing device aimed to be used in humans skin to track an infection by one or more antigens	2018	WO2018026931A1	Beech et al., 2018
United States	Aviana molecular technologies, LLC	Multiplex acoustical biosensor with higher sensitivity	2011	US20110136262A1	Ragavan et al., 2011
United States	Purdue research foundation	Electrochemical biosensor for RNA and DNA sensing	2017	US20170107565A1	Marinero-Caceres et al., 2017
United States	Aviana molecular technologies, LLC	Biocoated piezoelectric biosensor platform for point-of-care diagnostic use	2015	US20150111765A1	Laury-Kleintop and Rutner, 2015
France/ United States	Cornell Research Foundation, Inc.	Microfluidic biosensor and methods of use	2005	WO2005084404A2	Baeumner et al., 2005
Australia/ United States	Lifepoint Australia Pty Ltd.	Auto-feedback loop biosensor—signal amplification auto-feedback loop for the detection of a target analyte in a sample	2009	WO2009152566A1	Fletcher and Milligan, 2009
United States	UT-Battelle, LLC	Biosensor which has multiple functions and broad spectrum and methods of utilization	2004	USOO6743581B1	Vo-Dinh, 2004

clearly demonstrated. Nanosensors have the potential to be allies in the early detection of *Aedes aegypti*-borne diseases by offering novel approaches to achieve sensitive, specific, and stable recognition in complex matrices in quick or real-time diagnostics. Although some variations in synthesis protocols can still be tested to improve the productivity and efficiency of nanomaterials for diagnostic applications, more research on the way lab-to-practical nanodiagnostics is needed. Not only more research must be done on nanosensors for *A. aegypti*-borne diseases, but the upscale of the applicability is urgently necessary, as shown by the few patents filed for this purpose (Table 2). One of the ways for this accomplishment is to narrow the scientific boundaries between disciplines such as chemistry, sociology, bio, and nanotechnology and information technology. A good example that results from this new multidisciplinary approach is the smartphones-based POC devices, as previously cited. They are a real and promising novel tool for flaviviruses detection without complex instruments, since, in a simple way, the blood sample can be analyzed under 40 min (Priye et al., 2017; Rong et al., 2019). Despite the advances, still, there is an urgent need for proper and precise use of nanosensors in hospitals, field, and to prevent these potential health risk diseases.

Insect Repellents

The development of new nanotechnology-based formulations for the encapsulation of natural and synthetic repellents is an important strategy for obtaining systems that are more effective and have fewer undesirable impacts. These sustained-release formulations provide controlled or slow release of active agents into the environment, increasing the duration of action and reducing human exposure to the agent (for example, by permeation through the skin). Encapsulation also protects the active agent against premature degradation caused by the effects of light, temperature, oxidation, and humidity, among others (Tavares et al., 2018). Numerous matrices (synthetic and natural) can be used for the preparation of carrier systems, including polymers, proteins, lipids, polysaccharides, and others. It should be noted that the main desirable characteristics of such matrices are biocompatibility and biodegradability, as well as low cost (Barradas et al., 2016).

Gomes et al. encapsulated DEET in polymeric nanospheres, resulting in particles with an average diameter of 114 ± 37 nm, low polydispersion index, and stability as a function of time. The sustained release of nanoencapsulated DEET provided repellency for over 9 h, which was longer than obtained using free DEET. The results showed that the release mechanism was temperature dependent, which the authors highlighted as having great potential, since the release rate could be adjusted by alteration of temperature (Gomes et al., 2018).

Silva et al. (2019) encapsulated essential oils of *Piper aduncum* L. and *Piper hispidinervum* in gelatin nanoparticles and evaluated effect against *Aedes aegypti* Linn. Results showed a high encapsulation efficiency of the EOs (around 80%), average size around 100–200 nm, zeta potential around -40 mV. Both encapsulated EOs reached lethal dosages within 24 h of exposure and total mortality of the tested pests (Silva et al., 2019).

Forgearin et al. prepared and characterized permethrin-loaded lipid nanocapsules and tested their application as repellents in clothes. The formulations presented a mean particle diameter of 201 ± 4 nm, with a monomodal size distribution and permethrin content of 4.6 ± 0.1 mg/mL. It was observed that even after washing and with the action of temperature, the polyester fabrics containing the nanoparticles had higher concentrations of permethrin, compared to those containing only the free compound. The results showed that the innovative repellent spray composed of the nanoparticle formulation was useful for the impregnation of clothes and was promising for the protection of an individual against insects (Forgearini et al., 2016).

Werdin González et al. prepared and characterized polymeric nanoparticles (composed of PEG and chitosan) for the encapsulation of essential oils (geranium and bergamot). Evaluation was also made of the acute and residual larvicidal activities of the formulations against *Culex pipiens*. Physicochemical characterization showed that the PEG nanoparticles containing the essential oils had a mean size of < 255 nm and provided encapsulation efficiencies between 68 and 77%, while the chitosan nanoparticles presented a mean size of < 535 nm and encapsulation efficiencies between 22 and 38%. Both systems showed high larvicidal activity (acute and residual), with the chitosan-based formulations having the best effects. These findings demonstrated the potential of polymeric nanoparticles containing essential oils for use as eco-friendly larvicidal products (Werdin González et al., 2017).

Silva et al. studied the encapsulation of the essential oils of *Piper aduncum* L. and *Piper hispidinervum* C. in gelatin nanoparticles, with evaluation of the biological effects against *Aedes aegypti*. The encapsulation efficiencies exceeded 80%, and the particles were spherical, monodispersed, and smaller than 100 nm in size. Both of the encapsulated essential oils provided lethal effects within 24 h of exposure, with *Aedes aegypti* mortality greater than 80% (Silva et al., 2019).

It should be noted that the search for new formulations has also led to patenting. For example, patent **BR1020180168665** describes the preparation and characterization of nanostructured lipid carriers (NLCs) and nanoemulsions containing citronella and neem oil, for the control of insects such as *Aedes aegypti*. The results showed that both the nanoemulsions and the NLCs loaded with the essential oils caused 100% mortality of *A. aegypti* larvae during the first day of exposure, while the NLCs without essential oil induced 100% mortality after 10 days of exposure. Both carriers showed satisfactory efficacy in the control of *A. aegypti* larvae (Fraceto et al., 2018).

The patent **WO2017143421A1** describes the invention of cosmetic formulations for use as topical insect repellents, employing polymeric micelles, nanoemulsions, and solid lipid nanoparticles containing active repellent substances. Nanoencapsulation techniques were used to produce systems consisting of nanostructures with stable hydrophobic and hydrophilic chains. The formulations presented sustained release of the active substances, consequently providing long duration of action of the repellents, together with greater safety (Paula et al., 2017).

The patent **US20190160016A1** present an invention to a nanoparticle composition comprising one or more β -triketones selected from *Leptospermum scoparium* botanical extract. The prepared system presented high insecticidal activity against ectoparasites, and according to the inventors can also be applied to repel insects like *Aedes aegypti* (Thomas, 2019).

Table 3 presents other studies concerning the use of formulations based on micro/nanotechnology for the encapsulation of compounds (natural and synthetic) presenting repellent activity.

DEET is currently considered a “gold standard” due to its outstanding protection against mosquitoes and other biting insects. It is the most common active ingredient in all commercially available repellents and is used as a comparative for other substances (Khater et al., 2019). However, due to indiscriminate use has suffered resistance effects, leading to loss of formulations effectiveness. In addition, due to its toxicity has raised health and environmental concerns. Thus, the search for natural alternative repellents as well as new molecules is necessary. This is the case of compound IR3535, one of the newest products with odorless and non-toxic characteristics, which is recommended for children over 6 months of age and pregnant women (Benelli et al., 2018a).

It is in this scenario of innovation and search for new solutions that nanotechnology applies. It is becoming increasingly necessary to develop formulations that increase the repellent's longevity by controlling delivery and evaporation rate. In addition, the different applications forms such as sprays, creams, lotions, aerosols, oils, adhesives, protective clothing, treated nets, among others, is very important in order to ensure options for people living in endemic areas (Tavares et al., 2018; Agnihotri et al., 2019). Thus, encapsulation in micro/nanoparticles, cyclodextrins, micelles, hydrogels among others constitutes an approach to modify the physicochemical properties of encapsulated repellents. When applied in topical formulations or in personal protective clothes, for example, they have been shown to be more effective in increasing repellency time and also in reducing dermal absorption, improving the safety profiles of these products (Ahmed et al., 2019; Osanloo et al., 2019). Innovative nanotechnology-based formulations should be followed by safety and efficacy studies, as this will increase consumer confidence in this new formulations (Hameed et al., 2019).

Larvicidal and Ovicidal Nanoparticles

The green or biological synthesis of nanoparticles, also called biogenic synthesis, can offer advantages over the classical nanotechnological techniques currently employed. The new synthesis methods are economical, fast, and less expensive, and are performed at ambient temperature and pressure. In contrast, standard physical and chemical techniques typically involve high energy consumption, due to the need for high pressures and temperatures (Benelli et al., 2018b). In addition, potentially harmful reagents and solvent are not used in green synthesis methods, because the reducing and stabilizing agents are substituted by molecules produced by living organisms (Kumar et al., 2015). These agents can be extracted from

bacteria, fungi, yeasts, algae, or plants (Sintubin et al., 2012). The technique can be used to produce nanoparticles composed of metals, metal oxides, silica, and carbon (Benelli et al., 2016).

Recent studies have reported the synthesis of various nanoparticles using different natural extracts, with demonstration of the larvicidal, ovicidal, and mosquitocidal activities of these nanoparticles. Udayabhanu et al. observed the larvicidal effect of titanium dioxide nanoparticles (TiO_2 NPs) synthesized using an aqueous extract of *Euphorbia hirta* leaves against the larvae of *Aedes aegypti* ($\text{LC}_{50} = 13.2 \text{ mg/L}$) and *Culex quinquefasciatus* ($\text{LC}_{50} = 6.89 \text{ mg/L}$) (Udayabhanu et al., 2018).

Another study proposed the green synthesis of Ag NPs for use as an environmentally-friendly alternative to pyrethroid and carbamate larvicides. Silver nanoparticles synthesized from extracts of the *Quisqualis indica* plant showed high toxicity against the vectors of filariasis, zika virus, and malaria. In addition, toxicity tests employing three non-target organisms indicated low toxicity of these systems (Govindarajan et al., 2016).

The patent databases include inventions that describe methods of green synthesis of metal nanoparticles and metal oxides, with broad applications in consumer products, medicines, pharmaceuticals, and other biomedical products (Hoag et al., 2011; Liu et al., 2013; Yujia et al., 2015; Awad et al., 2016). There have been several reports concerning vector control using biogenic nanoparticles (**Table 4**), indicating the promising potential of these nanoparticles that are both environmentally-friendly and highly effective for vector control.

GAPS, OBSTACLES, AND CONCLUSIONS

The potential application of nano-based formulations in the field of arboviruses management was investigated by analyzing the number of publications in the last ten years. The results revealed that around 1000 articles were published worldwide, which an exponential trend in publication numbers after 2016, mainly for researches related to zika and chikungunya. Overall, many publications have shown that nanotechnology has led to rapid advancements in the development of pesticides, repellents, drug delivery system and diagnostic devices for arboviruses management. However, the clinical translational of nano-based formulations has some bottlenecks that hinders the broad acceptance and commercialization of these products (Hua et al., 2018; Soares et al., 2018). Also, it's already known, that nanomaterials, independently of their composition and method of production, have new properties, which are not observed by their bulk materials (Laux et al., 2018). These novel properties have brought innovative solutions due their flexibility, responsiveness and possibility of functionalization, the last one, which is very useful for drug targeting delivery (Jeevanandam et al., 2018). However, the behavior, fate, bioavailability nanomaterials in the environment and toxicity of non-target organisms should be better understood the possible environmental impacts (Dinda, 2018).

Much debate still exists regarding the legislation of nanomaterials, which is in an early stage of development.

TABLE 3 | Formulations based on modified release systems for the encapsulation of active agents with insect repellent properties.

Systems	Matrices	Active agents	Main characteristics	References
Polymeric nanoparticles	Poly(ethylene glycol) (PEG)	Diethylphenylacetamide (DEPA)	Diameter: 149 ± 1.06 nm; Properties: 5-fold decrease of median lethal indices (LC_{50}), compared to free DEPA	Balaji et al., 2017
Polymeric nanospheres	Poly(n-butyl methacrylate-co-methyl methacrylate)	N,N-diethyl-m-toluamide (DEET)	Diameter: 114 ± 37 nm. Properties: release rate of the encapsulated DEET provides repellency for over 9 h and is more controlled when compared to the free DEET	Gomes et al., 2018
Nanoemulsion	Polaxamer 407	Ethyl butylacetylaminopropionate (IR3535)	Diameter: ± 200 nm; Properties: Nanoemulsion less retained by the epidermis and not toxic to the cells	Pinto et al., 2017
Gel/Nanoparticle	Chitosan	<i>Zanthoxylum acanthopodium</i> essential oil (ZA EO)	Encapsulation efficiency of 96.64%; Properties: Reduction in essential oil permeation in <i>in vitro</i> membrane study and mosquito repellent activity against <i>Aedes aegypti</i> with protection time of 2 h	Sharma, 2019
Polymeric nanoparticles	Polyethylene glycol	Quercetin	Diameter: 124.0 ± 1.1 nm; Properties: Stability at 4°C, affected larval <i>Aedes aegypti</i> development, less toxic than non-encapsulated quercetin toward <i>C. vulgaris</i> (green alga)	Pessoa et al., 2018
Polymeric micelles	PEG and PLGA	Pyrethrins	Diameter: 140–320 nm. Properties: Protection against ultraviolet degradation (at 26°C) and high larvicidal activity against <i>Culex pipiens pallens</i>	Zhang et al., 2018
Polymeric microparticles	Gum arabic	Essential oils and DEET	Diameter: 1–68 nm; Properties: Spherical shapes and cotton fabric impregnated with system presentend better insect repellency, compared to DEET	Eyupoglu et al., 2018
Nanoparticles	Chitosan	<i>Siparuna guianensis</i> essential oil	Diameter: 268 ± 3.4 nm; Encapsulation efficiency 84.8–88.0% Properties: 100% mortality during the first week and provides Against <i>Aedes aegypti</i> mosquito larvae	Ferreira et al., 2019
Polymeric nanoparticles	Polaxamer 407	Eugenol, 1,8-cineole, geraniol, linalool, carvacrol, α -terpineol, citronellol, thymol, and menthol	Diameter: Around 40 nm; Properties: Mortalities ranging from 30 to 60% against insects with linalool and 1,8-cineole being most effective	Lucia et al., 2017
Polymeric microparticles	Cellulose	N,N-diethyl-m-toluamide (DEET)	Encapsulation efficiency of 98%. Properties: A significant reduction in release rate of DEET	Kadam et al., 2019
Inclusion complexes	β -Cyclodextrin	<i>Lippia gracilis</i> essential oil	Inclusion complex formation by kneading and co-evaporation with essential oil content $\sim 15\%$; Properties: LC_{50} of 33 ppm toward <i>Aedes aegypti</i> larvae and inclusion complex was not harmful to non-target organisms	Galvão et al., 2018
Polymeric microparticles	Carboxy-methylcellulose (CMC)	Essential oils (<i>Alpinia galanga</i> , <i>Citrus grandis</i> , and <i>C. aurantifolia</i>), and DEET	Diameter: 4–200 μ m. Properties: The same period of repellent activity for essential oil encapsulated in comparison with microencapsulated DEET. Extended duration of repellent activity (between 1 and 2 h) compared with commercial formulations	Misni et al., 2017
Nanoemulsion	Tween 80	<i>Vitex negundo</i> L. essential oil	Diameter: < 200 nm; Properties: Nanoemulsion with higher larvicidal activity (<i>Aedes aegypti</i>) compared with only essential oil;	Balasubramani et al., 2017
Nanoemulsion	Tween 80	<i>Ocimum sanctum</i> essential oil	Diameter: 50–300 nm; Properties: Nanoemulsion with potential insecticidal effect against <i>Aedes aegypti</i> and <i>C. quinquefasciatus</i> adults	Ramar et al., 2017
Nanofibrous	Cellulose	Citriodiol (CD)	Properties: Nanofibrous presented more prolonged repellency (34 days) than monolithic ones in experiments using <i>Aedes aegypti</i>	Muñoz et al., 2019

In addition, there is a lack of a clear definition of nanomaterials, lack of standard methods for assessment of pharmacology, toxicology and efficacy evaluation of nano-based formulations and lack of worldwide network for gathering and sharing pertinent information. According to Schnell-Inderst et al. (2018) 12% of the documents related to the test of medical devices are written by academics and the regulators write

the rest. In addition, there is a low number of experts in nanomaterials in regulatory agencies, resulting in the delayed development of these documents. Another issue is that FDA use the traditional regulatory frameworks to approve products, which contains nanomaterials (Jones et al., 2019). Nano-based formulations are evaluated by FDA using case-by-case approach, through combination product framework

TABLE 4 | Biogenic nanoparticles tested for the control of disease vectors.

Name	Action	Organism species	LC ₅₀ / LC ₉₀ in <i>A. aegypti</i>	Treatment time	References
AgNP	Larvicidal property against fourth instar larvae of <i>Aedes aegypti</i>	Apple extract	AgNPs -T 15.76/27.7 ppm AgNPs -RT 29.81/42.3 ppm	24 h	Ali et al., 2017
AgNP	Ovicidal activity against <i>Aedes aegypti</i>	<i>Bauhinia acumiata</i> leaf powder aqueous extract	27.19/52.32 µg.mL ⁻¹	24 h	Alharbi et al., 2018
AgNP	Larvicidal activity against <i>Aedes aegypti</i> , <i>Anopheles stephensi</i> , and <i>Culex quinquefasciatus</i>	Leaf extracts of <i>Leucas aspera</i> and <i>Hyptis suaveolens</i>	4.02/11.22 mg.mL ⁻¹	24 h	Elumalai et al., 2017
AgNP	Ovicidal activity against <i>A. aegypti</i> eggs	<i>Holarrhena antidysenterica</i> bark extract	5.53/12.01 ppm	72 h	Kumar et al., 2018
ZnONP	Larvicidal and ovicidal activities against <i>Aedes aegypti</i>	<i>Scadoxus multiflorus</i> leaf powder aqueous extract	34.04/78.06 ppm	24 h	Al-Dhabi and Valan Arasu, 2018
ZnONP	Larvicidal activity against of <i>Aedes aegypti</i> (4th instar)	Extract of the seaweed <i>Ulva lactuca</i>	22.38/41.94 µg.mL ⁻¹	24 h	Ishwarya et al., 2018
ZnONP	Larvicidal activity against fourth instar of <i>Aedes aegypti</i>	<i>Petalium murex</i> seed extract	34.88/64.56 µg.mL ⁻¹	24 h	Ishwarya et al., 2017
AgNP	Larvicidal property against <i>Anopheles stephensi</i> and <i>A. aegypti</i>	<i>Belosynapsi Kewensis</i> leaf extract	84.2/117.3 ppm	24 h	Bhuvaneswari et al., 2016
AgNP	Potential larvicidal activity against larvae of <i>Aedes aegypti</i> (3rd instar), <i>Anopheles stephensi</i> , and <i>Culex quinquefasciatus</i>	Aqueous leaf extract of <i>Heliotropium indicum</i>	72.72/126.86 µg.mL ⁻¹	24 h	Veerakumar et al., 2014
AgNP	Larvicidal activity against third and larvae of <i>Aedes aegypti</i> (4th instar)	Leaf extract of <i>Derris trifoliata</i>	3rd instar: 7.0/17.76 mg.mL ⁻¹ 4th instar: 5.87/12.11 mg.mL ⁻¹	24 h	Kumar et al., 2017
AgNP	Larvicidal activity against 1st–4th instar larvae dengue vector	3,5 di-t-butyl-4 hidroxyanisole isolated from <i>Cynodon dactylon</i> leaf	1st–4th instar: 2.5; 2.78; 3.02; 3.05/8.28; 7.47; 8.13; 8.74 µg.mL ⁻¹	24 h	Ramanibai and Velayutham, 2016
AgNP	Larvicidal and pupicidal against <i>Aedes aegypti</i> and <i>Anopheles stephensi</i>	Aqueous leaf filtrate from <i>Artemisia nilagirica</i>	1st–4th instar: 0.46; 0.35; 0.33; 0.21% Pupa: 0.16% LC ₉₀ : N/A	24 h	Nalini et al., 2017

N/A- data not provided by the autor; LC₅₀, Lethal Concentration that kills 50% of the exposed larvae; LC₉₀, Lethal Concentration that kills 90% of the exposed larvae; AgNPs—T, prepared by heating; AgNPs—RT, prepared by non-heating.

in order to determine regulatory framework will be used. Three main challenges should be addressed by FDA to boost the market of nano-based formulations: (i) development of a regulatory framework specific to nanomaterials; (ii) development of methods capable of characterize and quantify the toxicological impacts of nanomaterials, and (iii) deal with public acceptance and understanding through awareness programs and product labeling (Hua et al., 2018; Jones et al., 2019).

Also, economic issues can limiting the broad commercialization of nano-based formulations (Ventola, 2017; Jones et al., 2019). In comparison to conventional therapies, the production of a nano-based formulations required higher initial investments that will only be worth it from a business perspective if brings good opportunities for the pharmaceutical company, justifying their investment of R&D sector and reduce substantially health care costs (Benelli et al., 2017; Hua et al., 2018). Future research should explore the possibility of (i)

determine the mechanism of action of nano-based formulations; (ii) understand the behavior and interaction of nanomaterial in complex biological matrix, such as human body; (iii) evaluate the possible toxicity and residual effects of nanomaterials in both target and non-target organisms; (iv) development of a international legislation; (v) create a standard definition of nanotechnology and nanomaterials, and (vi) standardized methods for establishing the risk-benefit of nanomaterials, in order to create large scale production of nanodevices to fight against of arboviruses as suggested in literature (Parisi et al., 2015; Kah et al., 2018a,b).

As conclusion, as indicated in this review, it is important to learn and appreciate the great potential offered by nanotechnology in relation to the development of new and more efficient tools and products. In this regard, it is extremely important that ongoing research involves all sectors: academia, industries, research centers, and government agencies, in order to turning this technology into a sustainable commercial reality and

REFERENCES

- Alfahi, S., Lerner, M. B., Goldstein, J. M., Lee, J., Tang, X., Bagarozzi, D. A., et al. (2018). Novel graphene-based biosensor for early detection of Zika virus infection. *Biosens. Bioelectron.* 100, 85–88. doi: 10.1016/j.bios.2017.08.051
- Agnihotri, A., Wazed Ali, S., Das, A., and Alagirusamy, R. (2019). "11 - Insect-repellent textiles using green and sustainable approaches," in *The Impact and Prospects of Green Chemistry for Textile Technology The Textile Institute Book Series*, eds S. Islam and B. S. Butola (Woodhead Publishing), 307–325. doi: 10.1016/B978-0-08-102491-1.00011-3
- Ahmed, T., Hyder, M. Z., Liaqat, I., and Scholz, M. (2019). Climatic conditions: conventional and nanotechnology-based methods for the control of mosquito vectors causing human health issues. *Int. J. Environ. Res. Public Health* 16:3165. doi: 10.3390/ijerph16173165
- Akahata, W., and Ueno, R. (2017). *Zika Virus Virus Like Particle*. Available online at: <https://patents.google.com/patent/WO2017150683A1/en> (accessed November 14, 2018).
- Al-Dhabi, N. A., and Valan Arasu, M. (2018). Environmentally-friendly green approach for the production of zinc oxide nanoparticles and their anti-fungal, ovidical, and larvicidal properties. *Nanomaterials* 8:500. doi: 10.3390/nano8070500
- Alharbi, B. S., Govindarajan, M., Kadaikunnan, S., Khaled, J. M., Almanaa, T. N., Alyahya, S. A., et al. (2018). Nanosilver crystals capped with *Bauhinia acuminata* phytochemicals as new antimicrobials and mosquito larvicides. *J. Trace Elem. Med. Biol.* 50, 146–153. doi: 10.1016/j.jtemb.2018.06.016
- Ali, Z. A., Roslan, M. A., Yahya, R., Wan Sulaiman, W. Y., and Puteh, R. (2017). Eco-friendly synthesis of silver nanoparticles and its larvicidal property against fourth instar larvae of *Aedes aegypti*. *IET Nanobiotechnol.* 11, 152–156. doi: 10.1049/iet-nbt.2015.0123
- Ariffin, S. A. B., Adam, T., Hashim, U., Faridah Sfaridah, S., Zamri, I., and Uda, M. N. A. (2013). Plant diseases detection using nanowire as biosensor transducer. *Adv. Mat. Res.* 832, 113–117. doi: 10.4028/www.scientific.net/AMR.832.113
- Awad, M. A. G., Hendi, A. A., Wagealla, M. A. E., Ortashi, K. M. O., and Virk, P. (2016). *Synthesis of Nanoparticles of Metals and Metal Oxides Using Plant Seed Extract*. Available online at: [https://patents.google.com/patent/US9428399B1/en?q=green+\\$+synthesis&q=%22silver+\\$+nanoparticle%22&oq=green+\\$+synthesis\\$\\$\\$%22silver+\\$+nanoparticle%22\\$+\\$](https://patents.google.com/patent/US9428399B1/en?q=green+$+synthesis&q=%22silver+$+nanoparticle%22&oq=green+$+synthesis$$$%22silver+$+nanoparticle%22$+$) (accessed October 18, 2018).
- Aytur, T., Foley, J., Anwar, M., Boser, B., Harris, E., and Beatty, P. R. (2006). A novel magnetic bead bioassay platform using a microchip-based sensor for infectious disease diagnosis. *J. Immunol. Methods* 314, 21–29. doi: 10.1016/j.jim.2006.05.006

to create alternatives for detection and control of *Aedes aegypti*-borne diseases.

AUTHOR CONTRIBUTIONS

EC, JO, and LF proposed the structure of the manuscript. EC, JO, DA, CR, CB, VM, and RM wrote the manuscript. EC and LF revised the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors are grateful for financial support provided by MCTIC-CNPq/MEC-CAPES/MS-Decit/FNDCT N° 14/2016–Prevenção e Combate ao vírus Zika—Processo CNPq: 440350/2016-6 e Processo CAPES: 88881.130834/2016-01. The authors thank the São Paulo State Research Foundation (FAPESP) for scholarships awarded to EC (#2017/24402-1), DA (#2018/14743-0), and CR (2018/02404-5).

- Baumner, A. J., Kwakye, S. D. S., Goral, V. N., and Zaytseva, N. V. (2005). *Microfluidic Biosensor and Methods of Use*. Available online at: <https://patents.google.com/patent/WO2005084404A2/en?q=WO2005084404A2> (accessed November 14, 2018).
- Balaji, A. P. B., Ashu, A., Manigandan, S., Sastry, T. P., Mukherjee, A., and Chandrasekaran, N. (2017). Polymeric nanoencapsulation of insect repellent: Evaluation of its bioefficacy on *Culex quinquefasciatus* mosquito population and effective impregnation onto cotton fabrics for insect repellent clothing. *J. King Saud University* 29, 517–527. doi: 10.1016/j.jksus.2016.12.005
- Balasubramani, S., Rajendhiran, T., Moola, A. K., and Diana, R. K. B. (2017). Development of nanoemulsion from *Vitex negundo* L. essential oil and their efficacy of antioxidant, antimicrobial and larvicidal activities (*Aedes aegypti* L.). *Environ. Sci. Pollution Res.* 24, 15125–15133. doi: 10.1007/s11356-017-9118-y
- Barradas, T., Perdiz Senna, J., Ricci Júnior, E., and Regina Elias Mansur, C. (2016). Polymer-based drug delivery systems applied to insects repellents devices: a review. *Curr. Drug Deliv.* 13, 221–235. doi: 10.2174/1567201813666151207110515
- Barrett, T. W., Gautam, S., Mitra, R., and Thorne, J. (2018). *Nano-Field Electrical Sensor*. U.S. Patent No 20180067107A1. Available online at: <https://patents.google.com/patent/US20180067107A1/en?q=Nano-field&q=electrical&q=Sensor&q=Biomarkes&q=targets&q=analytes&q=determining&q=impedance&q=bodily+fluid&q=nanoporous+membrane&q=Nano-field+electrical+Sensor+for+Biomarkes+and+other+targets+analytes+by+determining+impedance+in+bodily+fluid+on+nanoporous+membrane> (accessed February 12, 2020).
- Basso, C. R., Tozato, C. C., Crulhas, B. P., Castro, G. R., Junior, J. P. A., and Pedrosa, V. A. (2018). An easy way to detect dengue virus using nanoparticle-antibody conjugates. *Virology* 513, 85–90. doi: 10.1016/j.virol.2017.10.001
- Batool, K., Alam, I., Wu, S., Liu, W., Zhao, G., Chen, M., et al. (2018). Transcriptomic analysis of *aedes aegypti* in response to mosquitoicidal *Bacillus thuringiensis* LLP29 toxin. *Sci. Rep.* 8:12650. doi: 10.1038/s41598-018-30741-x
- Beech, R., WHITE, E., and Katchman, B. (2018). *Biosensor Disease and Infection Screening*. Available online at: <https://patents.google.com/patent/WO2018026931A1/en?q=WO2018026931A1> (accessed November 14, 2018).
- Benelli, G. (2016). Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. *Parasitol. Res.* 115, 23–34. doi: 10.1007/s00436-015-4800-9
- Benelli, G., Caselli, A., and Canale, A. (2017). Nanoparticles for mosquito control: challenges and constraints. *J. King Saud Univ.* 29, 424–435. doi: 10.1016/j.jksus.2016.08.006
- Benelli, G., Jeffries, C. L., and Walker, T. (2016). Biological control of mosquito vectors: past, present, and future. *Insects* 7:52. doi: 10.3390/insects7040052

- Benelli, G., Maggi, F., Pavea, R., Murugan, K., Govindarajan, M., Vaseeharan, B., et al. (2018a). Mosquito control with green nanopesticides: towards the one health approach? A review of non-target effects. *Environ. Sci. Pollut. Res.* 25, 10184–10206.
- Benelli, G., Maggi, F., Pavea, R., Murugan, K., Govindarajan, M., Vaseeharan, B., et al. (2018b). Mosquito control with green nanopesticides: towards the one health approach? A review of non-target effects. *Environ. Sci. Pollut. Res. Int.* 25, 10184–10206. doi: 10.1007/s11356-017-9752-4
- Benelli, G., and Mehlhorn, H. (2016). Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitol. Res.* 115, 1747–1754. doi: 10.1007/s00436-016-4971-z
- Berini, P. S. J., and Wong, W. R. (2018). *Long-Range Surface Plasmon-Polariton Biosensor*. Available online at: [https://patents.google.com/patent/WO2018090125A1/fr?oq=W2018090125A1\\$+\\$\[Accessed\\$+\\$November\\$+\\$14%2c\\$+\\$2018](https://patents.google.com/patent/WO2018090125A1/fr?oq=W2018090125A1$+$[Accessed$+$November$+$14%2c$+$2018 (accessed January 15, 2020).) (accessed January 15, 2020).
- Bhuvaneswari, R., Xavier, R. J., and Arumugam, M. (2016). Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (*Anopheles stephensi* and *Aedes aegypti*). *J. King Saud Univ. Sci.* 28, 318–323. doi: 10.1016/j.jksus.2015.10.006
- Bosak, A., Saraf, N., Willenberg, A., Kwan, M. W. C., Alto, B. W., Jackson, G. W., et al. (2019). Aptamer-gold nanoparticle conjugates for the colorimetric detection of arboviruses and vector mosquito species. *RSC Adv.* 9, 23752–23763. doi: 10.1039/C9RA02089F
- Braga, L., Marini, B., and Ippodrino, R. (2015). *Biosensors for the Detection of Infection and Associated Maladies*. Available online at: [https://patents.google.com/patent/WO2015114506A3/en](https://patents.google.com/patent/WO2015114506A3/en (accessed November 14, 2018).) (accessed November 14, 2018).
- Brister, M., Neale, P. V., Saint, S., Petisce, J. R., McGee, T. F., Codd, D. S., et al. (2012). *Transcutaneous Analyte Sensor*. Available online at: [https://patents.google.com/patent/US8229534B2/en](https://patents.google.com/patent/US8229534B2/en (accessed November 14, 2018).) (accessed November 14, 2018).
- Cabarcos, E. J. L., González, D. M., Laurenti, M., Cristóbal, P. A., and Retama, J. R. (2017). *Biosensor for the Detection of Nucleic Acids, Preparation Method and Use*. Available online at: [https://patents.google.com/patent/ES2580138A1/en](https://patents.google.com/patent/ES2580138A1/en (accessed November 14, 2018).) (accessed November 14, 2018).
- Carrell, C., Kava, A., Nguyen, M., Menger, R., Munshi, Z., Call, Z., et al. (2019). Beyond the lateral flow assay: a review of paper-based microfluidics. *Microelectron. Eng.* 206, 45–54. doi: 10.1016/j.mee.2018.12.002
- Cecchetto, J., Fernandes, F. C. B., Lopes, R., and Bueno, P. R. (2017). The capacitive sensing of NS1 Flavivirus biomarker. *Biosens. Bioelectron.* 87, 949–956. doi: 10.1016/j.bios.2016.08.097
- Dias, A. C. M. S., Gomes-Filho, S. L. R., Silva, M. M. S., and Dutra, R. F. (2013). A sensor tip based on carbon nanotube-ink printed electrode for the dengue virus NS1 protein. *Biosens. Bioelectron.* 44, 216–221. doi: 10.1016/j.bios.2012.12.033
- Dinda, S. C. (2018). “Bioaccumulation and toxic profiling of nanostructured particles and materials,” in *Unraveling the Safety Profile of Nanoscale Particles and Materials - From Biomedical to Environmental Applications*, eds A. F. de Castro Gomes (University of Minho). doi: 10.5772/intechopen.74802
- Durán, N., Islan, G. A., Durán, M., and Castro, G. R. (2016). Nanobiotechnology Solutions against *Aedes aegypti*. *J. Braz. Chem. Soc.* 27, 1139–1149. doi: 10.5935/0103-5053.20160122
- Eboigbodin, K. E., Brummer, M., Ojalehto, T., and Hoser, M. (2016). Rapid molecular diagnostic test for Zika virus with low demands on sample preparation and instrumentation. *Diagn. Microbiol. Infect. Dis.* 86, 369–371. doi: 10.1016/j.diagmicrobio.2016.08.027
- Elumalai, D., Hemavathi, M., Deepaa, C. V., and Kaleena, P. K. (2017). Evaluation of phytosynthesized silver nanoparticles from leaf extracts of *Leucas aspera* and *Hyptis suaveolens* and their larvicidal activity against malaria, dengue and filariasis vectors. *Parasite Epidemiol. Control* 2, 15–26. doi: 10.1016/j.parepi.2017.09.001
- Eyupoglu, S., Kut, D., Girisgin, A. O., Eyupoglu, C., Ozuicli, M., Dayioglu, H., et al. (2018). Investigation of the bee-repellent properties of cotton fabrics treated with microencapsulated essential oils. *Textile Res. J.* 89, 1417–1435. doi: 10.1177/0040517518773370
- Ferreira, T. P., Haddi, K., Corrêa, R. F. T., Zapata, V. L. B., Piau, T. B., Souza, L. F. N., et al. (2019). Prolonged mosquitocidal activity of *Siparuna guianensis* essential oil encapsulated in chitosan nanoparticles. *PLoS Negl. Trop. Dis.* 13:e0007624. doi: 10.1371/journal.pntd.0007624
- Fletcher, S. J., and Milligan, A. S. (2009). *Auto-Feedback Loop Biosensor*. Available online at: [https://patents.google.com/patent/WO2009152566A1/en?q=W2009152566A1](https://patents.google.com/patent/WO2009152566A1/en?q=W2009152566A1 (accessed November 14, 2018).) (accessed November 14, 2018).
- Forgearini, J. C., Michalowski, C. B., Assumpção, E., Pohlmann, A. R., and Guterres, S. S. (2016). Development of an insect repellent spray for textile based on permethrin-loaded lipid-core nanocapsules. *J. Nanosci. Nanotechnol.* 16, 1301–1309. doi: 10.1166/jnn.2016.11665
- Fraceto, L. F., Barbosa, M. C. M., Oliveira, J. L., and Campos, E. V. R. (2018). *Sistemas Nanoestruturados E Seu Uso*. Available online at: [https://patents.google.com/patent/WO2017143421A1/pt?oq=repelentes+aedes+aegypti](https://patents.google.com/patent/WO2017143421A1/pt?oq=repelentes+aedes+aegypti (accessed February 12, 2020).) (accessed February 12, 2020).
- Galvão, J. G., Cerpe, P., Santos, D. A., Gonsalves, J. K., Santos, A. J., Nunes, R. K., et al. (2018). *Lippia gracilis* essential oil in β -cyclodextrin inclusion complexes: an environmentally safe formulation to control *Aedes aegypti* larvae: *Lippia gracilis* essential oil in β -cyclodextrin inclusion complexes. *Pest Manag. Sci.* 75, 452–459. doi: 10.1002/ps.5138
- García, A. A., Franco, L. S., Pirez-Gomez, M. A., Pech-Pacheco, J. L., Mendez-Galvan, J. F., Machain-Williams, C., et al. (2017). Feasibility study of an optical caustic plasmonic light scattering sensor for human serum anti-dengue protein E antibody detection. *Diagnostics* 7:47. doi: 10.3390/diagnostics7030047
- Gomes, G. M., Bigon, J. P., Montoro, F. E., and Lona, L. M. F. (2018). Encapsulation of *N,N*-diethyl-*meta*-toluamide (DEET) via miniemulsion polymerization for temperature controlled release: encapsulation of *N,N*-diethyl-*meta*-toluamide (DEET) via miniemulsion polymerization for temperature controlled release. *J. Appl. Polym. Sci.* 139:47139. doi: 10.1002/app.47139
- Govindarajan, M., Vijayan, P., Kadaikunnan, S., Alharbi, N. S., and Benelli, G. (2016). One-pot biogenic fabrication of silver nanocrystals using *Quisqualis indica*: effectiveness on malaria and Zika virus mosquito vectors, and impact on non-target aquatic organisms. *J. Photochem. Photobiol. Biol.* 162, 646–655. doi: 10.1016/j.jphotobiol.2016.07.036
- Hameed, A., Fatima, G. R., Malik, K., Muqadas, A., and Fazal-ur-Rehman, M. (2019). Scope of nanotechnology in cosmetics: dermatology and skin care products. *J. Med. Chem. Sci.* 2, 9–16. doi: 10.26655/jmchemsci.2019.6.2
- Hao, J., Yang, M., Tsang, M.-K., and Ye, W. (2018). *Heterogeneous Microarray Based Hybrid Upconversion Nanoprobe/Nanoporous Membrane System*. U.S. Patent No 20180246084. Available online at: [https://patents.google.com/patent/US20180246084A1/en?q=US20180246084++Heterogeneous+microarray+based+hybrid+upconversion+nanoprobe%2fnanoporous+membrane+system](https://patents.google.com/patent/US20180246084A1/en?q=US20180246084++Heterogeneous+microarray+based+hybrid+upconversion+nanoprobe%2fnanoporous+membrane+system (accessed February 12, 2020).) (accessed February 12, 2020).
- Hoag, G. E., Collins, J. B., Varma, R. S., and Nadagouda, M. N. (2011). *Green Synthesis of Nanometals Using Plant Extracts and Use Thereof*. Available online at: [https://patents.google.com/patent/US8057682B2/en?q=green+\\$+sinthesis&q=%22silver+\\$+nanoparticle%22&oq=green+\\$+sinthesis\\$++\\$%22silver+\\$+nanoparticle%22\\$+\\$](https://patents.google.com/patent/US8057682B2/en?q=green+$+sinthesis&q=%22silver+$+nanoparticle%22&oq=green+$+sinthesis$++$%22silver+$+nanoparticle%22$+$ (accessed October 18, 2018).) (accessed October 18, 2018).
- Hua, S., de Matos, M. B. C., Metselaar, J. M., and Storm, G. (2018). Current trends and challenges in the clinical translation of nanoparticulate nanomedicines: pathways for translational development and commercialization. *Front. Pharmacol.* 9:14. doi: 10.3389/fphar.2018.00790
- Huang, S., Li, C., Lin, B., and Qin, J. (2010). Microvalve and micropump controlled shuttle flow microfluidic device for rapid DNA hybridization. *Lab Chip* 10, 2925–2931. doi: 10.1039/c005227b
- Ishwarya, R., Vaseeharan, B., Anuradha, R., Rekha, R., Govindarajan, M., Alharbi, N. S., et al. (2017). Eco-friendly fabrication of Ag nanostructures using the seed extract of *Petalium murex*, an ancient Indian medicinal plant: histopathological effects on the Zika virus vector *Aedes aegypti* and inhibition of biofilm-forming pathogenic bacteria. *J. Photochem. Photobiol. B Biol.* 174, 133–143. doi: 10.1016/j.jphotobiol.2017.07.026
- Ishwarya, R., Vaseeharan, B., Kalyani, S., Banumathi, B., Govindarajan, M., Alharbi, N. S., et al. (2018). Facile green synthesis of zinc oxide nanoparticles using *Ulva lactuca* seaweed extract and evaluation of their photocatalytic, antibiofilm and insecticidal activity. *J. Photochem. Photobiol. Biol.* 178, 249–258. doi: 10.1016/j.jphotobiol.2017.11.006
- Iswardy, E., Tsai, T.-C., Cheng, I.-F., Ho, T.-C., Perng, G. C., and Chang, H.-C. (2017). A bead-based immunofluorescence-assay on a microfluidic dielectrophoresis platform for rapid dengue virus detection. *Biosens. Bioelectron.* 95, 174–180. doi: 10.1016/j.bios.2017.04.011
- Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., and Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history,

- sources, toxicity and regulations. *Beilstein J. Nanotechnol.* 9, 1050–1074. doi: 10.3762/bjnano.9.98
- Jones, A. A. D., Mi, G., and Webster, T. J. (2019). A status report on FDA approval of medical devices containing nanostructured materials. *Trends Biotechnol.* 37, 117–120. doi: 10.1016/j.tibtech.2018.06.003
- Kadam, S. L., Yadav, P., Bhutkar, S., Patil, V. D., Shukla, P. G., and Shanmuganathan, K. (2019). Sustained release insect repellent microcapsules using modified cellulose nanofibers (mCNF) as pickering emulsifier. *Colloids Surfaces A*. 582:123883. doi: 10.1016/j.colsurfa.2019.123883
- Kah, M., Kookana, R. S., Gogos, A., and Bucheli, T. D. (2018a). A critical evaluation of nanopesticides and nanofertilizers against their conventional analogues. *Nat. Nanotech* 13, 677–684. doi: 10.1038/s41565-018-0131-1
- Kah, M., Walch, H., and Hofmann, T. (2018b). Environmental fate of nanopesticides: durability, sorption and photodegradation of nanoformulated clothianidin. *Environ. Sci.* 5, 882–889. doi: 10.1039/C8EN00038G
- Kaushik, A., and Nair, M. (2018). *Rapid Zika Virus Detection Using Nano-Enabled Electrochemical Sensing System*. Available online at: <https://patents.google.com/patent/US20180059099A1/en> (accessed November 14, 2018).
- Khater, H. F., Selim, A. M., Abouelella, G. A., Abouelella, N. A., Murugan, K., Vaz, N. P., et al. (2019). “Commercial mosquito repellents and their safety concerns,” in *Malaria*, eds F. H. Kasenga (Malawi Adventist University). doi: 10.5772/intechopen.87436
- Koczula, K. M., and Gallotta, A. (2016). Lateral flow assays. *Essays Biochem.* 60, 111–120. doi: 10.1042/EBC20150012
- Kumar, D., Kumar, G., and Agrawal, V. (2018). Green synthesis of silver nanoparticles using *Holarrhena antidysenterica* (L.) Wall. bark extract and their larvicidal activity against dengue and filariasis vectors. *Parasitol. Res.* 117, 377–389. doi: 10.1007/s00436-017-5711-8
- Kumar, S., Lather, V., and Pandita, D. (2015). Green synthesis of therapeutic nanoparticles: an expanding horizon. *Nanomedicine* 10, 2451–2471. doi: 10.2217/nnm.15.112
- Kumar, V. A., Ammani, K., Jobina, R., Subhaswaraj, P., and Siddhardha, B. (2017). Photo-induced and phytomediated synthesis of silver nanoparticles using *Derris trifoliata* leaf extract and its larvicidal activity against *Aedes aegypti*. *J. Photochem. Photobiol. B Biol.* 171, 1–8. doi: 10.1016/j.jphotobiol.2017.04.022
- Laury-Kleintop, L., and Rutner, H. (2015). *Biocoated Piezoelectric Biosensor Platform for Point-Of-Care Diagnostic Use*. Available online at: <https://patents.google.com/patent/US20150111765A1/en?q=US20150111765A1> (accessed November 14, 2018).
- Laux, P., Tentschert, J., Riebeling, C., Braeuning, A., Creutzenberg, O., Epp, A., et al. (2018). Nanomaterials: certain aspects of application, risk assessment and risk communication. *Arch. Toxicol.* 92, 121–141. doi: 10.1007/s00204-017-2144-1
- Li, J., Yang, K., Wu, Z., Li, X., and Duan, Q. (2019). Nitrogen-doped porous carbon-based fluorescence sensor for the detection of ZIKV RNA sequences: fluorescence image analysis. *Talanta* 205:120091. doi: 10.1016/j.talanta.2019.06.091
- Liu, S., Liu, L., Tan, X., and Meng, B. (2013). *Environmentally-Friendly Synthetic Method for Metal Nanoparticle*. Available online at: [https://patents.google.com/patent/CN103071808A/en?q=green&q=synthesis&q=nanoparticle&oq=\\$+green\\$+\\$synthesis\\$+\\$nanoparticle\\$](https://patents.google.com/patent/CN103071808A/en?q=green&q=synthesis&q=nanoparticle&oq=$+green$+$synthesis$+$nanoparticle$) (accessed October 18, 2018).
- Lucia, A., Toloza, A. C., Guzmán, E., Ortega, F., and Rubio, R. G. (2017). Novel polymeric micelles for insect pest control: encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control. *PeerJ* 5:e3171. doi: 10.7717/peerj.3171
- Magro, M., Bramuzzo, S., Baratella, D., Ugoletti, J., Zoppellaro, G., Chemello, G., et al. (2019). Self-assembly of chlorin-e6 on γ -Fe₂O₃ nanoparticles: application for larvicidal activity against *Aedes aegypti*. *J. Photochem. Photobiol.* 194, 21–31. doi: 10.1016/j.jphotobiol.2019.03.004
- Marinero-Caceres, E. E., Kuhn, R. J., Stanciu, L. A., and Jin, S.-A. (2017). *Functionalized Particles for Label-Free DNA Impedimetric Biosensor for DNA and RNA Sensing*. Available online at: [https://patents.google.com/patent/US20170107565A1/en?q=US\\$+2017%2f0107565\\$+\\$A1](https://patents.google.com/patent/US20170107565A1/en?q=US$+2017%2f0107565$+$A1) (accessed November 14, 2018).
- Mazan, N.-F., Tan, L. L., Karim, N. H. A., Heng, L. Y., Jamaluddin, N. D., Yusof, N. Y. M., et al. (2019). Acrylic-based genosensor utilizing metal salphen labeling approach for reflectometric dengue virus detection. *Talanta* 198, 358–370. doi: 10.1016/j.talanta.2019.02.036
- Misni, N., Nor, Z. M., and Ahmad, R. (2017). Repellent effect of microencapsulated essential oil in lotion formulation against mosquito bites. *J. Vector Borne Dis.* 54, 44–53.
- Muñoz, V., Buffa, F., Molinari, F., Hermida, L. G., García, J. J., and Abraham, G. A. (2019). Electrospun ethylcellulose-based nanofibrous mats with insect-repellent activity. *Mater. Lett.* 253, 289–292. doi: 10.1016/j.matlet.2019.06.091
- Murugan, K., Dinesh, D., Paulpandi, M., Althbyani, A. D. M., Subramaniam, J., Madhiyazhagan, P., et al. (2015). Nanoparticles in the fight against mosquito-borne diseases: bioactivity of *Bruguiera cylindrica*-synthesized nanoparticles against dengue virus DEN-2 (*in vitro*) and its mosquito vector *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.* 114, 4349–4361. doi: 10.1007/s00436-015-4676-8
- Nakata, Y., and Röst, G. (2015). Global analysis for spread of infectious diseases via transportation networks. *J. Math. Biol.* 70, 1411–1456. doi: 10.1007/s00285-014-0801-z
- Nalini, M., Lena, M., Sumathi, P., and Sundaravadevelan, C. (2017). Effect of phyto-synthesized silver nanoparticles on developmental stages of malaria vector, *Anopheles stephensi* and dengue vector, *Aedes aegypti*. *Egyptian J. Basic Appl. Sci.* 4, 212–218. doi: 10.1016/j.ejbas.2017.04.005
- Nawaz, M. H., Hayat, A., Catanante, G., Latif, U., and Marty, J. L. (2018). Development of a portable and disposable NSI based electrochemical immunosensor for early diagnosis of dengue virus. *Anal. Chim. Acta* 1026, 1–7. doi: 10.1016/j.aca.2018.04.032
- Nicolini, A. M., McCracken, K. E., and Yoon, J.-Y. (2017a). Future developments in biosensors for field-ready Zika virus diagnostics. *J. Biol. Eng.* 11:7.
- Nicolini, A. M., McCracken, K. E., and Yoon, J.-Y. (2017b). Future developments in biosensors for field-ready Zika virus diagnostics. *J. Biol. Eng.* 11:7. doi: 10.1186/s13036-016-0046-z
- Omar, N. A. S., Fen, Y. W., Abdullah, J., Chik, C. E. N., and Mahdi, M. A. (2018). Development of an optical sensor based on surface plasmon resonance phenomenon for diagnosis of dengue virus E-protein. *Sens. Bio-Sensing Res.* 20, 16–21. doi: 10.1016/j.sbsr.2018.06.001
- Osanloo, M., Arish, J., and Sereshti, H. (2019). Developed methods for the preparation of electrospun nanofibers containing plant-derived oil or essential oil: a systematic review. *Polym. Bull.* doi: 10.1007/s00289-019-03042-0. [Epub ahead of print].
- Parisi, C., Viganì, M., and Rodríguez-Cerezo, E. (2015). Agricultural nanotechnologies: what are the current possibilities? *Nano Today* 10, 124–127. doi: 10.1016/j.nantod.2014.09.009
- Pashchenko, O., Shelby, T., Banerjee, T., and Santra, S. (2018). A comparison of optical, electrochemical, magnetic, and colorimetric point-of-care biosensors for infectious disease diagnosis. *ACS Infect. Dis.* 4, 1162–1178. doi: 10.1021/acsinfecdis.8b00023
- Patterson, J., Sammon, M., and Garg, M. (2016). Dengue, Zika and Chikungunya: emerging arboviruses in the new world. *West. J. Emerg. Med.* 17, 671–679. doi: 10.5811/westjem.2016.9.30904
- Paula, C. A. D., Dias, A. M. R., and Heffliger, R. A. (2017). *Sistema Nanométrico De Liberação Prolongada De Ativos Cosméticos e/ou Repelentes*. Available online at: <https://patents.google.com/patent/WO2017143421A1/pt?q=WO2017143421A1> (accessed November 14, 2018).
- Pessoa, L. Z., da Duarte, J. L., Ferreira, R. M., dos Oliveira, A. E. M., Cruz, R. A. S. Faustino, S. M. M., et al. (2018). Nanosuspension of quercetin: preparation, characterization and effects against *Aedes aegypti* larvae. *Revista Brasileira de Farmacog.* 28, 618–625. doi: 10.1016/j.bjrp.2018.07.003
- Pinto, I. C., Cerqueira-Coutinho, C. S., Santos, E. P., Carmo, F. A., and Ricci-Junior, E. (2017). Development and characterization of repellent formulations based on nanostructured hydrogels. *Drug Dev. Ind. Pharm.* 43, 67–73. doi: 10.1080/03639045.2016.1220564
- Priye, A., Bird, S. W., Light, Y. K., Ball, C. S., Negrete, O. A., and Meagher, R. J. (2017). A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. *Sci. Rep.* 7:44778. doi: 10.1038/srep44778
- Ragavan, V. V., Roy, A., and Rutner, H. (2011). *Integrated Microchip Sensor System for Detection of Infectious Agents*. Available online at: [https://patents.google.com/patent/US20110136262A1/en?q=US\\$+2011%2f0136262\\$+\\$A1](https://patents.google.com/patent/US20110136262A1/en?q=US$+2011%2f0136262$+$A1) (accessed November 14, 2018).
- Ramanibai, R., and Velayutham, K. (2016). Synthesis of silver nanoparticles using 3,5-di-t-butyl-4-hydroxyanisole from *Cynodon dactylon* against *Aedes*

- aegypti* and *Culex quinquefasciatus*. *J. Asia Pac. Entomol.* 19, 603–609. doi: 10.1016/j.aspen.2016.06.007
- Ramar, M., Manonmani, P., Arumugam, P., Kannan, S. K., Erusan, R. R., and Murugan, K. (2017). Nano-insecticidal formulations from essential oil (*Ocimum sanctum*) and fabricated in filter paper on adult of *Aedes aegypti* and *Culex quinquefasciatus*. *J. Entomol. Zool. Stud.* 5, 1769–1774.
- Rashid, J. I. A., and Yusof, N. A. (2018). Laboratory diagnosis and potential application of nucleic acid biosensor approach for early detection of dengue virus infections. *Biosci. Biotechnol. Res. Asia* 15, 245–255. doi: 10.13005/bbra/2628
- Rong, Z., Wang, Q., Sun, N., Jia, X., Wang, K., Xiao, R., et al. (2019). Smartphone-based fluorescent lateral flow immunoassay platform for highly sensitive point-of-care detection of Zika virus nonstructural protein 1. *Anal. Chim. Acta* 1055, 140–147. doi: 10.1016/j.aca.2018.12.043
- Roni, M., Murugan, K., Panneerselvam, C., Subramaniam, J., Nicoletti, M., Madhiyazhagan, P., et al. (2015). Characterization and biotoxicity of Hypnea musciformis-synthesized silver nanoparticles as potential eco-friendly control tool against *Aedes aegypti* and *Plutella xylostella*. *Ecotoxicol. Environ. Saf.* 121, 31–38. doi: 10.1016/j.ecoenv.2015.07.005
- Sabalza, M., Yasmin, R., Barber, C. A., Castro, T., Malamud, D., Kim, B. J., et al. (2018). Detection of Zika virus using reverse-transcription LAMP coupled with reverse dot blot analysis in saliva. *PLoS ONE* 13:e0192398. doi: 10.1371/journal.pone.0192398
- Sajid, M., Ilyas, M., Basheer, C., Tariq, M., Daud, M., Baig, N., et al. (2015). Impact of nanoparticles on human and environment: review of toxicity factors, exposures, control strategies, and future prospects. *Environ. Sci. Pollut. Res. Int.* 22, 4122–4143. doi: 10.1007/s11356-014-3994-1
- Sánchez-Purrà, M., Carré-Camps, M., de Puig, H., Bosch, I., Gehrke, L., and Hamad-Schifferli, K. (2017). Surface-enhanced raman spectroscopy-based sandwich immunoassays for multiplexed detection of Zika and Dengue viral biomarkers. *ACS Infect. Dis.* 3, 767–776. doi: 10.1021/acsinfectdis.7b00110
- Schnell-Inderst, P., Hunger, T., Conrads-Frank, A., Arvandi, M., and Siebert, U. (2018). Recommendations for primary studies evaluating therapeutic medical devices were identified and systematically reported through reviewing existing guidance. *J. Clin. Epidemiol.* 94, 46–58. doi: 10.1016/j.jclinepi.2017.10.007
- Sethi, R. S. (1994). Transducer aspects of biosensors. *Biosens. Bioelectr.* 9, 243–264. doi: 10.1016/0956-5663(94)80127-4
- Sharma, D. H. K. (2019). Formulation and evaluation of controlled release herbal mosquito repellent gel containing encapsulated essential oils obtained from natural sources indigenous to Northeast India. *Asian J. Pharmaceut.* 13. doi: 10.22377/ajp.v13i01.3005
- Silva, L. S., Mar, J. M., Azevedo, S. G., Rabelo, M. S., Bezerra, J. A., Campelo, P. H., et al. (2019). Encapsulation of *Piper aduncum* and *Piper hispidinervum* essential oils in gelatin nanoparticles: a possible sustainable control tool of *Aedes aegypti*, *Tetranychus urticae* and *Cerataphis lataniae*. *J. Sci. Food Agric.* 99, 685–695. doi: 10.1002/jsfa.9233
- Singhal, C., Dubey, A., Mathur, A., Pundir, C. S., and Narang, J. (2018). Paper based DNA biosensor for detection of chikungunya virus using gold shells coated magnetic nanocubes. *Proc. Biochem.* 74, 35–42. doi: 10.1016/j.procbio.2018.08.020
- Sintubin, L., Verstraete, W., and Boon, N. (2012). Biologically produced nanosilver: current state and future perspectives. *Biotechnol. Bioeng.* 109, 2422–2436. doi: 10.1002/bit.24570
- Soares, S., Sousa, J., Pais, A., and Vitorino, C. (2018). Nanomedicine: principles, properties, and regulatory issues. *Front. Chem.* 6:360. doi: 10.3389/fchem.2018.00360
- Song, J., Mauk, M. G., Hackett, B. A., Cherry, S., Bau, H. H., and Liu, C. (2016). Instrument-free point-of-care molecular detection of Zika virus. *Anal. Chem.* 88, 7289–7294. doi: 10.1021/acs.analchem.6b01632
- Soutschek, E., Boecher, O., and Noelting, C. (2019). *Method for the Immunological Diagnosis of a Sample with a Potential Infection with an Arbovirus and Test Kits Suitable for This Purpose*. U.S. Patent No 20190227065. Available online at: <http://www.freepatentsonline.com/y2019/0227065.html> (accessed February 12, 2020).
- Starpharma (2016). *Starpharma VivaGel® active against Zika virus*. Starpharma. Available online at: <https://www.starpharma.com/news/281> (accessed December 31, 2019).
- Suganya, P., Vaseeharan, B., Vijayakumar, S., Balan, B., Govindarajan, M., Alharbi, N. S., et al. (2017). Biopolymer zein-coated gold nanoparticles: synthesis, antibacterial potential, toxicity and histopathological effects against the Zika virus vector *Aedes aegypti*. *J. Photochem. Photobiol.* 173, 404–411. doi: 10.1016/j.jphotobiol.2017.06.004
- Tancharoen, C., Sukjee, W., Thepparit, C., Jaimipuk, T., Auewarakul, P., Thitithanyanont, A., et al. (2019). Electrochemical biosensor based on surface imprinting for Zika virus detection in serum. *ACS Sens.* 4, 69–75. doi: 10.1021/acssensors.8b00885
- Tavares, M., da Silva, M. R. M., de Oliveira de Siqueira, L. B., Rodrigues, R. A. S., Bodjolle-d'Almeida, L., dos Santos, E. P., et al. (2018). Trends in insect repellent formulations: a review. *Int. J. Pharm.* 539, 190–209. doi: 10.1016/j.ijpharm.2018.01.046
- Thanh, B. T., Van Sau, N., Ju, H., Bashir, M. J. K., Jun, H. K., Phan, T. B., et al. (2019). Immobilization of protein a on monodisperse magnetic nanoparticles for biomedical applications. *J. Nanomater.* 2019:2182471. doi: 10.1155/2019/2182471
- Theillet, G., Rubens, A., Foucault, F., Dalbon, P., Rozand, C., Leparç-Goffart, I., et al. (2018). Laser-cut paper-based device for the detection of dengue non-structural NS1 protein and specific IgM in human samples. *Arch. Virol.* 163, 1757–1767. doi: 10.1007/s00705-018-3776-z
- Thomas, J. (2019). *Anti-Parasitic Compositions and Methods*. Available online at: <https://patents.google.com/patent/US20190160016A1/en> (accessed January 6, 2020).
- Tian, B., Qiu, Z., Ma, J., Zardán Gómez de la Torre, T., Johansson, C., Svedlindh, P., et al. (2016). Attomolar Zika virus oligonucleotide detection based on loop-mediated isothermal amplification and AC susceptometry. *Biosens. Bioelectron.* 86, 420–425. doi: 10.1016/j.bios.2016.06.085
- Tripathy, S., Krishna Vanjari, S. R., Singh, V., Swaminathan, S., and Singh, S. G. (2017). Electrospun manganese (III) oxide nanofiber based electrochemical DNA-nanobiosensor for zeptomolar detection of dengue consensus primer. *Biosens. Bioelectr.* 90, 378–387. doi: 10.1016/j.bios.2016.12.008
- Udayabhanu, J., Kannan, V., Tiwari, M., Natesan, G., Giovanni, B., and Perumal, V. (2018). *Nanotitania crystals* induced efficient photocatalytic color degradation, antimicrobial and larvicidal activity. *J. Photochem. Photobiol. B Biol.* 178, 496–504. doi: 10.1016/j.jphotobiol.2017.12.005
- Veerakumar, K., Govindarajan, M., Rajeswary, M., and Muthukumar, U. (2014). Mosquito larvicidal properties of silver nanoparticles synthesized using *Heliotropium indicum* (Boraginaceae) against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.* 113, 2363–2373. doi: 10.1007/s00436-014-3895-8
- Ventola, C. L. (2017). Progress in nanomedicine: approved and investigational nanodrugs. *P T* 42, 742–755.
- Vinayagam, S., Rajaiah, P., Mukherjee, A., and Natarajan, C. (2018). DNA-triangular silver nanoparticles nanoprobe for the detection of dengue virus distinguishing serotype. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 202, 346–351. doi: 10.1016/j.saa.2018.05.047
- Vo-Dinh, T. (2004). *Multifunctional and Multispectral Biosensor Devices and Methods of Use*. Available online at: <https://patents.google.com/patent/US6743581B1/en> (accessed November 14, 2018).
- Wasik, D., Mulchandani, A., and Yates, M. V. (2017). A heparin-functionalized carbon nanotube-based affinity biosensor for dengue virus. *Biosens. Bioelectron.* 91, 811–816. doi: 10.1016/j.bios.2017.01.017
- Werdin González, J. O., Jesser, E. N., Yeguerman, C. A., Ferrero, A. A., and Fernández Band, B. (2017). Polymer nanoparticles containing essential oils: new options for mosquito control. *Environ. Sci. Pollut. Res.* 24, 17006–17015. doi: 10.1007/s11356-017-9327-4
- Wilder-Smith, A., Gubler, D. J., Weaver, S. C., Monath, T. P., Heymann, D. L., and Scott, T. W. (2017). Epidemic arboviral diseases: priorities for research and public health. *Lancet Infect. Dis.* 17:e101–e106. doi: 10.1016/S1473-3099(16)30518-7
- Willenberg, B. J., and Seal, S. (2018). *Passive Insect Surveillance Sensor Device*. Available online at: <https://patents.google.com/patent/WO2017027677A1/en?q=Passive&q=Insect&q=Surveillance&q=Sensor&q=Device&oq=+Passive+Insect+Surveillance+Sensor+Device> (accessed February 11, 2020).
- Wolkowicz, R. (2018). *Compositions and Cell-Based Methods for Monitoring the Activity of a Dengue Virus Protease*. Available online at: <https://patents.google.com/patent/US20180100000A1/en> (accessed February 11, 2020).

- com/patent/US10006077B2/en?oq=US10006077B2 (accessed November 14, 2018).
- Yaren, O., Alto, B. W., Gangodkar, P. V., Ranade, S. R., Patil, K. N., Bradley, K. M., et al. (2017). Point of sampling detection of Zika virus within a multiplexed kit capable of detecting dengue and chikungunya. *BMC Infect. Dis.* 17:293. doi: 10.1186/s12879-017-2382-0
- Yen, C.-W., de Puig, H., Tam, J., Gómez-Márquez, J., Bosch, I., Hamad-Schifferli, K., et al. (2015). Multicolored silver nanoparticles for multiplexed disease diagnostics: distinguishing dengue, yellow fever, and ebola viruses. *Lab Chip* 15, 1638–1641. doi: 10.1039/C5LC00055F
- Yrad, F. M., Castañares, J. M., and Alocilja, E. C. (2019). Visual detection of dengue-1 RNA using gold nanoparticle-based lateral flow biosensor. *Diagnostics* 9:74. doi: 10.3390/diagnostics9030074
- Yujia, G. H., Wu, Y., and Chi, D. (2015). *Green Synthesis Method of Gold Nanoparticles*. Available online at: [https://patents.google.com/patent/CN105057692A/en?q=green&q=synthesis&q=nanoparticle&oq\\$=+\\$green\\$+\\$synthesis\\$+\\$nanoparticle](https://patents.google.com/patent/CN105057692A/en?q=green&q=synthesis&q=nanoparticle&oq$=+$green$+$synthesis$+$nanoparticle) (accessed October 18, 2018).
- Yusoh, S. N., Yaacob, K. A., and Alias, N. N. (2019). Trapezoidal SiNWs array fabricated by AFM-LAO for Dengue virus DNA oligomer detection. *Mater. Res. Express* 6:115005. doi: 10.1088/2053-1591/ab3571
- Yuzon, Ma. K., Kim, J.-H., and Kim, S. (2019). A novel paper-plastic microfluidic hybrid chip integrated with a lateral flow immunoassay for dengue nonstructural protein 1 antigen detection. *BioChip J.* 13, 277–287. doi: 10.1007/s13206-019-3305-5
- Zhang, B., Pinsky, B. A., Ananta, J. S., Zhao, S., Arulkumar, S., Wan, H., et al. (2017). Diagnosis of Zika virus infection on a nanotechnology platform. *Nat. Med.* 23, 548–550. doi: 10.1038/nm.4302
- Zhang, Y., Chen, W., Jing, M., Liu, S., Feng, J., Wu, H., et al. (2018). Self-assembled mixed micelle loaded with natural pyrethrins as an intelligent nano-insecticide with a novel temperature-responsive release mode. *Chem. Eng. J.* 361, 1381–1391. doi: 10.1016/j.cej.2018.10.132

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Campos, de Oliveira, Abrantes, Rogério, Bueno, Miranda, Monteiro and Fraceto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

CHAPTER II
DEVELOPMENT OF A MOSQUITO REPELLENT FORMULATION BASED ON
NANOSTRUCTURED LIPID CARRIERS

Frontiers in Pharmacology, V. 12, 2021

doi: 10.3389/fphar.2021.760682



Development of a Mosquito Repellent Formulation Based on Nanostructured Lipid Carriers

Daniele Carvalho Abrantes¹, Carolina Barbara Rogerio¹, Jhones L. de Oliveira², Estefânia V. R. Campos³, Daniele Ribeiro de Araújo³, Laurindo Cesar Pampana⁴, Marcelo José Duarte⁴, Geórgio Freesz Valadares⁴ and Leonardo Fernandes Fraceto^{1*}

¹Institute of Science and Technology, São Paulo State University (UNESP), São Paulo, Brazil, ²Faculty of Agronomy and Veterinary Sciences, São Paulo State University (UNESP), São Paulo, Brazil, ³Federal University of ABC, São Paulo, Brazil, ⁴Veterinary Products Institute, Araçoiaba da Serra, Brazil

OPEN ACCESS

Edited by:

Roberto Paganelli,
University of Studies G d'Annunzio
Chieti and Pescara, Italy

Reviewed by:

Whitney Qualls,
Anastasia Mosquito Control District,
United States
Devanathan Sukumaran,
Defence Research and Development
Establishment (DRDE), India

*Correspondence:

Leonardo Fernandes Fraceto
leonardo.fraceto@unesp.br

Specialty section:

This article was submitted to
Translational Pharmacology,
a section of the journal
Frontiers in Pharmacology

Received: 18 August 2021

Accepted: 27 September 2021

Published: 11 October 2021

Citation:

Abrantes DC, Rogerio CB,
de Oliveira JL, Campos EVR,
de Araújo DR, Pampana LC,
Duarte MJ, Valadares GF and
Fraceto LF (2021) Development of a
Mosquito Repellent Formulation Based
on Nanostructured Lipid Carriers.
Front. Pharmacol. 12:760682.
doi: 10.3389/fphar.2021.760682

Arboviral diseases are a threat to global public health systems, with recent data suggesting that around 40% of the world's population is at risk of contracting arboviruses. The use of mosquito repellents is an appropriate strategy to avoid humans coming into contact with vectors transmitting these viruses. However, the cost associated with daily applications of repellents can make their use unfeasible for the low-income populations that most need protection. Therefore, the development of effective formulations offers a way to expand access to this means of individual protection. Consequently, research efforts have focused on formulations with smaller quantities of active agents and sustained release technology, aiming to reduce re-applications, toxicity, and cost. The present study investigates the development of nanostructured lipid carriers (NLCs) loaded with a mixture of the compounds icaridin (synthetic) and geraniol (natural), incorporated in cellulose hydrogel. The NLCs were prepared by the emulsion/solvent evaporation method and were submitted to physicochemical characterization as a function of time (at 0, 15, 30, and 60 days). The prepared system presented an average particle size of 252 ± 5 nm, with encapsulation efficiency of 99% for both of the active compounds. The stability profile revealed that the change of particle size was not significant ($p > 0.05$), indicating high stability of the system. Rheological characterization of the gels containing NLCs showed that all formulations presented pseudoplastic and thixotropic behavior, providing satisfactory spreadability and long shelf life. Morphological analysis using atomic force microscopy (AFM) revealed the presence of spherical nanoparticles (252 ± 5 nm) in the cellulose gel matrix. Permeation assays showed low fluxes of the active agents through a Strat-M[®] membrane, with low permeability coefficients, indicating that the repellents would be retained on the surface to which they are applied, rather than permeating the tissue. These findings open perspectives for the use of hybrid formulations consisting of gels containing nanoparticles that incorporate repellents effective against arthropod-borne virus. These systems could potentially provide improvements considering the issues of effectiveness, toxicity, and safety.

Keywords: *Aedes aegypti*, repellent, arboviruses, nanoparticles, nanostructure lipid carriers

INTRODUCTION

Arthropod-borne virus (arboviruses) are a global public health concern since most of them are related to neglected tropical diseases. Several arthropod can transmit arboviruses to human, including dengue, chikungunya, Zika, yellow fever among others (Guarner and Hale, 2019). Although *Aedes aegypti* is the most popularly known vector, arboviruses can also be transmitted to humans by ticks, midges, flies, and fleas. Arboviral diseases are a global problem for public health systems, with recent data suggesting that 40% of the human population is at risk of such diseases (Leta et al., 2018). Hence, the development of ways to prevent contact between the mosquito and humans continues to be one of the strategies strongly recommended by national and international health protection agencies. Among these methods, insect repellents are used as the first line of defense against mosquito bites and arbovirus transmission (CDC, 2019; Ministério da Saúde, 2019). However, the cost of daily repellent applications may be unfeasible for low-income populations that most need protection. Therefore, the development of effective and inexpensive formulations is one way to expand access to this method of individual protection.

The synthetic repellents currently most widely used against *Aedes aegypti* are DEET (considered as the gold standard), icaridin (KB3023), and IR3535. Icaridin (ICA) is a promising repellent derived from pepper, which was developed in the 1990s and began to be used in 1998, and has been shown to be effective in protecting against insect bites (Naucke et al., 2007). However, there are concerns about the biological impacts of these compounds and their potential toxicity towards nontarget organisms (Webb and Hess, 2016). Another option is to use botanical repellents such as the essential oil of citronella grass (*Cymbopogon nardus*), which has high contents of geraniol and citronellal. However, the duration of its protection is very short, compared to synthetic repellents, due to its high volatility, and direct application to the skin is not recommended, due to possible irritation (Maia and Moore, 2011; Rehman et al., 2014). Therefore, the development of formulations that include suitable carrier systems can contribute to the use of such compounds.

An ideal repellent formulation should provide low permeation through the skin; a pleasant odor; prolonged repellent action; broad activity against insects, especially the main vectors of interest; agreeable aesthetic and sensory characteristics; resistance to water and perspiration; high chemical stability; no staining of fabrics and clothing; and low cost (Tavares et al., 2018). Generally, higher concentrations of active agents provide longer-lasting effects (Kuri-Morales et al., 2017), but they can cause local toxicity, in addition to increasing costs. A promising solution is to use formulations containing repellents encapsulated in nanoparticles, which can decrease the dosages required and prolong the duration of release of active agents, consequently reducing toxicity and the number of applications required (Tavares et al., 2018). Furthermore, the formulations can become more affordable, due to the smaller amounts of active agents used.

Studies have reported the encapsulation of botanical repellents in different controlled release systems, resulting in positive effects such as prolonged action and low permeation (Chang and Dobashi, 2003; Maia and Moore, 2011; Solomon et al., 2011; Misni et al., 2016; Lammari et al., 2020). Neem oil, citronella, and icaridin, encapsulated separately in nanoparticles, have recently been marketed by a company supplying high-performance products (NanoScoping, 2020a; 2020b). Previously, another similar company encapsulated 5.5% icaridin in lipid nanoparticles (Nanovetores, 2017). Up to now, there have been no commercial formulations based on nanoparticles containing a mixture of botanical and synthetic agents, and there have been no studies in the literature concerning this type of formulation. Therefore, in the present work, attention was focused on the synthesis of repellents containing a mixture of icaridin and geraniol. These compounds belong to different chemical classes, suggesting that the spectrum of action could be enhanced, with possible synergistic effects, as observed in studies of the larvicidal effects of botanical extracts in combination with synthetic insecticides (Shaan et al., 2005; Raghavendra et al., 2013). This could enable the development of an innovative product for use as a repellent.

In the last 2 decades, nanostructured lipid carriers (NLCs) have emerged as innovative systems for topical and transdermal applications (Sharma et al., 2017). These materials were developed as a second generation of lipid nanoparticles, with a composition consisting of a mixture of solid and liquid (oil) lipids. The solubility of active agents is usually much higher in oils than in solid lipids, enabling higher loading capacity and prolongation of the release of active agents (Fang et al., 2008). Various approaches have been used to improve performance in dermal and transdermal applications. The pharmaceuticals and cosmetics industry has employed a wide range of NLCs, due to their ease of preparation, feasibility of scale-up, biocompatibility, and prolonged release of the encapsulated substances (Doktorovova and Souto, 2009; Junyaprasert et al., 2009; Khosa et al., 2018).

The majority of cosmetics and topical medications exhibit viscoelastic behavior, which facilitates application and spreadability. For this purpose, hydrogels are widely used as gelling agents in the formulations (Binder et al., 2019). Hydroxypropylmethylcellulose (HPMC), a hydrophilic polymer derived from cellulose, has the characteristic of forming chemical interactions between the chains, creating a three-dimensional network. It is frequently used in biomedical applications, due to its biodegradability and biocompatibility. In low concentrations of around 2%, it can be used to form a film at the application site, enhancing the local persistence of the active agent (Desfrancois et al., 2018; Hay et al., 2018).

Therefore, the aim of this work was to develop, characterize, and evaluate the applicability of repellent formulations based on geraniol (GRL) and icaridin (ICA) encapsulated in nanostructured lipid carriers (NLCs) in suspension or incorporated in HPMC hydrogel. The NLCs were characterized in terms of their size distribution, polydispersity index (PDI), zeta potential (ZP), and encapsulation efficiency. Analyses of the nanocarriers were performed using differential

scanning calorimetry (DSC), atomic force microscopy (AFM), and physicochemical stability measurements. Release assays, *in vitro* permeation tests, and accelerated stability studies were also performed. The system was shown to be a potential repellent that could contribute to reducing diseases caused by arboviruses transmitted by *Aedes aegypti*.

MATERIALS AND METHODS

Materials

Icaridin 97% (ICA) was obtained from Chemidin (China). Geraniol 98% (GRL) was obtained from Quinari (Ponta Grossa, Paraná, Brazil). Tripalmitin, polyvinyl alcohol (PVA), and hydroxypropylmethylcellulose (HPMC) were obtained from Sigma-Aldrich (St. Louis, United State). Myrytol was obtained from BASF. The ethanol used was from Labsynth (*Diadema*, São Paulo, Brazil). Acetonitrile (HPLC grade) was from J.T. Baker (United State). Other reagents were obtained from local suppliers in Sorocaba (São Paulo, Brazil).

Preparation of the Nanostructured Lipid Carriers

The NLCs were prepared by the emulsion/solvent evaporation method (Vitorino et al., 2011). The organic phase was composed of lipids (myritol and tripalmitin) and the active agents (ICA and GRL), diluted in chloroform. This phase was mechanically mixed (using a Turrax homogenizer at 14,000 rpm) with the aqueous phase composed of 1.25% PVA (polyvinyl alcohol) surfactant and ethyl alcohol. The solvent was evaporated until the formulation reached a final volume of 10 ml, resulting in active agent concentrations of 5% (w/v) for ICA and 2% (w/v) for GRL. As a control, empty NLCs were synthesized using the same procedure, but without addition of the active agents in the organic phase. The emulsion was used as a control in specific comparative analyses. Due to the low solubilities of the repellent chemicals in water, the mixture of 5% ICA +2% GRL was emulsified using 1.25% PVA, the same concentration used in preparation of the nanoparticles.

Incorporation of the Lipid Carriers in Hydrogel

Incorporation of the lipid carriers in HPMC was performed after synthesis of the nanostructured lipid carriers loaded with 5% ICA and 2% GRL, and the empty NLCs. For this, 2% of HPMC powder was slowly added to the suspension of NLCs, with homogenization for 10 min (Turrax, 10,000 rpm). A control gel was prepared similarly, dissolving 2% of HPMC powder in distilled water.

Physicochemical Characterization of the Nanostructured Lipid Carriers in Suspension

Size distribution and polydispersity index (PDI) measurements were performed using the dynamic light scattering (DLS) technique. Zeta potential determination was performed by microelectrophoresis.

These analyses employed a ZetaSizer Nano ZS90 system (Malvern Instruments, United Kingdom) operated with a fixed angle of 90°, at 25°C, with the samples diluted 200 times. In addition, measurements of nanoparticle concentration, size distribution, and polydispersity were performed by nanoparticle tracking analysis, with the samples diluted 20,000 times, employing a NanoSight instrument (Malvern Instruments, United Kingdom) equipped with a green laser (532 nm) and a sCMOS camera controlled by NanoSight v.3 software. The results were obtained as the average of three analyses. The formulations were kept at ambient temperature, with evaluation of their stability over time (at 0, 15, 30, 60, 90, and 120 days). The encapsulation efficiency was determined using the microcentrifugation technique, with quantification of the non-encapsulated active compounds in the filtrate by high performance liquid chromatography (HPLC), using an Ultimate 3,000 instrument (Thermo Fisher Scientific, Waltham, United State), with Chromeleon 7.2 software for acquisition and analysis of the chromatograms. The mobile phase used for analysis of icaridin was methanol:water (65:35 v/v), while acetonitrile:water (60:40 v/v) was used for geraniol, in both cases employing a Phenomenex Gemini C18 column (150 × 4.60 mm, 5 µm). The tests were performed in triplicate. Linear regression applied to the points of the calibration curves resulted in the following equations: $y = 4.97732x + 0.04689$ (GRL, **Supplementary Figure S2**) and $y = 0.26518x - 0.01767$ (ICA, **Supplementary Figure S3**).

Morphology of the Nanostructured Lipid Carriers in Suspension and in the Gel

The morphology and size distribution of the nanoparticles were analyzed by atomic force microscopy (AFM), using a Nanosurf EasyScan 2 Basic BT02217 instrument. The suspension of nanoparticles was diluted 20,000 times, deposited on silicon plates, and placed in a desiccator. In the case of the nanoparticles incorporated in gel, a smear of the formulation was used, without dilution. The images (256 × 256 pixels, TIFF format) were obtained using a TapA1-G probe (BudgetSensors, Izgrev, Bulgaria) in tapping mode at 90 Hz. The images were processed using Gwyddion software (Dublin, Ireland).

Differential Scanning Calorimetry

The differential scanning calorimetry technique was used to investigate the interactions among the components of the nanoparticle formulations and the hydrogel matrix. The calorimetric curves were obtained using a Q20 system (TA Instruments), with heating from 0 to 250°C, at 10°C/min, under a flow of nitrogen at 50 ml/min. The results were presented as thermograms showing the melting temperature of a reference material, compared to the studied sample.

Mechanical Properties: Rheological Analysis

The rheological properties of the formulations were analyzed using an oscillatory rheometer (Kinexus lab, Malvern Instruments, United Kingdom) with cone and plate geometry (20 mm diameter, 0.5 rad angle, and 1 mm gap). The repellent

formulations (1 g) were placed in the sample holder and evaluated at skin temperature ($32.0 \pm 0.5^\circ\text{C}$) and using a temperature ramp (from 10 to 50°C , at $5^\circ\text{C}/\text{min}$), at a frequency of 1 Hz and shear stress of 1 Pa. The aim was to determine whether the rheological properties changed after application on the skin or during shelf storage. The samples were carefully applied to the lower plate of the rheometer and allowed to equilibrate for 1 min, prior to analysis. In addition, oscillatory analyses were performed with frequency scanning in the range 0.1–10 Hz, with shear stress of 1 Pa and constant temperature of 32.5°C . Continuous rheological measurements were performed using shear rates in the range $0.01\text{--}500\text{ s}^{-1}$, for both the ascending and descending curves, with each step having duration of 120 s.

Determination of the Release Profiles and Mechanisms of Release of the Repellents From the Formulations

Investigation of the *in vitro* release profiles for the liquid and gel NLCs formulations employed a vertical diffusion system with a regenerated cellulose membrane (MWCO 12000–14000 pores) coupled to two compartments (donor and receptor). The system was operated under sink conditions. A volume of 100 ml of 3% PVA was used as co-eluent in the receptor solution. The system was kept in a closed environment, at a controlled temperature of 32.5°C , under constant magnetic stirring during the assay (Ikura et al., 2019). The NLC formulations (5% ICA +2% GRL) were applied (1 g of gel, or 1 ml of suspension) in the donor compartment, after which 1 ml aliquots were periodically removed from the receptor compartment, replacing the volume removed by co-eluent solution. Geraniol and icaridin were quantified by the HPLC method, as described above.

The release profile data for the compounds were analyzed using different mathematical models (zero order, first order, Higuchi, and Korsmeyer-Peppas), in order to investigate the mechanisms of release of the active agents from the different systems.

In vitro Determination of Permeation Profiles

Assays of the permeation kinetics were performed using a two-compartment vertical diffusion cell system (Microette Plus, Hanson Research, Chatsworth, CA, United State) with donor compartment (1.72 cm^2 permeation area) and receptor compartment (7 ml) separated by a synthetic membrane (Strat-M[®], 25 mm diameter, Millipore Co., United State) (Uchida et al., 2015; Kaur et al., 2018).

Samples of the liquid formulations (0.6 ml) or hydrogels (0.6 g) were placed in the donor compartment, in contact with the membrane, and the receptor compartment was filled with 0.1 M sodium phosphate buffer (pH 7.4). The system was kept under magnetic stirring (350 rpm), at $32.5 \pm 0.5^\circ\text{C}$. Aliquots were removed at times of 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h, for analysis using HPLC. All the formulations were evaluated in triplicate. The cumulative amount of repellent permeated, Q ($\mu\text{g}/\text{cm}^2$), that permeated through the Strat-M per unit area was plotted as a function of time (min). For data analysis, the flux values were obtained from the slope of the concentration versus

time curve in the linear portion of plot (interval between 15 and 450 min). The data were analyzed according to Equation 1.

$$J = P \times C_d \quad (1)$$

where, J ($\%.\text{cm}^{-2}.\text{min}^{-1}$) is the flux of the compound through the membrane, P ($\text{cm}.\text{min}^{-1}$) is the permeability coefficient, and C_d ($\%.\text{cm}^{-3}$) is the concentration of the compound in the donor compartment (de Araujo et al., 2010). Lag time (t_{lag}) was calculated from the intercept ($Y = 0$).

Accelerated Stability Assays and Microbiological Control of the Nanostructured Lipid Carriers in Gel

The accelerated stability study of the NLC formulations in gel was performed according to the Harmonized Tripartite Guideline for Stability Testing of New Drug Substances and Products Q1A (R2) established by the International Conference on Harmonization, Geneva, Switzerland (European Medicines Agency, 2018) at the following conditions: $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for 6 months. The parameters analyzed during this period were color, odor, density, pH, and viscosity (ISO 6658, 2005). The color and odor were evaluated by observation of macroscopic changes. The hydrogen ion potential (pH) was determined using an electrode immersed in an aqueous dispersion containing 10% of the sample. The specific density was determined from the mass and volume of the sample. In addition, the stabilities of the formulations were periodically evaluated by oscillatory frequency analysis, considering the elastic (G') and viscous (G'') moduli. The formulations were stored in tubes (80% LDPE +20% HDPE), under controlled conditions of temperature ($40 \pm 2^\circ\text{C}$) and relative humidity ($75 \pm 5\%$ RH).

Microbiological control of non-sterile cosmetic formulations requires determination of the total number of microorganisms present (ISO 16212, 2017; ISO 21149, 2017), as well as identification of pathogens such as *Salmonella* sp., *Escherichia coli*, *Staphylococcus* sp. (ISO 22718, 2015), and *Pseudomonas aeruginosa* (ISO 22717, 2015), which should not be present (Dao et al., 2018; Sharma Bora et al., 2019). Analyses performed on the day of preparation and after 6 months.

Statistical Analysis

The data were presented as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used for evaluation of normality, followed by application of the two-way ANOVA test for multiple variables, considering a significance level of 0.05. The software used was GraphPad Prism 8.0.1.

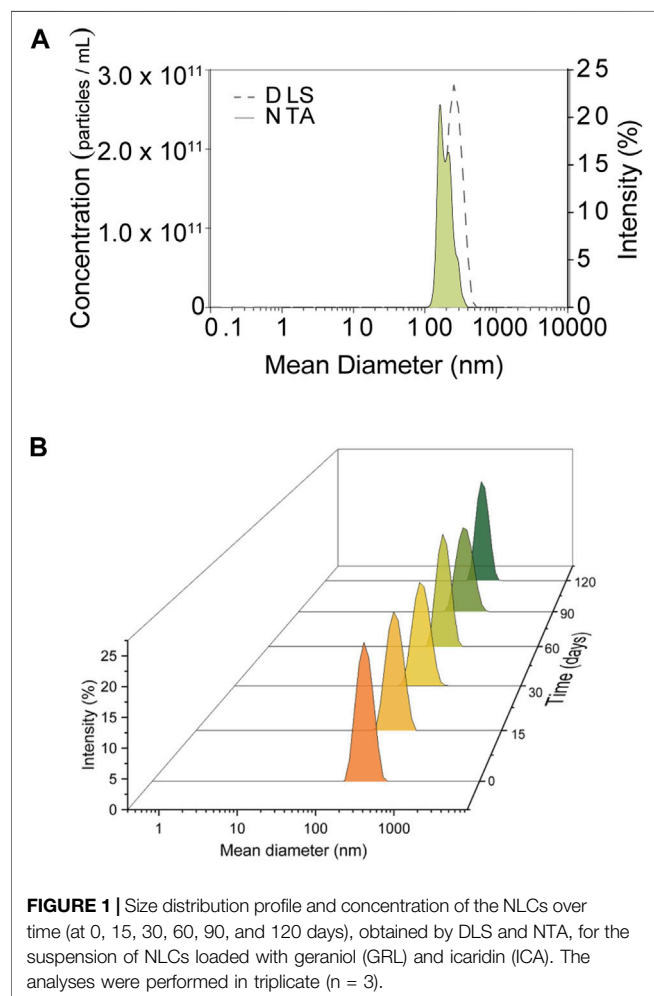
RESULTS AND DISCUSSION

Characterization and Stability of the Nanostructured Lipid Carriers in Suspension

The results of the physicochemical characterizations of the systems are provided in Table 1 and Figure 1. The control formulation (NLC) presented an average diameter of $271 \pm$

TABLE 1 | Characterization of the NLCs without repellents and containing geraniol (GRL) and icaridin (ICA): Dynamic light scattering (DLS) analyses of mean diameter (MD) and polydispersity index (PDI); nanotracking analysis (NTA) of mean diameter (MD) and NLCs concentration (CT); microelectrophoresis analysis of zeta potential (ZP); and ultracentrifugation/HPLC analysis of encapsulation efficiency (EE). The analyses were performed in triplicate (mean $\pm$ SD).

Formulations	MD		PDI	ZP (mV)	CT ($\times 10^{13}$ particles/mL)	EE (%)
	DLS	NTA				
NLC	272 $\pm$ 5	211.3 $\pm$ 2	0.064 $\pm$ 0.04	-17 $\pm$ 1.2	2.0 $\pm$ 0.08	—
NLC_ICA + GRL	252 $\pm$ 5	209.4 $\pm$ 1	0.065 $\pm$ 0.02	-14 $\pm$ 0.2	2.5 $\pm$ 0.1	ICA 99.4 GRL 99.9



5 nm, while the value for the repellent formulation (NLC_ICA + GRL) was 252 ± 5 nm, both with low polydispersity index (<0.1). Use of the DLS and NTA techniques resulted in different mean sizes of the nanoparticles loaded with repellents, although similar size distributions were obtained using the two techniques, with low polydispersity of 0.46 ± 0.07 , in agreement with the polydispersity calculated from the DLS measurements (Figure 1A). The size distribution profile analyzed for 120 days showed good stability of the system, with no significant differences ($p < 0.05$) in formulation homogeneity (Figure 1B). The negative ZP values were due to the type of surfactant used (PVA), with the stabilization mechanism being

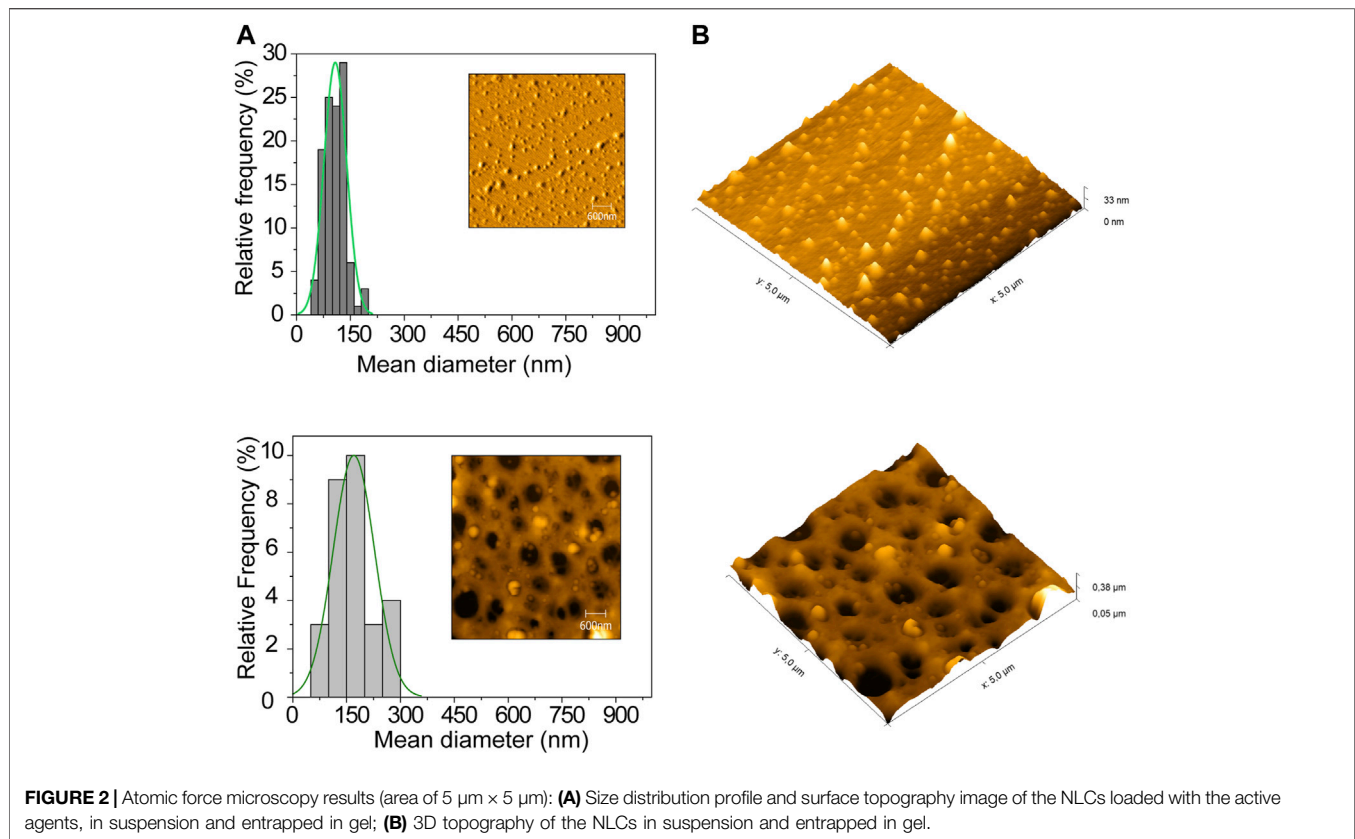
according to steric hindrance, rather than electrostatic effects, which commonly leads to lower ZP values. In a stability study of PLGA nanoparticles loaded with insulin, high molecular weight PVA had a greater stabilizing effect than Tween 20:Tween 80 or low molecular weight PVA (Rawat et al., 2015).

The results obtained for reproducibility and repeatability over 60 days are shown in **Supplementary Figure S1** (Supplementary Material). The stability profile over 60 days revealed insignificant ($p < 0.05$) changes of the mean particle diameter and polydispersity index, indicating a monodisperse formulation and high stability of the system for the five batches analyzed. During the same period, the EE for icaridin ranged between $99.4 \pm 0.3\%$ and $87 \pm 0.1\%$, while the EE for geraniol ranged between $99.9 \pm 0.04\%$ and $97 \pm 0.06\%$. The NLC characterization results agreed with some previous studies. For example, Song et al. (2016) investigated the effect of the liquid/solid lipid ratio on NLCs loaded with furbiprofen, which had average size <200 nm, zeta potential < -20 mV, and encapsulation efficiency $>78\%$. No changes in these parameters were observed after 90 days of storage at temperatures of 4 and 25°C .

Pinto et al. (2014) investigated NLCs loaded with methotrexate, for topical use in the treatment of psoriasis. In agreement with the present work, the average particle size was 274–298 nm, with PDI <0.25 . The mean pH of the formulations was 6.2 ± 0.3 , with no significant changes observed over time. Similarly, Motawea et al. (2018) developed an NLCs formulation for topical use, obtaining pH values between 5.6 and 6.4, which were within the permissible range.

Morphologies of the Nanostructured Lipid Carriers in Suspension and Gel

In addition to the DLS and NTA analyses, atomic force microscopy was used to evaluate the size distributions and morphologies of the nanoparticles. The nanoparticles presented mean diameters between 90 and 150 nm, as shown in **Figure 2A** (analysis of 100 particles using Gwyddion software). In this case, the size was smaller than obtained using DLS and NTA, due to the specific characteristics of the different techniques. For analysis using AFM, the sample must be dried, which can lead to changes in the morphology, especially regarding particle organization and distribution, depending on the concentration and osmolarity of the particles (Sitterberg et al., 2010). On the other hand, the low polydispersity index was in agreement with the DLS and NTA results. **Figure 2B** shows the 3D topography of the NLCs in gel (HPMC), confirming that the NLCs were inserted in the polymeric gel network.



It should be noted that the mean size of the NLCs incorporated in the gel was compatible with that of the NLCs in suspension. Therefore, the incorporation process did not lead to alteration of the NLC structure, size, or morphology, and there was no effect on particle aggregation, showing that the NLCs remained stable after incorporation in the hydrogel. There have been various reports of AFM used to analyze the topography of hydrogels (Helfricht et al., 2017). El-Mekawy and Jassas (2017) described the synthesis of insulin systems employing ZnO nanoparticles in chitosan hydrogel, with analyses using AFM, transmission electron microscopy (TEM), and scanning electron microscopy (SEM), showing that the film exhibited porosity and that nanoparticles were present in the matrix, corroborating the present findings.

Differential Scanning Calorimetry Analyses

The DSC thermograms were used to investigate the interactions of the various formulation components, as well as the effect of the preparation process on the components. The thermogram obtained for tripalmitin, the main component of the NLC lipid matrix, showed an endothermic peak at around 66°C (Supplementary Figure S4 I, Supplementary Material). The other thermograms corresponding to the components of the control NLCs (Supplementary Figure S4 II), the NLCs in suspension (Supplementary Figure S4 III), and the NLCs in gel (Supplementary Figure S4 V) showed a shift of the phase transition temperature, due to interaction between the charges of the polymeric components and the formation of nanoparticles. In

addition, the presence of the active agents (icaridin and geraniol) led to a reduction of the endothermic peak temperature from 61°C (without the active agents) to 56°C (with icaridin and geraniol) (Supplementary Table S1). The thermogram for HPMC showed a broad endothermic signal in the temperature range between 60 and 130°C, which could be attributed to the water loss (Supplementary Figure S4 IV). However, this peak did not appear in the thermogram for the formulation of NLCs incorporated in the HPMC gel, which could have been related to the complete dispersion of the NLCs in the polymeric matrix of the gel (McPhillips et al., 1999; Jayaramudu et al., 2021).

According to the literature, this behavior could be indicative of changes in the structural organization of the lipid crystalline network following interaction with the active agents, leading to a reduction of the tripalmitin melting point (Sarangi et al., 2018). Furthermore, the mixing of two lipids usually leads to a lower melting temperature and lower enthalpy, due to structural disruption of the solid lipid (Doktorovova and Souto, 2009). A similar finding was reported by Kudarha et al. (2015), who analyzed the structure of NLCs with encapsulated bicalutamide (a drug used to treat prostate cancer) and observed a slight change in the melting point of the lipid matrix after interaction with the drug.

In another study evaluating the nanoencapsulation of geraniol in chitosan/gum arabic nanoparticles, it was also observed that the presence of geraniol decreased the nanoparticle endothermic peak, probably due to interaction of the matrix with the hydrophobic encapsulated component (de Oliveira et al.,

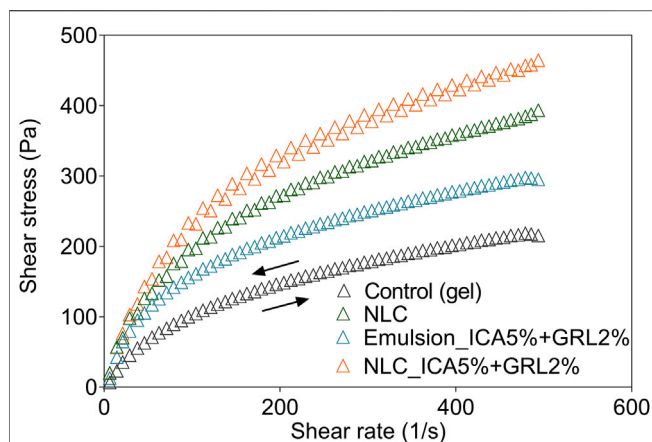


FIGURE 3 | Rheograms of shear stress variation according to shear rate for the control HPMC hydrogel, the NLCs formulation without active ingredients, the emulsion with 2% geraniol (GRL) and 5% icaridin, and the NLCs formulation with 2% geraniol and 5% icaridin.

2018). Teeranachaideekul et al. (2007) used DSC to investigate the interaction of ascorbyl palmitate with the NLC lipid matrix, observing a shift of the maximum peak (56.9°C), because the active agent altered the crystallization process of the NLC lipid matrix.

Rheological Analysis

Rheological analysis of the formulations provided information concerning the effects of composition and storage time on the mechanical properties of the hydrogels before and after incorporation of the NLCs. For this, flow and oscillatory analyses were used to obtain parameters including the **Table 2** elastic modulus (G'), viscous modulus (G''), and apparent viscosity (η^*).

The flow curve profiles were indicative of pseudoplastic behavior, characteristic of some non-Newtonian fluids, where high shear rates result in low flow resistance of the polymer particles. The graph of the flow curves shows the ascending curves, when the shear rate is increased over time, and the descending curves, when the inverse process occurs. This is known as the hysteresis cycle (Lee et al., 2009). In the present case, the ascending and descending curves were almost superimposed (**Figure 3**).

The calculated flux index (n) values were all lower than 1, indicating that the formulations presented thixotropic behavior, even after incorporation of the NLCs. Transient and reversible interactions between the NLC surfaces and hydrophilic groups in the HPMC structure may act to modulate the self-organization of hydrogels, so that there is complete recovery of their mechanical properties, even under high applied shear stress, as shown by a small hysteresis area (Appel et al., 2015).

It should be noted that pseudoplastic and thixotropic behaviors are essential for topical formulations, so that they deform during application, becoming more fluid and easy to spread, and then return to their initial structural organization, preventing flow of the product away from the application site

TABLE 2 | Rheological parameters G' (elastic modulus), G'' (viscous modulus), and η (viscosity), measured at a frequency of 1 Hz for the control hydrogel (HPMC), the emulsion with 2% GRL and 5% ICA, the NLCs formulation (hydrogel) without active ingredients, and the NLCs formulation (hydrogel) with 2% GRL and 5% ICA.

Parameters	Formulation in HPMC			
	Control	Emulsion_ICA + GRL	NLC	NLC_ICA + GRL
G' (Pa)	10.62	17.120	35.49	45.14
G'' (Pa)	22.11	33.58	51.5	62.73
G'/G''	0.480	0.509	0.689	0.719
η (Pa.s) 32.5°C	5,531	5,999	9,954	12,300

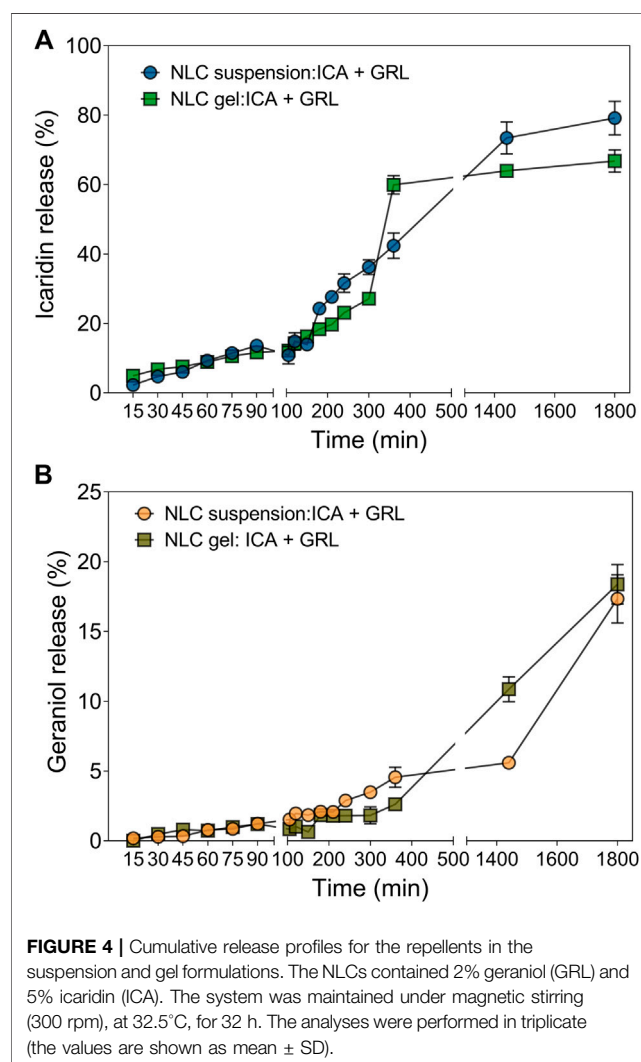
(Malkin and Isayev, 2017). Thixotropic fluids are known to decrease the apparent viscosity with the time of application at a given shear rate, which is reversible phenomena. This decrease in viscosity can be explained by the gradual loss of the structure formed by particles in the dispersed phase, whose bond strength does not resist the action of the applied shear. In addition, when the shear force ceases, the fluid returns to its initial structural conformation (Ghica et al., 2016). A thixotropic formulation tends to have greater physicochemical stability, with little separation of the components during storage (Gaspar and Maia Campos, 2003). The oscillatory temperature analysis and the G' and G'' values show that the NLCs incorporated in gel showed greater stability than emulsion in gel. It was possible to observe that a disruption of the gel occurs at temperatures above 35°C (**Supplementary Figure S5**, Supplementary Material). Corroborating with a study that evaluated the thermo-responsive behavior of HPMC, which verified the abrupt drop in modulus on heating. It also can account for the decreased stability of the fibrils, such that they melt over a range of temperatures ($\geq 40^\circ\text{C}$) which resulted in a decrease in viscosity that was also possible to verify in our study (Lodge et al., 2018). The oscillatory frequency analysis and the G' and G'' values showed that the fluidity decreased in the presence of the NLCs (increase of the G'/G'' ratio), while the viscosity increased at higher shear rates, due to the dispersion of the particulate phase in the gel polymeric matrix (**Table 3**; **Supplementary Figure S6**, Supplementary Material). The values of η , at 1 Hz, were from 5,531 to 12,300 Pa.s, in the order: control < gel emulsion < empty NLCs gel < loaded NLCs gel (**Supplementary Table S4**). Previous work has described the effects of incorporation of solid lipid nanoparticles in gels to increase the viscosity of topical formulations (Lippacher et al., 2004).

Release Behaviors of the Nanostructured Lipid Carriers in Suspension and Gel

The profiles of release of icaridin and geraniol from the NLCs are shown in **Figure 4**. For icaridin, the release percentages were $79 \pm 5\%$ and $67 \pm 3\%$ for the NLCs in the suspension and gel formulations, respectively, after 30 h. For geraniol, the release percentages were $17 \pm 2\%$ and $18 \pm 1\%$ for the NLCs in the suspension and gel formulations, respectively, after 30 h. This difference between the release profiles of the two compounds could have been due to their different physicochemical

TABLE 3 | Parameters for permeation of geraniol (GRL) and icaridin (ICA) across Strat-M[®] membranes from the suspension and gel formulations of NLCs containing 5% ICA +2% GRL.

Formulations	Flux (10^{-4} . μ g.cm $^{-2}$.min $^{-1}$)	Permeability coefficient (10^{-4} .cm.min $^{-1}$)	Lag time (min)
NLC suspension	—	—	—
GRL	1.0	0.5	591.4
ICA	109.1	21.8	23.8
NLC gel	—	—	—
GRL	44.4	22.2	43.9
ICA	1,545	309	24.3



characteristics, since the solubility of icaridin in water is 8.2 g/L, at 20°C, while that of geraniol is 0.1 g/L, at 20°C (PubChem, 2019a; 2019b).

Furthermore, it is important to mention that although the encapsulation of both active ingredients led to a delayed release profile when compared to unencapsulated systems (emulsions) for both suspension and gel formulations this is not a problem from the point of view of the start of repellent activity, since it is a system that co-encapsulates two active ingredients, it is possible to observe that the icaridin that is released more quickly would be

responsible for the immediate repellent effect, while the geraniol would be responsible for the longer repellent effect.

The release of icaridin from the NLCs in suspension was significantly higher ($p < 0.05$) than from the NLCs in the gel. This difference in the release rate was probably due to the entrapment of the nanoparticles in the HPMC polymeric matrix, which has a dilation rate (SR) of 96 h, enabling the integrity of the matrix to be maintained for an extended period (Notario-Pérez et al., 2019). A similar effect was observed for a vaginal film for sustained release of the drug tenofovir (TFV), which prevents HIV infection. In this case, the combination of HPMC and zein containing TFV, at a ratio of 1:5, provided sustained release of the drug for 24 h, while addition of 40% PEG increased the release time to 120 h, due to greater plasticity of the matrix (Notario-Pérez et al., 2019).

Comparison was also made between the NLCs and the emulsion containing the repellents, with sustained release profiles observed in both cases (Supplementary Figure S7, Supplementary Material). The profile of sustained release from the emulsion could be explained by the formation of micellar structures during the process of emulsification with the surfactant (PVA). Recent work has described the use of these micellar structures with drugs that have low water solubility, with the aim of reducing adverse effects (Yukuyama et al., 2017).

Mathematical modeling was used to describe the dissolution/release profiles (Caccavo, 2019), in order to obtain a better understanding of the release kinetics of the repellents. Based on the correlation coefficient (r^2) values, the Korsmeyer-Peppas model provided the best description of the release of icaridin in both liquid and gel formulations (Supplementary Table S5). This model is a derivation of the Higuchi model, but in addition to Fickian release, the release mechanism also includes the contribution from relaxation of the polymeric chains of the gel matrix. The value of n (0.97) obtained for the release of icaridin from the NLCs in the suspension corresponded to non-Fickian release kinetics, probably because the release depended only on diffusion from the NLCs, not involving diffusion through the medium. On the other hand, the value of n (1.48) obtained for icaridin in the gel formulation was indicative of Super-Case II transport, mainly governed by relaxation of the polymeric chains. For geraniol in the NLCs in suspension, the Higuchi model provided the best description of the release, indicating that the release mechanism was predominantly by diffusion. For the release of geraniol present in the gel formulation, the zero-order mathematical model provided the best fit. This model is most suitable when the rate of release of a substance from a hydrophilic matrix such as HPMC is slow.

It could be concluded from application of the mathematical models that the kinetics of icaridin release from both formulations presented a non-Fickian profile, with a higher n value for the gel, probably due to the influence of two release systems, involving the nanoparticles and the hydrogel. In contrast, the release kinetics of geraniol followed Fick's law, although incorporation in the gel led to slower diffusion, according to the zero order model. de Oliveira et al., 2019 evaluated the release mechanisms of botanical compounds encapsulated in zein, where the Korsmeyer-Peppas model provided the best representation of the release profiles. For zein nanoparticles containing geraniol and eugenol, $n < 0.45$ indicated that diffusion was the main release mechanism, while zein nanoparticles containing geraniol and cinnamaldehyde showed an anomalous release profile (a combination of diffusion and case II transport). Another mechanism of geraniol release was studied in the development of a portable device for external use, loaded with geraniol (Kwan et al., 2019). In this case, the zero-order mathematical model was most representative, due to the slow release of geraniol, as also observed in the present work.

Notario-Pérez et al. (2019) reported that for the combination of HPMC and zein with tenofovir, the best description of the profile of release from films was provided by the Higuchi and Hopfenberg model, indicating the importance of diffusion of the drug and erosion of the polymer. Variation of the value of n was observed, where the highest values were attributed to structural alteration of the polymer matrix, while lower values indicated the predominance of diffusion of the drug, in agreement with the present findings.

In vitro Permeation Profiles

Comparison was made of the permeation profiles of the emulsions (5% ICA +2% GRL) and the NLCs (5% ICA +2% GRL) in suspension and gel. There have been many studies concerning the optimization of transdermal permeation systems employing formulations in the form of sprays, gels, and creams. In the case of a repellent formulation, good performance is indicated by prolonged release, low doses, low frequency of applications, and low permeation rates.

The Strat-M[®] synthetic membrane system has been used in cutaneous permeation experiments, due to its easy availability, reproducible results, and similarity to human skin (Uchida et al., 2015; Grillo et al., 2019). Here, the permeation of the repellents was evaluated using the NLCs formulations in suspension and gel. The results are summarized in **Figure 5** and **Table 3**. All the formulations presented gradual permeation during the experiment (24 h). There was a significant difference ($p < 0.05$) between the permeation (during 24 h) of icaridin from the NLCs in suspension ($9.5 \pm 0.1 \mu\text{g}/\text{cm}^2$) and from the gel formulation ($171 \pm 9 \mu\text{g}/\text{cm}^2$). A possible explanation was greater membrane hydration of the NLCs in the hydrogel, which acted to increase penetration of the compound. The degree of porosity and gel hydration can influence the solubility of active compounds in water, leading to rapid release and reduced bioavailability (Doktorovova and Souto, 2009; Desfrancois et al., 2018; Iikura et al., 2019).

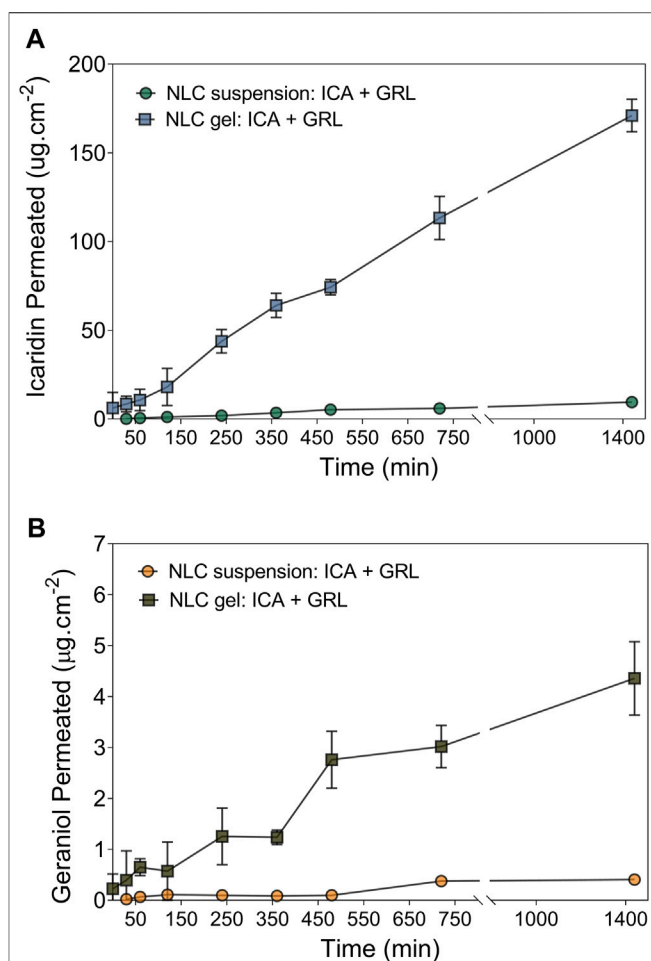
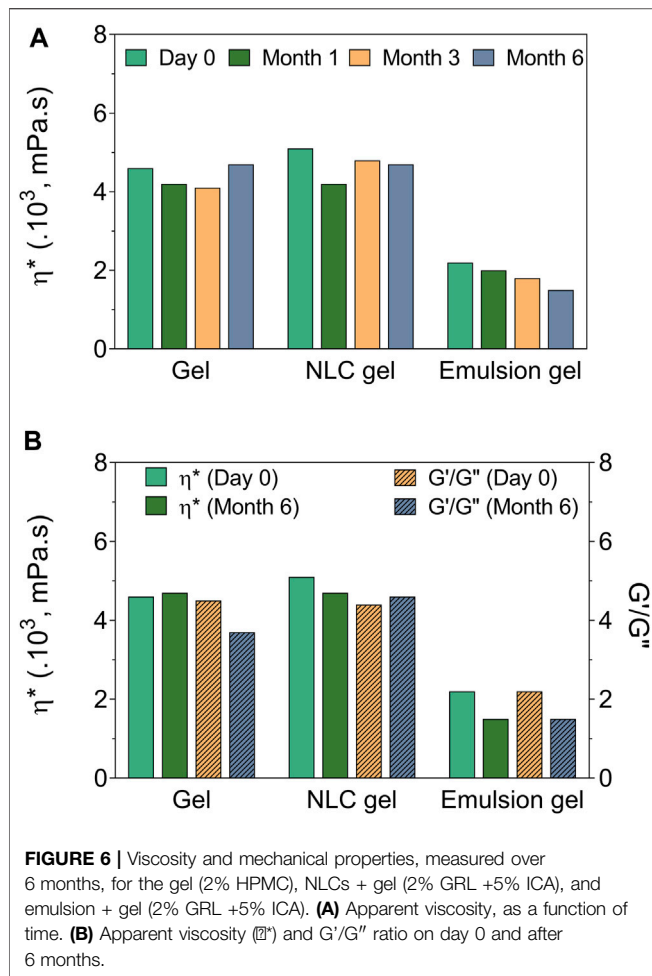


FIGURE 5 | Permeation profiles of the repellents across Strat-M[®] membranes (mean $\pm$ SD, $n = 3$ per formulation), obtained using a vertical Franz diffusion cell apparatus with the formulations of NLCs containing 5% ICA +2% GRL. **(A)** Icaridin permeated in suspension and gel. **(B)** Geraniol Permeated in suspension and gel.

Furthermore, HPMC provides film formation increasing the surface area available for permeation (Aho et al., 2017).

The lag time of icaridin to be similar in both formulations. On the other hand, the lag time of geraniol in NLC suspension (591.4 min) was higher than NLC gel (44.4 min). The fluxes and permeability coefficients obtained for icaridin showed significant differences between the formulations in suspension ($109.1 \times 10^{-4} \mu\text{g}/\text{cm}^2 \text{min}^{-1}$ and $21.8 \times 10^{-4} \text{cm}^2 \text{min}^{-1}$) and gel ($1,545 \times 10^{-4} \mu\text{g}/\text{cm}^2 \text{min}^{-1}$ and $309 \times 10^{-4} \text{cm}^2 \text{min}^{-1}$). Similarly, there were significant differences for the permeation of geraniol in suspension ($1.0 \times 10^{-4} \mu\text{g}/\text{cm}^2 \text{min}^{-1}$ and $0.5 \times 10^{-4} \text{cm}^2 \text{min}^{-1}$) and gel ($44.4 \times 10^{-4} \mu\text{g}/\text{cm}^2 \text{min}^{-1}$ and $22.2 \times 10^{-4} \text{cm}^2 \text{min}^{-1}$), although the values were lower, compared to icaridin. These results were consistent with the release profiles and could be explained by the fact that the water solubility of icaridin (8.2 mg/ml) is 80 times higher than that of geraniol (0.1 mg/ml) (PubChem, 2019b; 2019a).



The action of a repellent mainly occurs by its volatilization. Therefore, since the vapor pressure of icaridin (4×10^{-4} mm Hg) is around 100-fold lower than that of geraniol (3×10^{-2} mm Hg), the vaporization of icaridin present in the NLCs gel formulation could proceed in a more sustained manner.

Although some studies have shown that NLCs can enhance the permeation of drugs through the skin (Junyaprasert et al., 2009; Na et al., 2020), the rates of release and permeation depend on the physicochemical properties of the compounds, the polarity of the solvents, the size of the nanoparticles, and the matrices within which they are dispersed. In the present work, low percentages of the compounds permeated through the Strat-M membrane, showing that these repellent formulations could potentially reduce the amounts of active agents permeated through the epidermal barrier, consequently reducing their toxicity. Wissing and Müller (2001) found that a topical formulation of SLNs loaded with tocopherol acetate formed a film containing the active agent, which reduced skin penetration. In other work by the same researchers, the penetration of oxybenzone was evaluated *in vitro* (using a Franz cell) and *in vivo* (by tape stripping), which showed that the permeation rate was related to the concentration of the active agent in the formulation and decreased by 30–60% when SLNs were used. Similar considerations could apply to the systems

developed here, where the retention of the NLCs in the HPMC matrix resulted in modulation of permeation of the compounds, so they would be retained in the uppermost layers of the skin, providing a prolonged repellent effect.

Accelerated Stability Study and Microbiological Control of Nanostructured Lipid Carriers in Gel

Evaluation was made of the mechanical properties and viscosity of the formulations, according to storage time, in order to correlate the results with those obtained previously. Figure 6 shows the temporal variation of the apparent viscosity for the formulations, revealing that for the NLCs incorporated in hydrogel, there was no significant alteration of this parameter after 6 months of storage. In contrast, the emulsion incorporated in hydrogel showed a progressive decrease of viscosity, indicating lower physicochemical stability of this system, compared to the NLCs in gel (Figure 6A). The G'/G'' ratios indicated a predominance of the elastic modulus (G'), relative to the viscous modulus (G''), after preparation and up to 6 months of storage, demonstrating the superior maintenance of the structural organization of the NLCs formulation, compared to the emulsion hydrogel (Figure 6B). The organoleptic characteristics of the NLCs formulation, such as color, odor, appearance, and homogeneity, did not change significantly during 6 months, as indicated in Supplementary Figure S8 (green arrow). On the other hand, the emulsion presented phase separation, in agreement with the viscosity analysis and indicating lower physicochemical stability (Supplementary Figure S6, white arrow).

All the formulations presented a significant ($p < 0.05$) decrease of the pH after 60 days (Supplementary Table S3). This could be explained by increased solubilization of the repellent compounds, or by interactions among the formulation components. It is also possible that the formulations could have been degraded by hydrolysis, which indicated the need for microbiological evaluation, for the purpose of quality control. It is known that the skin has a slightly acidic pH, which provides bactericidal and fungicidal activity on the surface. Therefore, it could be concluded that the NLCs and emulsion formulations complied with established safety standards (pH 4.00–7.00) (Hashem et al., 2011; Sharma Bora et al., 2019).

The densities of the formulations were evaluated during the same period (Supplementary Table S4), revealing significant increases ($p < 0.05$). This could have been due to the loss of water, consequently decreasing the volume. The stability was not affected by the incorporation of air (which would increase the volume), considering the increases in density from 1.04 to 1.11 g/cm³ and from 0.99 to 1.11 g/cm³ for the NLCs and emulsion formulations, respectively. Although stability guidelines do not provide a default density value, a typical value is around 1.003 g/cm³. Sensorial analysis showed that there were no significant changes in the organoleptic characteristics of the NLCs formulation (considering color, odor, appearance, and homogeneity). As noted by Isaac et al. (2008), the homogeneity and color of cosmetic products are important aspects from the commercial perspective, since they can influence the decision of the consumer at the time of purchase.

The assays of microbial growth showed an absence of aerobic bacteria and the presence of fungi and yeasts (<1 CFU/g) at levels within the limits established for non-sterile products (Supplementary Table 5S). The results for microbial counts and pathogens showed compliance with quality assurance specifications. Therefore, the increase of pH in the formulations was not associated with contamination.

CONCLUSION

A system was developed for simultaneous loading with two repellent compounds, based on the incorporation of NLCs in a polymeric hydrogel. Analyses were made of the compositions, characteristics, and physicochemical stabilities of formulations containing NLCs loaded with geraniol and icaridin, in suspension and incorporated in a hydrogel. The stability profile obtained during 120 days for the NLCs formulation revealed that there were no significant ($p > 0.05$) changes of the mean particle diameter or polydispersity index, indicating high colloidal stability of the system. The high efficiency of encapsulation (>99%) of the compounds in the NLCs resulted in a prolonged release system, with the synthetic and natural repellents being released at different rates, favoring possible synergistic action and an increased spectrum of repellent activity. The formulations presented suitable rheological characteristics, with pseudoplastic and thixotropic behavior that ensured easy spreading, as well as satisfactory physicochemical stability. The *in vitro* permeation profiles showed gradual penetration of the compounds, but with low flux and permeation coefficient values for both geraniol and icaridin, suggesting that the rate of penetration through the epidermal barrier would be low.

REFERENCES

- Aho, J., Halme, A., Boetker, J., Water, J. J., Bohr, A., Sandler, N., et al. (2017). The Effect of HPMC and MC as Pore Formers on the Rheology of the Implant Microenvironment and the Drug Release *In Vitro*. *Carbohydr. Polym.* 177, 433–442. doi:10.1016/j.carbpol.2017.08.135
- Appel, E. A., Tibbitt, M. W., Webber, M. J., Mattix, B. A., Veisheh, O., and Langer, R. (2015). Self-assembled Hydrogels Utilizing Polymer-Nanoparticle Interactions. *Nat. Commun.* 6, 6295. doi:10.1038/ncomms7295
- Binder, L., Mazál, J., Petz, R., Klang, V., and Valenta, C. (2019). The Role of Viscosity on Skin Penetration from Cellulose Ether-Based Hydrogels. *Skin Res. Technol.* 25, 725–734. doi:10.1111/srt.12709
- Caccavo, D. (2019). An Overview on the Mathematical Modeling of Hydrogels' Behavior for Drug Delivery Systems. *Int. J. Pharm.* 560, 175–190. doi:10.1016/j.jipharm.2019.01.076
- CDC (2019). Prevention Dengue. Available at: <https://www.cdc.gov/dengue/prevention/index.html> (Accessed June 11, 2021).
- Chang, C. P., and Dobashi, T. (2003). Preparation of Alginate Complex Capsules Containing eucalyptus Essential Oil and its Controlled Release. *Colloids Surf. B: Biointerfaces* 32, 257–262. doi:10.1016/j.colsurfb.2003.07.002
- da Saúde, M. (2019). *Guia de Vigilância em Saúde*. 3ª Edição. Brasília: Executiva. Available at: https://bvsms.saude.gov.br/bvs/publicacoes/guia_vigilancia_saude_3ed.pdf (Accessed June 8, 2021).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

DCA, CR, DRA and LF designed the experiment. LP, MD and GV performed the accelerated stability assay and microbiological control. EC and JD helped in the rheological and permeation studies. DCA conducted the rest of the experiment. DCA, EC, JD and LF drafted and revised the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors are grateful for financial support provided by MCTIC-CNPq/MEC-CAPES/MS-Decit/FNDCT No 14/2016—Prevenção e Combate ao Vírus Zika (process #440350/2016-6). The authors thank the São Paulo State Research Foundation (FAPESP) for scholarships awarded to DA (#2018/14734-0), CR (#2018/02404-5), EC (2017/24402-1), JO (#2018/21142-1 #2020/12779-6). We also thank the Ipanema Veterinary Institution for collaboration.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2021.760682/full#supplementary-material>

- Dao, H., Lakhani, P., Police, A., Kallakunta, V., Ajjarapu, S. S., Wu, K. W., et al. (2018). Microbial Stability of Pharmaceutical and Cosmetic Products. *AAPS PharmSciTech* 19, 60–78. doi:10.1208/s12249-017-0875-1
- de Araujo, D. R., Padula, C., Cereda, C. M., Tófoli, G. R., Brito, R. B., de Paula, E., et al. (2010). Bioadhesive Films Containing Benzocaine: Correlation between *In Vitro* Permeation and *In Vivo* Local Anesthetic Effect. *Pharm. Res.* 27, 1677–1686. doi:10.1007/s11095-010-0151-5
- de Oliveira, J. L., Campos, E. V. R., Germano-Costa, T., Lima, R., Vechia, J. F. D., Soares, S. T., et al. (2019). Association of Zein Nanoparticles with Botanical Compounds for Effective Pest Control Systems. *Pest Manag. Sci.* 75, 1855–1865. doi:10.1002/ps.5338
- de Oliveira, J. L., Campos, E. V. R., Pereira, A. E. S., Nunes, L. E. S., da Silva, C. C. L., Pasquoto, T., et al. (2018). Geraniol Encapsulated in Chitosan/Gum Arabic Nanoparticles: A Promising System for Pest Management in Sustainable Agriculture. *J. Agric. Food Chem.* 66, 5325–5334. doi:10.1021/acs.jafc.8b00331
- Desfrancois, C., Auzély, R., and Texier, I. (2018). Lipid Nanoparticles and Their Hydrogel Composites for Drug Delivery: A Review. *Pharmaceuticals (Basel)* 11. doi:10.3390/ph11040118
- Doktorovova, S., and Souto, E. B. (2009). Nanostructured Lipid Carrier-Based Hydrogel Formulations for Drug Delivery: a Comprehensive Review. *Expert Opin. Drug Deliv.* 6, 165–176. doi:10.1517/17425240802712590
- El-Mekawy, R. E., and Jassas, R. S. (2017). Recent Trends in Smart and Flexible Three-Dimensional Cross-Linked Polymers: Synthesis of Chitosan-ZnO Nanocomposite Hydrogels for Insulin Drug Delivery. *Medchemcomm* 8, 897–906. doi:10.1039/c6md00580b

- European Medicines Agency (2018). ICH Q1A (R2) Stability Testing of New Drug Substances and Products. *European Medicines Agency*. Available at: <https://www.ema.europa.eu/en/ich-q1a-r2-stability-testing-new-drug-substances-drug-products> (Accessed August 12, 2021).
- Fang, J. Y., Fang, C. L., Liu, C. H., and Su, Y. H. (2008). Lipid Nanoparticles as Vehicles for Topical Psoralen Delivery: Solid Lipid Nanoparticles (SLN) versus Nanostructured Lipid Carriers (NLC). *Eur. J. Pharm. Biopharm.* 70, 633–640. doi:10.1016/j.ejpb.2008.05.008
- Gaspar, L. R., and Maia Campos, P. M. (2003). Rheological Behavior and the SPF of Sunscreens. *Int. J. Pharm.* 250, 35–44. doi:10.1016/s0378-5173(02)00462-3
- Ghica, M. V., Hirjau, M., Lupuleasa, D., and Dinu-Pirvu, C. E. (2016). Flow and Thixotropic Parameters for Rheological Characterization of Hydrogels. *Molecules* 21, 786. doi:10.3390/molecules21060786
- Grillo, R., Dias, F. V., Querobino, S. M., Alberto-Silva, C., Fraceto, L. F., de Paula, E., et al. (2019). Influence of Hybrid Polymeric Nanoparticle/thermosensitive Hydrogels Systems on Formulation Tracking and *In Vitro* Artificial Membrane Permeation: A Promising System for Skin Drug-Delivery. *Colloids Surf. B Biointerfaces* 174, 56–62. doi:10.1016/j.colsurfb.2018.10.063
- Guarner, J., and Hale, G. L. (2019). Four Human Diseases with Significant Public Health Impact Caused by Mosquito-Borne Flaviviruses: West Nile, Zika, Dengue and Yellow Fever. *Semin. Diagn. Pathol.* 36, 170–176. doi:10.1053/j.semdp.2019.04.009
- Hashem, F. M., Shaker, D. S., Ghorab, M. K., Nasr, M., and Ismail, A. (2011). Formulation, Characterization, and Clinical Evaluation of Microemulsion Containing Clotrimazole for Topical Delivery. *AAPS PharmSciTech* 12, 879–886. doi:10.1208/s12249-011-9653-7
- Hay, W. T., Fanta, G. F., Peterson, S. C., Thomas, A. J., Utt, K. D., Walsh, K. A., et al. (2018). Improved Hydroxypropyl Methylcellulose (HPMC) Films through Incorporation of Amylose-Sodium Palmitate Inclusion Complexes. *Carbohydr. Polym.* 188, 76–84. doi:10.1016/j.carbpol.2018.01.088
- Helfricht, N., Mark, A., Behr, M., Bernet, A., Schmidt, H. W., and Papastavrou, G. (2017). Writing with Fluid: Structuring Hydrogels with Micrometer Precision by AFM in Combination with Nanofluidics. *Small* 13. doi:10.1002/sml.201700962
- Iikura, H., Uchida, K., Ogawa-Fuse, C., Bito, K., Naitou, S., Hosokawa, M., et al. (2019). Effects of Temperature and Humidity on the Skin Permeation of Hydrophilic and Hydrophobic Drugs. *AAPS PharmSciTech* 20, 264. doi:10.1208/s12249-019-1481-1
- Isaac, V., Cefali, L. C., Chiari, B. G., Oliveira, C., Salgado, H. R., and Corrêa, M. (2008). Protocolo para ensaios físico-químicos de estabilidade de fitocosméticos. *undefined*. Available at: <http://paper/Protocolo-para-ensaios-f%C3%ADsico-qu%C3%ADmicos-de-de-Isaac-Cefali/fa0ba805b342e0aae6b4db73c30c8c4a1e033f> [Accessed January 21, 2021].
- ISO 10993-5 (2009). Biological Evaluation of Medical Devices — Part 5: Tests for *In Vitro* Cytotoxicity. *ISO*. Available at: <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/03/64/36406.html> (Accessed September 15, 2021).
- ISO 16212 (2017). Cosmetics — Microbiology — Enumeration of Yeast and Mould. *ISO 16212* Available at: <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/07/22/72241.html> (Accessed August 16, 2021).
- ISO 21149 (2017). Cosmetics — Microbiology — Enumeration and Detection of Aerobic Mesophilic Bacteria. International Organization for Standardization. Available at: <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/07/22/72240.html> (Accessed August 13, 2021).
- ISO 22717 (2015). Cosmetics — Microbiology — Detection of *Pseudomonas aeruginosa*. International Organization For Standardization. Available at: <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/06/83/68312.html> (Accessed August 13, 2021).
- ISO 22718 (2015). Cosmetics — Microbiology — Detection of *Staphylococcus aureus*. International Organization For Standardization. Available at: <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/06/83/68313.html> (Accessed August 13, 2021).
- ISO 6658 (2005). Sensory Analysis — Methodology — General Guidance. International Organization For Standardization. Available at: <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/06/55/65519.html> (Accessed August 13, 2021).
- Jayaramudu, T., Varaprasad, K., Pyarasi, R. D., Reddy, K. K., Akbari-Fakhrabadi, A., Carrasco-Sánchez, V., et al. (2021). Hydroxypropyl Methylcellulose-Copper Nanoparticle and its Nanocomposite Hydrogel Films for Antibacterial Application. *Carbohydr. Polym.* 254, 117302. doi:10.1016/j.carbpol.2020.117302
- Junyaprasert, V. B., Teeranachaideekul, V., Souto, E. B., Boonme, P., and Müller, R. H. (2009). Q10-loaded NLC versus Nanoemulsions: Stability, Rheology and *In Vitro* Skin Permeation. *Int. J. Pharm.* 377, 207–214. doi:10.1016/j.jipharm.2009.05.020
- Kaur, L., Singh, K., Paul, S., Singh, S., and Jain, S. K. (2018). A Mechanistic Study to Determine the Structural Similarities between Artificial Membrane Strat-M™ and Biological Membranes and its Application to Carry Out Skin Permeation Study of Amphotericin B Nanoformulations. *AAPS PharmSciTech* 19, 1606–1624. doi:10.1208/s12249-018-0959-6
- Khosa, A., Reddi, S., and Saha, R. N. (2018). Nanostructured Lipid Carriers for Site-specific Drug Delivery. *Biomed. Pharmacother.* 103, 598–613. doi:10.1016/j.biopha.2018.04.055
- Kudharha, R., Dhas, N. L., Pandey, A., Belgamwar, V. S., and Ige, P. P. (2015). Box-Behnken Study Design for Optimization of Bicalutamide-Loaded Nanostructured Lipid Carrier: Stability Assessment. *Pharm. Dev. Technol.* 20, 608–618. doi:10.3109/10837450.2014.908305
- Kuri-Morales, P. A., Correa-Morales, F., González-Acosta, C., Sánchez-Tejeda, G., Moreno-García, M., Dávalos-Becerril, E., et al. (2017). Repellency of 29 Synthetic and Natural Commercial Topical Insect Repellents against *Aedes aegypti* (Diptera: Culicidae) in Central Mexico. *J. Med. Entomol.* 54, 1305–1311. doi:10.1093/jme/tjx076
- Kwan, M. W. C., Bosak, A., Kline, J., Pita, M. A., Giel, N., Pereira, R. M., et al. (2019). A Low-Cost, Passive Release Device for the Surveillance and Control of Mosquitoes. *Int. J. Environ. Res. Public Health* 16. doi:10.3390/ijerph16091488
- Lammari, N., Louaer, O., Meniai, A. H., and Elaissari, A. (2020). Encapsulation of Essential Oils via Nanoprecipitation Process: Overview, Progress, Challenges and Prospects. *Pharmaceutics* 12. doi:10.3390/pharmaceutics12050431
- Lee, C. H., Moturi, V., and Lee, Y. (2009). Thixotropic Property in Pharmaceutical Formulations. *J. Control. Release* 136, 88–98. doi:10.1016/j.jconrel.2009.02.013
- Leta, S., Beyene, T. J., De Clercq, E. M., Amenu, K., Kraemer, M. U. G., and Revie, C. W. (2018). Global Risk Mapping for Major Diseases Transmitted by *Aedes aegypti* and *Aedes albopictus*. *Int. J. Infect. Dis.* 67, 25–35. doi:10.1016/j.ijid.2017.11.026
- Lippacher, A., Müller, R. H., and Mäder, K. (2004). Liquid and Semisolid SLN Dispersions for Topical Application: Rheological Characterization. *Eur. J. Pharm. Biopharm.* 58, 561–567. doi:10.1016/j.ejpb.2004.04.009
- Lodge, T. P., Maxwell, A. L., Lott, J. R., Schmidt, P. W., McAllister, J. W., Morozova, S., et al. (2018). Gelation, Phase Separation, and Fibril Formation in Aqueous Hydroxypropylmethylcellulose Solutions. *Biomacromolecules* 19, 816–824. doi:10.1021/acs.biomac.7b01611
- Maia, M. F., and Moore, S. J. (2011). Plant-based Insect Repellents: a Review of Their Efficacy, Development and Testing. *Malar. J.* 10 (Suppl. 1), S11. doi:10.1186/1475-2875-10-S1-S11
- Malkin, A. Y., and Isayev, A. I. (2017). *Rheology: Concepts, Methods, and Applications*. Elsevier.
- McPhillips, H., Craig, D. Q., Royall, P. G., and Hill, V. L. (1999). Characterisation of the Glass Transition of HPMC Using Modulated Temperature Differential Scanning Calorimetry. *Int. J. Pharm.* 180, 83–90. doi:10.1016/S0378-5173(98)00407-4
- Misni, N., Nor, Z. M., and Ahmad, R. (2016). New Candidates for Plant-Based Repellents against *Aedes aegypti*. *J. Am. Mosq. Control. Assoc.* 32, 117–123. doi:10.2987/moco-32-02-117-123.1
- Motawea, A., Borg, T., and Abd El-Gawad, A. E. H. (2018). Topical Phenytoin Nanostructured Lipid Carriers: Design and Development. *Drug Dev. Ind. Pharm.* 44, 144–157. doi:10.1080/03639045.2017.1386204
- Na, Y. G., Huh, H. W., Kim, M. K., Byeon, J. J., Han, M. G., Lee, H. K., et al. (2020). Development and Evaluation of a Film-Forming System Hybridized with Econazole-Loaded Nanostructured Lipid Carriers for Enhanced Antifungal Activity against Dermatophytes. *Acta Biomater.* 101, 507–518. doi:10.1016/j.actbio.2019.10.024
- NanoScoping (2020a). Nanodefense. *NanoScoping*. Available at: <https://www.nanoscoping.com.br/produtos> (Accessed June 11, 2021).
- NanoScoping (2020b). NanoNeem. *NanoScoping*. Available at: <https://www.nanoscoping.com.br/produtos> (Accessed June 11, 2021).

- Nanovetores (2017). Nano Icaridin. Available at: <https://www.nanovetores.com.br/nanoicaridina> (Accessed February 23, 2021).
- Naucke, T. J., Kröpke, R., Benner, G., Schulz, J., Wittern, K. P., Rose, A., et al. (2007). Field Evaluation of the Efficacy of Proprietary Repellent Formulations with IR3535 and Picaridin against *Aedes aegypti*. *Parasitol. Res.* 101, 169–177. doi:10.1007/s00436-006-0450-2
- Notario-Pérez, F., Martín-Illana, A., Cazorla-Luna, R., Ruiz-Caro, R., Bedoya, L. M., Peña, J., et al. (2019). Development of Mucoadhesive Vaginal Films Based on HPMC and Zein as Novel Formulations to Prevent Sexual Transmission of HIV. *Int. J. Pharm.* 570, 118643. doi:10.1016/j.jipharm.2019.118643
- Pinto, M. F., Moura, C. C., Nunes, C., Segundo, M. A., Costa Lima, S. A., and Reis, S. (2014). A New Topical Formulation for Psoriasis: Development of Methotrexate-Loaded Nanostructured Lipid Carriers. *Int. J. Pharm.* 477, 519–526. doi:10.1016/j.jipharm.2014.10.067
- PubChem (2019a). Geraniol. Available at: <https://pubchem.ncbi.nlm.nih.gov/compound/637566> (Accessed October 7, 2019)
- PubChem (2019b). Icaridin. Available at: <https://pubchem.ncbi.nlm.nih.gov/compound/125098> (Accessed October 7, 2019).
- Raghavendra, B. S., Prathibha, K. P., and Vijayan, V. A. (2013). Synergistic Effect of *Eugenia Jambolana* Linn. And *Solidago canadensis* Linn. Leaf Extracts with Deltamethrin against the Dengue Vector *Aedes aegypti* Linn. at Mysore. *Environ. Sci. Pollut. Res. Int.* 20, 3830–3835. doi:10.1007/s11356-012-1308-z
- Rawat, S., Gupta, P., Kumar, A., Garg, P., Suri, C. R., and Sahoo, D. K. (2015). Molecular Mechanism of Poly(vinyl Alcohol) Mediated Prevention of Aggregation and Stabilization of Insulin in Nanoparticles. *Mol. Pharm.* 12, 1018–1030. doi:10.1021/mp5003653
- Rehman, J. U., Ali, A., and Khan, I. A. (2014). Plant Based Products: Use and Development as Repellents against Mosquitoes: A Review. *Fitoterapia* 95, 65–74. doi:10.1016/j.fitote.2014.03.002
- Sarangi, B., Jana, U., Sahoo, J., Mohanta, G. P., and Manna, P. K. (2018). Systematic Approach for the Formulation and Optimization of Atorvastatin Loaded Solid Lipid NANOARTICLES Using Response Surface Methodology. *Biomed. Microdevices* 20, 53. doi:10.1007/s10544-018-0285-5
- Shalan, E. A., Canyon, D. V., Younes, M. W., Abdel-Wahab, H., and Mansour, A. H. (2005). Synergistic Efficacy of Botanical Blends with and without Synthetic Insecticides against *Aedes aegypti* and *Culex Annulirostris* Mosquitoes. *J. Vector Ecol.* 30, 284–288.
- Sharma Bora, N., Mazumder, B., Patowary, P., Kishor, S., Doma Bhutia, Y., Chattopadhyay, P., et al. (2019). Formulation Development and Accelerated Stability Testing of a Novel Sunscreen Cream for Ultraviolet Radiation protection in High Altitude Areas. *Drug Dev. Ind. Pharm.* 45, 1332–1341. doi:10.1080/03639045.2019.1616750
- Sharma, G., Thakur, K., Raza, K., Singh, B., and Katore, O. P. (2017). Nanostructured Lipid Carriers: A New Paradigm in Topical Delivery for Dermal and Transdermal Applications. *Crit. Rev. Ther. Drug Carrier Syst.* 34, 355–386. doi:10.1615/CritRevTherDrugCarrierSyst.2017019047
- Sitterberg, J., Ozcetin, A., Ehrhardt, C., and Bakowsky, U. (2010). Utilising Atomic Force Microscopy for the Characterisation of Nanoscale Drug Delivery Systems. *Eur. J. Pharm. Biopharm.* 74, 2–13. doi:10.1016/j.ejpb.2009.09.005
- Solomon, B., Sahle, F. F., Gebre-Mariam, T., Asres, K., and Neubert, R. H. (2011). Microencapsulation of Citronella Oil for Mosquito-Repellent Application: Formulation and *In Vitro* Permeation Studies. *Eur. J. Pharm. Biopharm.* 80, 61–66. doi:10.1016/j.ejpb.2011.08.003
- Song, A., Zhang, X., Li, Y., Mao, X., and Han, F. (2016). Effect of Liquid-To-Solid Lipid Ratio on Characterizations of Flurbiprofen-Loaded Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) for Transdermal Administration. *Drug Dev. Ind. Pharm.* 42, 1308–1314. doi:10.3109/03639045.2015.1132226
- Tavares, M., da Silva, M. R. M., de Oliveira de Siqueira, L. B., Rodrigues, R. A. S., Bodjolle-d'Almeida, L., Dos Santos, E. P., et al. (2018). Trends in Insect Repellent Formulations: A Review. *Int. J. Pharm.* 539, 190–209. doi:10.1016/j.jipharm.2018.01.046
- Teeranachaidekul, V., Souto, E. B., Junyaprasert, V. B., and Müller, R. H. (2007). Cetyl Palmitate-Based NLC for Topical Delivery of Coenzyme Q(10) - Development, Physicochemical Characterization and *In Vitro* Release Studies. *Eur. J. Pharm. Biopharm.* 67, 141–148. doi:10.1016/j.ejpb.2007.01.015
- Uchida, T., Kadhun, W. R., Kanai, S., Todo, H., Oshizaka, T., and Sugibayashi, K. (2015). Prediction of Skin Permeation by Chemical Compounds Using the Artificial Membrane, Strat-M™. *Eur. J. Pharm. Sci.* 67, 113–118. doi:10.1016/j.ejps.2014.11.002
- Vitorino, C., Carvalho, F. A., Almeida, A. J., Sousa, J. J., and Pais, A. A. (2011). The Size of Solid Lipid Nanoparticles: an Interpretation from Experimental Design. *Colloids Surf. B Biointerfaces* 84, 117–130. doi:10.1016/j.colsurfb.2010.12.024
- Webb, C. E., and Hess, I. M. (2016). A Review of Recommendations on the Safe and Effective Use of Topical Mosquito Repellents. *Public Health Res. Pract.* 26. doi:10.17061/phrp2651657
- Wissing, S. A., and Müller, R. H. (2001). A Novel Sunscreen System Based on Tocopherol Acetate Incorporated into Solid Lipid Nanoparticles. *Int. J. Cosmet. Sci.* 23, 233–243. doi:10.1046/j.1467-2494.2001.00087.x
- Yukuyama, M. N., Kato, E. T., Lobenberg, R., and Bou-Chacra, N. A. (2017). Challenges and Future Prospects of Nanoemulsion as a Drug Delivery System. *Curr. Pharm. Des.* 23, 495–508. doi:10.2174/1381612822666161027111957

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Abrantes, Rogerio, de Oliveira, Campos, de Araújo, Pampana, Duarte, Valadares and Fraceto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

CHAPTER III

**REPELLENT ACTIVE INGREDIENTS ENCAPSULATED IN POLYMERIC
NANOPARTICLES: POTENTIAL ALTERNATIVE FORMULATIONS TO CONTROL
ARBOVIRUSES**

Submitted to Journal of Nanobiotechnology

**REPELLENT ACTIVE INGREDIENTS ENCAPSULATED IN POLYMERIC
NANOPARTICLES: POTENTIAL ALTERNATIVE FORMULATIONS TO CONTROL
ARBOVIRUSES**

Daniele Carvalho Abrantes¹, Carolina Barbara Rogerio¹, Estefânia V. R Campos¹, Tais Germano-Costa², Aryane Alves Vigato³, Ian Pompermeyer Machado³, Anderson Ferreira Sepulveda³, Renata Lima², Daniele Ribeiro de Araujo³, and Leonardo Fernandes Fraceto^{1*}

¹ São Paulo State University (UNESP), Institute of Science and Technology, Avenida Três de Março 511, Alto da Boa Vista, Sorocaba, São Paulo, 18087-180, Brazil

² Laboratory of Bioactivity Assessment and Toxicology of Nanomaterials, University of Sorocaba, Sorocaba, São Paulo, Brazil

³ Human and Natural Sciences Center, Federal University of ABC, Santo André, São Paulo, 09210-580, Brazil

*Corresponding author: L. F. Fraceto. E-mail: leonardo.fraceto@unesp.br

Abstract

Dengue, yellow fever, Chikungunya, Zika virus, and West Nile fever have infected millions and killed a considerable number of humans since their emergence. These arboviruses are transmitted by mosquito bites and topical chemical repellents are the most commonly used method to protect against vector arthropod species. This study aimed to develop a new generation of repellent formulations to promote improved arboviruses transmission control. A repellent system based on polycaprolactone (PCL)-polymeric nanoparticles was developed for the dual encapsulation of IR3535 and geraniol and further incorporation into a thermosensitive hydrogel. The physicochemical and morphological parameters of the prepared formulations were evaluated by dynamic light scattering (DLS), nano tracking analysis (NTA), atomic force microscopy (AFM). *In vitro* release mechanisms and permeation performance were evaluated before and after nanoparticles incorporation into the hydrogels. FTIR analysis was performed to evaluate the effect of formulation epidermal contact. Potential cytotoxicity was evaluated using the MTT reduction test and disc diffusion methods. The nanoparticle formulations were stable over 120 days with encapsulation efficiency (EE) of 60% and 99% for IR3535 and geraniol, respectively. AFM analysis revealed a spherical nanoparticle morphology. After 24 h, $7 \pm 0.1\%$ and $83 \pm 2\%$ of the GRL and IR3535, respectively, were released while the same formulation incorporated in poloxamer 407 hydrogel released $11 \pm 0.9\%$ and $29 \pm 3\%$ of the loaded GRL and IR3535, respectively. GRL permeation from PCL nanoparticles and PCL nanoparticles in the hydrogel showed similar profiles, while IR3535 permeation was modulated by formulation compositions. Differences in IR3535 permeated amounts were higher for PCL nanoparticles in the hydrogels ($36.9 \pm 1.1 \text{ mg/cm}^2$) compared to the IR3535-PCL nanoparticles ($29.2 \pm 1.5 \text{ mg/cm}^2$). However, both active permeation concentrations were low at 24 h, indicating that the formulations (PCL nanoparticles and PCL in hydrogel) controlled the bioactive percutaneous absorption. Minor changes in the stratum corneum (SC) caused by interaction with the formulations may not represent a consumer safety risk. The cytotoxicity results presented herein indicate the carrier systems based on poly-epsilon caprolactone (PCL) exhibited a reduced toxic effect when compared to emulsions, opening perspectives for these systems to be used as a tool to prolong protection times with lower active repellent concentrations.

Keywords: Arboviruses, insect repellent, PCL nanoparticle, Geraniol, IR3535

1. Introduction

Arboviruses, or arthropod-borne viruses, are diseases transmitted by blood-feeding arthropod saliva and replicated/amplified in vertebrates hosts. Approximately 530 types of these viruses have been identified, of which 100 can affect humans and originate from families such as Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae, Rhabdoviridae, Orthomyxoviridae, and Asfarviridae [1,2].

In humans these viruses are typically transmitted by mosquito bites, with dengue, yellow fever, chikungunya, Zika virus and West Nile fever as representative examples. These diseases have infected millions and killed a considerable number of humans since their emergence. Arboviruses are present worldwide as consequence of rapid urbanization, climate change, and intense population flow associated with travel and migration [3,1,4].

Only a few vaccines have been developed for these diseases, indicating that other prevention methods are important. Protection measures including the use of clothes, avoiding activities outdoors during times with higher mosquito activity, protective screens, and chemical repellents have been used for arboviruses prevention [5].

In addition, the use of repellents as protection for humans can be obtained from natural or synthetic sources. Ideally, these repellents should be safe for humans, with low toxicity, chemical stability, low odor, and bug bite prevention properties that transform skin into an undesirable place for the insect vectors [6, 7].

The most common synthetic repellents include N,N-diethyl-3-methylbenzamide (DEET), 1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropylester (picaridin), and ethyl 3-[acetyl(butyl)amino]propanoate (IR3535). DEET is the gold standard synthesized in 1956 in the USA by the Department of Agriculture and has been applied worldwide used against flies, mosquitoes and fleas in formulations as aerosols, creams and sprays. Although the US Environmental Protection Agency determined in 2014 that DEET poses no risks to humans, the environment, and non-target species [6, 8, 9], it can cause itchy skin, inflammation, and seizures in children [10,11].

Picaridin was developed in the 1990s by Bayer AG and is commercialized in 50 countries as a promising compound against flies, mosquitoes, bees, fleas, and ticks in sprays, lotions and wet wipe formulations. It is indicated for use in children >2 years old in concentrations ranging from 7 to 25 vol% presenting light stability, resistance against water and sweat, and skin compatibility [9, 12, 13]. IR3535 was developed by Merck and commercialized in USA in the 1970s. It is commercially available as aerosols, sprays, and lotions in concentrations up to 30% for use against flies, mosquitoes, ticks,

lice, wasps, and bees. IR3535 is recommended for use in children <2 years old and pregnant women in concentrations up to 12.5% [14, 15, 7] .

DEET exhibits demonstrated cytotoxicity by decreasing in cell viability in human BE(2)-M17 cell line at 500–4000 mg/L with increased apoptotic protein expression that was not dose dependent [16]. Another study showed that picaridin and DEET pose no toxicological risks for humans, characterized by margin of exposure (MOE) and no-observed-effect-levels (NOEL) results [17]. DEET was also studied in red blood cells and induced hemolysis when present at 4–5 mM, causing premature eryptosis death, cell shrinkage, membrane damage, and altered Ca^{2+} influx [10].

Natural repellent alternatives based on essential oils are typically extracted from plants and exhibit low toxicity consisting mainly of volatile organic compounds. In particular, lemon eucalyptus and citronella oils are commonly used with major active components including p-Menthane-3,8-diol (PMD) and trans-3,7-dimethyl-2,6-octadiene-1-ol (geraniol), respectively [18,7]. PMD can be extracted from leaves of the *Corymbia citriodora* tree and is the only natural repellent endorsed by the Centers of Disease Control and Prevention, with comparable efficacy as DEET. Geraniol is a monoterpene found in several essential oils but primarily isolated from Palmarosa oil that can also be used as a repellent [19–22]

The mixture of different repellents can produce a formulation with improved repellent properties by combining various properties. Alavez-rosas et al. (2022) studied citronella, mint, rosemary, and clove oils against *Aedes aegypti* and observed that a ternary mixture of citronella:mint:clove (1:1:1) showed similar repellency as 5 and 10% commercial DEET. Noosidum et al. (2014) studied oils extracted from *Litsea cubeba*, *Litsea salicifolia*, and *Melaleuca leucadendron* against *Ae. aegypti*. The mixture of *Litsea cubeba* and *Litsea salicifolia* (0.075%) showed pronounced synergistic effects against the mosquitoes and non-contact repellency, which were similar to the DEET response.

Although natural repellents exhibit many benefits, essential oils are easily volatilized and their instability necessitates the development of novel technologies that can control their release when applied topically. Nanotechnology is a promising strategy for pollution reduction, sustainability, as well as biocompatible and biodegradable material production for formulations containing essential oils. These formulations can protect the actives and decrease environmental losses through encapsulation of the repellents, providing prolonged active ingredient release [25,26] Polymeric nanoparticles are widely used as topical drug delivery systems, especially nanospheres or nanocapsules that range in size from 100 to 1000 nm. Nanospheres form a dense matrix with dispersed actives or a matrix with surface-adhered actives,

whereas nanocapsules act as a reservoir protecting the actives in the capsule interior or in their polymeric membrane [26,27]. The shell formers poly lactic acid, polylactide-co-glycolide, and poly (ϵ -caprolactone) are commonly used for these purposes. Polycaprolactone (PCL) is approved by the US-FDA and is used to prepare different nanocapsules [25]. These materials are interesting for topical applications because of their suspension stability and high surface area [26] and have been essential oil carriers [25,30].

In addition, hydrogels are attractive delivery vehicles as they promote skin hydration and act as a protective barrier while facilitating repellent application. The use of nanoparticles incorporated into hydrogels (NP-gels) can improve mechanical characteristics of the base hydrogels [31]. Hydrogels are 3D grids composed of hydrophobic or hydrophilic polymers that can act as nanoparticle carriers while absorbing significant amounts of water, with similar characteristics as the extracellular matrix and other biocompatible materials [28–30]

. Hydrogels can be physically (reversible) or chemically (irreversible) [33] crosslinked, obtained from natural or synthetic [35] sources, and carry nanomaterials loaded with essential oils [36,37].

Therefore, a novel repellent hydrogel formulation was developed herein and its performance as a repellent system was examined. The formulation was based on PCL polymeric nanoparticles encapsulating a mixture of IR3535 + geraniol in suspension and subsequent incorporation into a hydrogel. This study aimed to provide potential alternatives for repellent formulations to promote improved arbovirus control.

2. Materials and Methods

2.1 Materials

Ethyl butyl acetylamine propionate butylacetylaminopropionate 98% (IR3535) and icaridin 97% (ICA) was obtained from Chemidin (China). Geraniol 98% (GRL) was obtained from Quinarí (Ponta Grossa, Paraná, Brazil). Tripalmitin, polyvinyl alcohol (PVA), and poloxamer 407 (PL407) were obtained from Sigma-Aldrich (St. Louis, USA). Mirytol was obtained from BASF. Ethanol was purchased from Labsynth (Diadema, São Paulo, Brazil). Acetonitrile (HPLC grade) was obtained from J.T. Baker (USA). All other reagents were obtained from local suppliers in Sorocaba (São Paulo, Brazil).

2.2 Preparation of polymeric poli-(epsilon-caprolactone) nanoparticles

The nanoparticles were prepared using the emulsion/solvent evaporation method [38]. The organic phase was composed of mirytol, poli-(epsilon-caprolactone), and active agents (IR3535 and GRL) diluted in dichloromethane. This phase was mechanically mixed (Turrax homogenizer, 14000 rpm) with the aqueous phase composed of 0.5% PVA (polyvinyl alcohol) surfactant. The solvent was evaporated until the formulation reached a final volume of 10 mL, resulting in active agent concentrations of 5 and 2% (w/v) for IR3535 and GRL, respectively. Empty PCLs were synthesized and an emulsion formulation composed of bioactives in a 1.25% PVA solution were used as controls.

2.3 Preparation of nanoparticle-poloxamer-based hydrogels

PCL nanoparticles were synthesized and incorporated into 30% (w/v) P407-based hydrogels. First, adequate amounts of P-407 were solubilized in water in an ice bath for 12 h and maintained at 8 °C until use. PCL nanoparticle suspensions were incorporated into hydrogels via overnight homogenization at refrigerator temperature (4°C).

2.4 Physicochemical characterization of the PCL nanoparticles in suspension

The size distribution and polydispersity index (PDI) measurements were performed using Dynamic Light Scattering (DLS). Zeta potential determination was performed by microelectrophoresis using a ZetaSizer Nano ZS90 system (Malvern Instruments, UK) operated with a fixed angle of 90° at 25 °C with 200-fold diluted samples. In addition, nanoparticle concentration, size distribution, and polydispersity measurements were performed by nanoparticle tracking analysis using a NanoSight instrument (Malvern Instruments, UK) equipped with a green laser (532 nm) and a sCMOS camera controlled by NanoSight v.3 software with the samples diluted 20,000 times. The results were obtained as the average of triplicate analyses. The formulations were maintained at room temperature (25°C) and their stability was evaluated over time with measurements taken at 0, 15, 30, 60, 90, and 120 days. The encapsulation efficiency was determined using microcentrifugation, with quantification of the non-encapsulated active compounds in the filtrate by high-performance liquid chromatography (HPLC) using an Ultimate 3000 instrument (Thermo Fisher Scientific, Waltham, USA) with Chromeleon 7.2 software for chromatogram acquisition and analysis. The mobile phases were methanol:water (65:35 v/v) and acetonitrile: water

(60:40 v/v) with a Phenomenex Gemini C18 column (150 x 4.60 mm, 5 μ m). All tests were performed in triplicate. The calculations were based on the GRL and IR3535 calibration curves with the equations $y = 1.28395x + 2.60255$ ($R^2 = 0.99894$) and $y = 0.52575x - 0.06279$ ($R^2 = 0.9997$), respectively.

2.5 Repellent formulation morphology

The nanoparticle morphology and size distributions were analyzed by atomic force microscopy (AFM) using a Nanosurf Easyscan 2 Basic BT02217 instrument. The nanoparticle suspensions were diluted 20,000 times, dispersed on silicon plates, and dried in a desiccator. For nanoparticles in the gel, a smear of the formulation was used without dilution. The images (256 x 256 pixels, TIFF format) were obtained using a TapA1-G probe (BudgetSensors, Izgrev, Bulgaria) in tapping mode at 90 Hz. The images were processed using Gwyddion software (Dublin, Ireland). All morphological analyzes were performed for both nanoparticle suspensions and hydrogel formulations.

2.6 Hydrogel mechanical properties: rheological analysis under frequency, temperature, and shear stress variation

Rheological studies were performed to analyze the formulation mechanical behavior under temperature, frequency, and shear stress variations to examine the system structural organization and stability. Hydrogel rheological properties under temperature and frequency sweeps were analyzed using an oscillatory rheometer (Kinexus lab, Malvern Instruments, Malvern, UK) with cone and plate geometry (20 mm diameter, 0.5 rad angle, and 1 mm gap). The repellent formulations (1 g) were placed in the sample holder and evaluated at skin temperature (32.0 ± 0.5 °C). The first analysis was performed under a temperature ramp from 10 to 50 °C at 5 °C/min and 1 Hz frequency and 1 Pa shear stress. In addition, oscillatory analyses were performed under frequency sweep from 0.1 to 10 Hz with a shear stress of 1 Pa at 32.5 °C.

In another approach, continuous rheological measurements were performed using a plate-plate geometry and 0.1 mm gap. The elastic G' and viscous moduli G'' were measured between 0 and 60 °C. G' and G'' measurements were also made with ω frequency from 0.1 to 10 Hz at 32.5 °C [39]. The flow curves $\sigma(\dot{\gamma})$ ("backward" and "forward") were obtained at 32.5 °C with a shear rate $\dot{\gamma}$ ranging from 0 to 100 s^{-1} . Self-healing curves were obtained at 32.5 °C, frequency of 1 Hz, varying the rate by 1% and 100% with equal time intervals.

With the G' data as a function of the frequency ω (in Hz) the adhesion parameter S was calculated from the following relationship:

$$G' = S \cdot \omega^n \quad (\text{Eq. 1})$$

Where n is an adimensional parameter that indicates material structuring. The calculation of S is related to material cohesion on a smooth surface, being proportional to the work required to remove it from that surface [40,41] and is calculated as follows (Eq. (2)):

$$\sigma = K \cdot \gamma^m \quad (\text{Eq. 2})$$

The consistency (K) and viscosity index (m) values are calculated from the flux curve data of σ (γ) in both directions. K is defined as a mechanical property, proportional to material stiffness, while the m index allows comparison of spreadability of each material [40,41].

2.7 Bioactive release profiles and mechanisms under *in vitro* release and permeation conditions

Investigation of the *in vitro* release profiles for the liquid and gel PCL formulations employed a vertical diffusion system with a regenerated cellulose membrane (MWCO 12000-14000 pores) coupled to donor and receptor compartments. The system was operated under sink conditions. First, 100 mL of 3% PVA was used as co-eluent in the receptor solution and the system was kept in a closed environment at 32.5 °C under constant magnetic stirring [42]. Formulations were applied (1 g of gel or 1 mL of suspension) in the donor compartment, after which 1 mL aliquots were periodically removed from the receptor compartment, replacing the volume removed by co-eluent solution. Geraniol and IR3535 were quantified via HPLC methods, as described above. The release profile data were analyzed considering different mathematical models (zero-order, first-order, Higuchi, and Korsmeyer-Peppas) to investigate the release mechanisms of the active agents from the different systems.

Permeation kinetic assays were performed in a two-compartment vertical diffusion cell system (Microette Plus, Hanson Research, Chatsworth, CA, USA) with donor (1.72 cm² permeation area) and receptor compartments (7 mL) separated by a synthetic membrane (Strat-M®, 25 mm diameter, Millipore Co., USA) [43,44].

Sample formulations (0.6 g) were placed in the donor compartment in contact with the membrane and the receptor compartment was filled with 0.1 M sodium phosphate buffer (pH 7.4). The system was kept under magnetic stirring (350 rpm) at

32.5 ± 0.5 °C. Aliquots were withdrawn at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h with bioactives quantified by HPLC. All formulations were evaluated in triplicate. The cumulative amount of repellent permeated, Q ($\mu\text{g}/\text{cm}^2$) the Strat-M per unit area was plotted as a function of time (min). For data analysis, the flux values were obtained from the slope of the concentration versus time curve in the linear portion (interval between 15 and 450 min). The data were analyzed according to Eq. (3).

$$J = P \times C_d \quad (3)$$

where J ($\%.\text{cm}^{-2}.\text{min}^{-1}$) is the compound flux through the membrane, P ($\text{cm}.\text{min}^{-1}$) is the permeability coefficient, and C_d ($\%.\text{cm}^{-3}$) is the compound concentration in the donor compartment [45]. Lag time (t_{lag}) was calculated from the intercept ($Y=0$).

2.8 Permeation mechanisms: epidermis structural analysis

Porcine ear samples were obtained from a local slaughterhouse and the experimental protocol was approved by the Federal University of ABC - Institutional Committee for the Care and Use of Animals (#8719010318). Blood vessels and subcutaneous tissue were removed and skin dermatomed at 0.45 mm (Nouvag, Rorschach, Switzerland). For epidermis separation, the skin samples were immersed in a water bath at 60 °C for 3 min and the isolated epidermis was subsequently detached. Epidermis samples were stored at -20 °C for a maximum of 3 months. For structural analysis, the epidermis surface (0.8 cm diameter) was treated with repellent formulations and kept in contact for 24 h at room temperature [39]. Formulations were then removed from the epidermis samples and analyzed by FTIR. FTIR spectra were obtained for the isolated formulation components, repellent formulations, while non-treated epidermis samples (0.8 cm diameter) were used as controls. FTIR spectra were recorded at 4000–650 cm^{-1} with 1 cm^{-1} resolution using a PerkinElmer Spectrum Two 160.000A in ATR mode [42–44]

2.9 *In vitro* cell viability assay

2.9.1 Cell viability based on mitochondrial activity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxic effects of the repellent formulations based on PCL nanoparticles in keratinocyte cell lines (HaCat) and fibroblasts (NIH/3T3). The cells were plated at 7000 and 8000 cells/well and incubated in a climate-controlled oven at 37 °C and 5% CO_2 . The cells were exposed for 24 h to either non-encapsulated or encapsulated repellents at 8 concentrations. The doses used for each activity ranged

from 0.02 to 200 $\mu\text{g/mL}$ for GRL and 0.05 to 500 $\mu\text{g/mL}$ for IR3535. The control nanoparticles (empty) were tested at the same dilution. After 24 h of exposure, the cells were incubated with 0.5 mg/mL MTT for 4 h. Subsequently, the supernatants were removed and DMSO was added and incubated for 10 min at 37 °C to dissolve the formazan crystals and their absorbance was measured at 540 nm using a microplate reader. In living cells, mitochondrial dehydrogenase oxidizes MTT to formazan, while damaged or dead cells show reduced or no dehydrogenase activity. Data were reported as means of two independent experiments (three replicates each) $\pm$ standard error of mean and were expressed as percent viability with respect to the control.

2.9.2 Disk diffusion test

Cells were plated in 60 mm Petri dishes at 1.5×10^5 cells/mL using DMEM High culture medium with 10% fetal bovine serum and were grown for 48 h at 37 °C in an oven with 5% CO_2 . Then the liquid medium was discarded and the solid overlay medium (2x concentrated Eagle medium, containing 1.8% agar and 0.01% neutral red as a vital dye) was added over the cell mat. After solidification, the test material was deposited in the center of the plate and incubated for 24 h at 37 °C in a 5% CO_2 environment. The inoculated plate readings were performed macroscopically, where cytotoxicity was confirmed by the presence of a clear halo around the material, corresponding to dead cells and microscopically for morphological alterations of the cells surrounding the sample.

2.10 Statistical analyses

The data were presented as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used for evaluation of normality, followed by application of the two-way ANOVA test for multiple variables considering a significance level of 0.05. The software used was GraphPad Prism 8.0.1.

3. Results

3.1. Nanoparticle physicochemical characterization

The mean diameters (nm) of the five batches of identical PCL formulations encapsulated with 5% IR3535 + 2% geraniol were 373 ± 11 , 378 ± 13 , 374 ± 12 , 371 ± 15 , 377 ± 21 , and 376 ± 5 nm (Figure 1A). The samples passed the Shapiro-Wilk normality test and the analysis of variance (ANOVA) did not indicate significant differences in particle size. The PDI and PZ were analyzed in the same manner where

the PDI values over 120 days were 0.21 ± 0.01 , 0.17 ± 0.02 , 0.21 ± 0.02 , 0.19 ± 0.03 , 0.20 ± 0.03 , and 0.21 ± 0.01 (Figure 1B).

PDI values of >0.2 are commonly deemed acceptable in practice for polymer-based nanoparticles in agreement with the DLS size distribution analysis over time shown in Figure 1E. The PZ values were -23 ± 5 , -20 ± 2 , -19 ± 2 , -18 ± 6 , -23 ± 0.5 , and -21 ± 0.7 mV with no significant differences as a function of time. The encapsulation efficiencies (EEs) of the active compounds IR3535 and GRL were evaluated over 0, 15, 30, 60, 90 and 120 days showing $60.1 \pm 1.9\%$, $60.4 \pm 0.1\%$, $59.3 \pm 1.3\%$, $57.8 \pm 1.4\%$, $60.7 \pm 1.5\%$, and $60.2 \pm 2\%$, respectively, for IR3535.

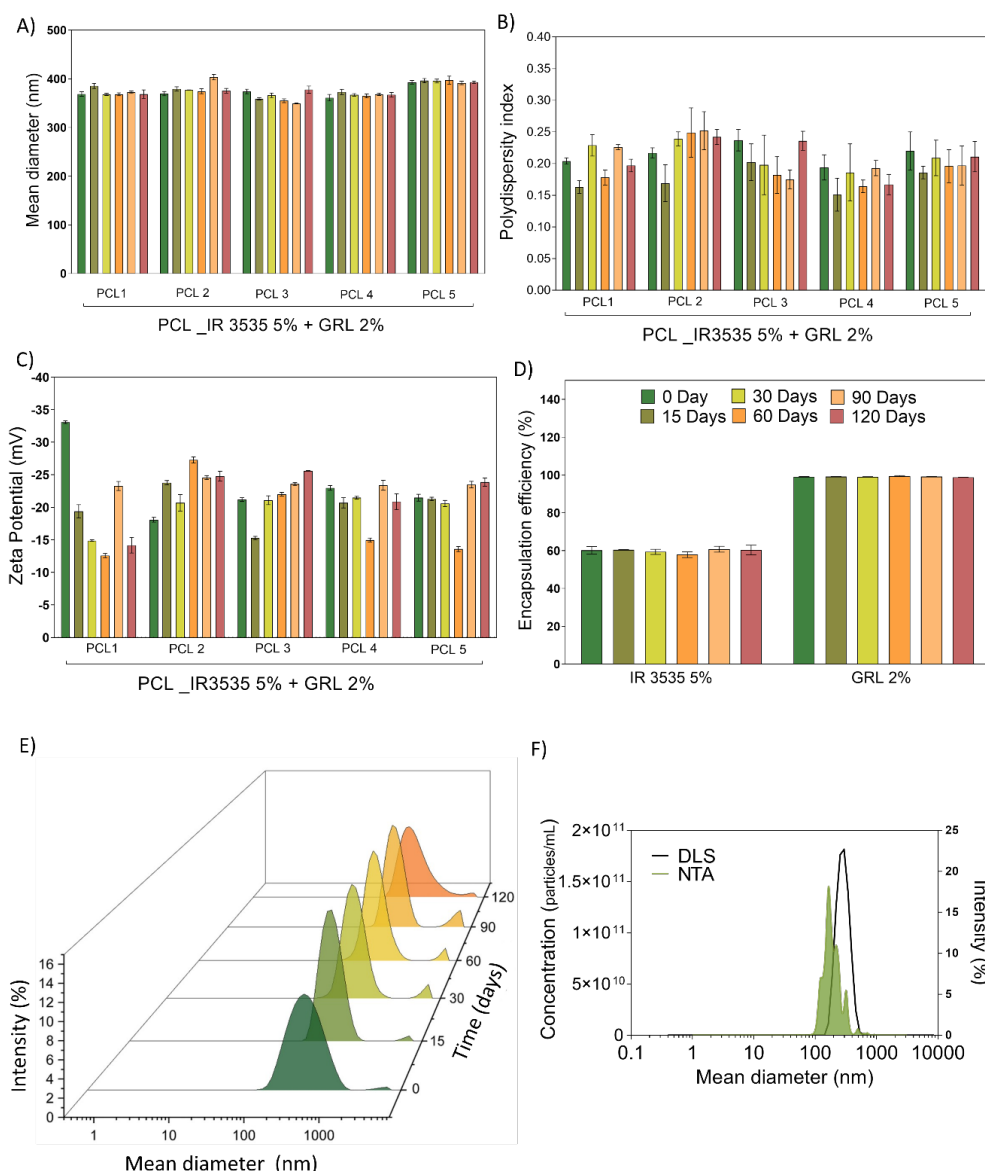


Figure 1. Analysis of the properties of PCLs along with the times 0, 15, 30, 60, 90, and 120 days for size (nm, A, and B); zeta potential (mV, C). The encapsulation efficiency (% , D) was expressed as the mean and standard deviation of the five batches of PCL formulation. E) Distribution as a function of intensity (DLS) over 120

days; F) Comparison between the diameter distribution as a function of concentration (NTA) and intensity (DLS). The respective values were expressed as mean and standard deviation ($n=3$) for five identical PCL formulation lots loaded with 5% IR3535 and 2% geraniol (GRL).

For geraniol, EE values were $99 \pm 0.1\%$, $99 \pm 0.03\%$, $99 \pm 0.09\%$, $99 \pm 0.3\%$, $99 \pm 0.1\%$, and $99 \pm 0.2\%$, respectively for each time point (Figure 1 A–D). Similarly, in a sunscreen formulation with PCL, the average nanoparticle size was determined to be 418 ± 56 and with a PDI of 0.36 ± 0.062 [49]. In addition, roxithromycin was encapsulated in PCL nanoparticles for hair follicle treatment, yielding a mean diameter of 300 nm and maximum EE of 61% [50]. Therefore, the evaluated parameters obtained herein agree with studies performed using similar systems.

The AFM size distribution histogram (Figure 1S-A) showed mean diameters between 200 and 800 nm (analysis of 100 particles using the Gwyddion software). In contrast, the average size of the PCLs was determined to be approximately 400 nm by DLS. The sample PDI which was evident in the size distribution determined by DLS (Figure 1E) and the aggregation of nanoparticles during the drying process (ubiquitous in morphological studies) explain this variation [51]. In addition, the topographic images demonstrate a spherical morphology, which is a characteristic related to the efficiency of nanoparticle synthesis.

3.2. Hydrogel rheological analysis

The incorporation of PCL nanoparticles or emulsions into hydrogel formulations tends to form structurally organized systems, as observed by the increased G' and viscosity values. Although emulsion-gel and PCL-hydrogel viscosities showed values close to $4.5 \cdot 10^6$ mPa.s, the emulsion-gel was the most structured material, as evidenced by the G'/G'' ratio of 351.9 (Table 1). Both emulsion-gel and PCL-gel formulations were stable between 20 and 40 °C and the incorporation of additives (emulsion and nanoparticles) decreased the sol-gel transition temperature for all systems.

Frequency sweep analysis allowed materials stability determination when exposed to oscillatory movement. This movement increased PCL-hydrogel stiffness compared to the emulsion-hydrogel. In addition, PCL hydrogels showed greater stability over the tested frequency range compared to the other systems (Figure 2A). Thus, it can be inferred that the PCL-hydrogel exhibited greater cohesion between the

formulation components, as indicated by the high adhesion value (S) compared to those obtained for the other formulations (Table 1).

As the PCL-hydrogel exhibited greater structural cohesion, this formulation is expected to exert greater flow resistivity than the emulsion-hydrogel. The frequency rheological results and flow curves showed that the PCL- and emulsion-gels were more responsive to movement than the emulsion and PCL alone, since the gel affected the molecular organization of both formulations. The viscosity index (m ; Table 1 and Figure 2B) showed that the emulsion and PCL alone exhibited more of a liquid character than the gel formulations, considered to be pseudoplastic behavior. This pseudoplastic character shows that the gel reduces materials outflow, a favorable indicator for topical applications [39,40,52,53] as repellents must remain on the skin surface at the application site. The consistency values (K) indicate that incorporation into the hydrogel increased the material stiffness. The emulsion-gel showed greater consistency than the PCL-hydrogel, confirming a greater elastic than viscous character when the emulsion is incorporated into the hydrogel. In fact, emulsion or PCL nanoparticle addition allowed structural recovery even under shear stress variation, as evidenced by the self-healing experiments results (Figure 2C and D). As shown by the flow curve of the PCL-gel, disruption occurs when subjected to voltage oscillation, decreasing the elastic modulus G' over time. In contrast, the emulsion-gel is more stable under stress variation, with G' values approximately the same as that at 1% shear stress.

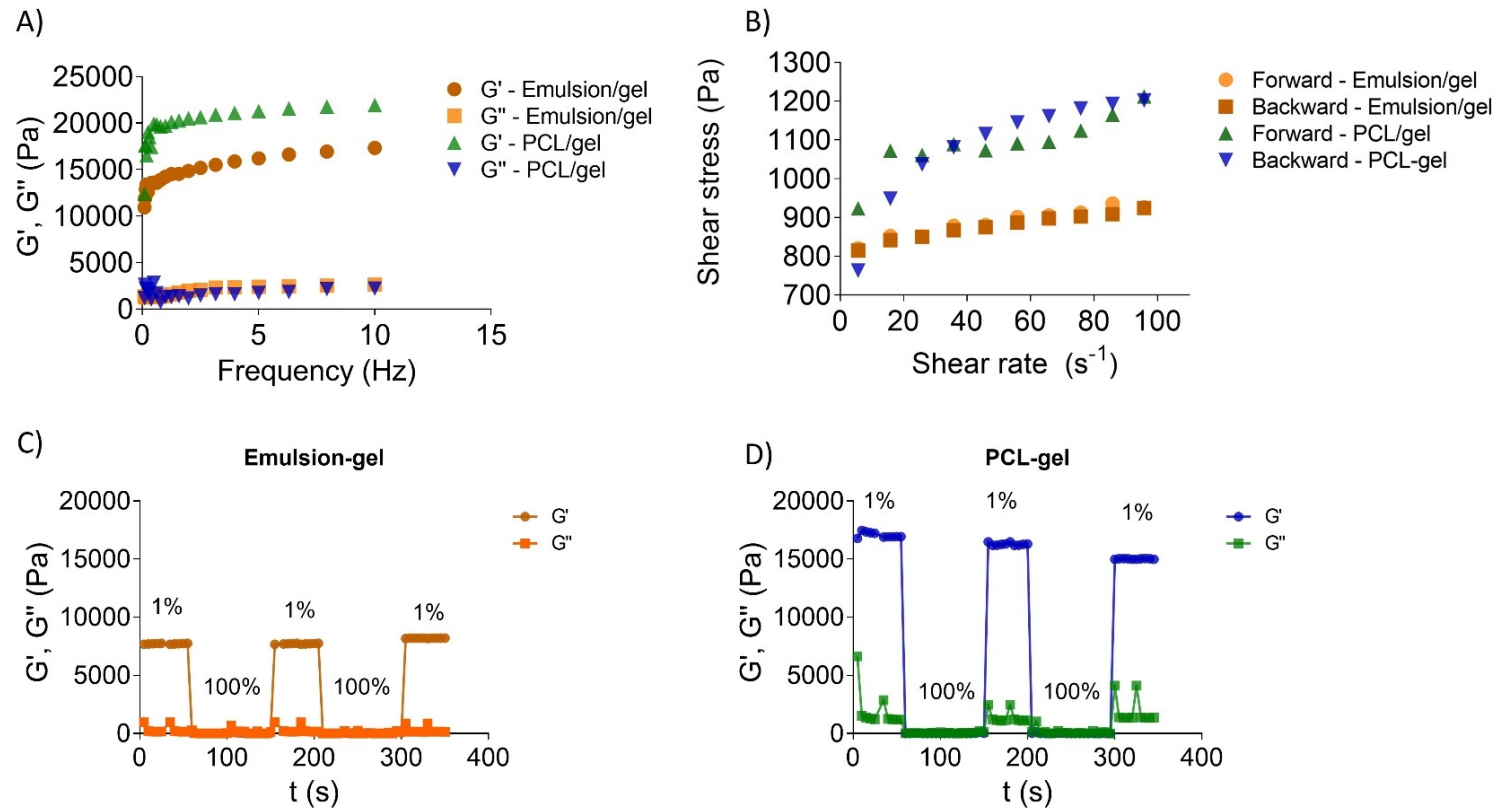


Figure 2- Rheological characterization of poloxamer hydrogels (30% w/v) containing non-encapsulated IR3535 nd GRL and hydrogel containing PCL nanoparticles loading IR3535 nd GRL. (A) Rheological properties as function of frequency sweep analysis (0.1 – 10 Hz) at 32.5 °C; (B) flow curves expressed as shear rate (0-100 s^{-1}) and applied shear stress; (C) Self-healins profiles obtained at 1Hz and at 32.5 °C for non-

encapsulated IR3535 and GRL in hydrogels and (C) PCL nanoparticles loading IR3535 and GRL incorporated in hydrogels.

Table 1- Rheological parameters of control emulsion, control emulsion incorporated into the hydrogel (emulsion-hydrogel), PCL nanoparticles (PCL) and PCL nanoparticles incorporated into hydrogels (PCL-hydrogel) measured at 32.5 °C.

Formulation	G'/G'' (32.5 °C)	η^* (mPa.s) (32.5 °C)	S	n	R^2	K
Emulsion	8.7 ± 0.8	40.6 ± 5.1	0.21 ± 0.01	2.12 ± 0.02	0.99	0.0597 ± 0.001
Emulsion-hydrogel	351.9 ± 7.1	4.2 ± 0.1 (.10 ⁶)	14266 ± 80	0.079 ± 0.004	0.95	745.8 ± 9.8
PCL	0.40 ± 0.15	226.6 ± 25.0	80.7 ± 1.3	0.39 ± 0.01	0.99	0.015 ± 0.002
PCL-hydrogel	52.3 ± 1.6	4.6 ± 0.9 (.10 ⁶)	19354 ± 261	0.07 ± 0.01	0.73	619.5 ± 23.0

Note: G'/G'' - elastic x viscous moduli relationship; η^* - viscosity; S – adhesion; n- flow index; K- consistency index. R^2 - linearity coefficient.

3.3 Repellent release profiles and mechanisms under *in vitro* release and permeation conditions

Nanoparticle suspensions and hydrogel formulations containing nanoparticles showed a sustained release profile for both IR3535 and GRL bioactives. A significant ($p < 0.05$) increase in IR3535 release from PCL nanoparticles was observed up to 6 h compared to IR3535 in the emulsion (liquid), whereas the concentrations released were similar after 24 h. However, the suspensions incorporated into poloxamer hydrogels showed no significant differences in release rates between IR3535 encapsulated in nanoparticles (PCL gel) and emulsified with PVA (Emulsion_gel). However, the GRL release profile from the emulsion formulation (Emulsion_liq.) showed significantly ($p < 0.05$) higher concentrations than the encapsulated GRL in nanoparticles (PCL liq.). Additionally, no significant differences in the release profiles between the emulsion and NPs over 12 h were observed after the suspensions were incorporated into poloxamer hydrogels. Although, a considerable increase in GRL was released from the emulsion_gel after 24 h (Figure 3B).

The hybrid systems (nanoparticles + hydrogel) showed a gradual IR3535 release profile compared to the nanoparticle suspension (PCL liq.) under the same release conditions. In contrast, there was significant increase in GRL release rate after 4 h (210 min, $p < 0.05$) in a hybrid system (PCL gel) compared to the nanoparticle suspension (PCL liq.).

Corroborating these results, Nnamani et al. (2014) reported that the release rate of artemether (ART), a malaria therapeutic, decreased after its incorporation into a hybrid system (NLC + Poloxamer 407) compared to encapsulation in liquid NLC. PCL encapsulated GRL and IR3535 released 7.3 ± 0.1 and $83.6 \pm 2.2\%$ at 24 h, respectively. The same formulation incorporated into a poloxamer 407 hydrogel released $11 \pm 0.9\%$ and $29.5 \pm 3\%$ of GRL and IR3535, respectively. All formulations demonstrated a sustained release pattern, indicating a high rate of polymer solubilization and hydration in the release medium. Table 1S shows the data from the release kinetic models applied to the liquid and gel PCL formulations. Based on the linear coefficient (R^2) values, the predominant GRL release model in the liquid and gel formulations was zero-order kinetics, suggesting a prolonged release model. The Higuchi model best described the release of IR3535 in the liquid formulation, indicating that diffusion was the predominant mechanism of drug release from the nanoparticles to the receptor medium. In the gel formulation, the Korsmeyer-Peppas model was a better fit to IR3535 release, with the values of the release exponent “n” of >1 . This is

characteristic of case II transport, showing the contribution of both drug diffusion and hydrogel erosion caused by polymeric chains hydration and relaxation [55]. These results are in agreement with previous reports related to polymeric nanocapsules and poloxamer 407 hydrogels [27,56].

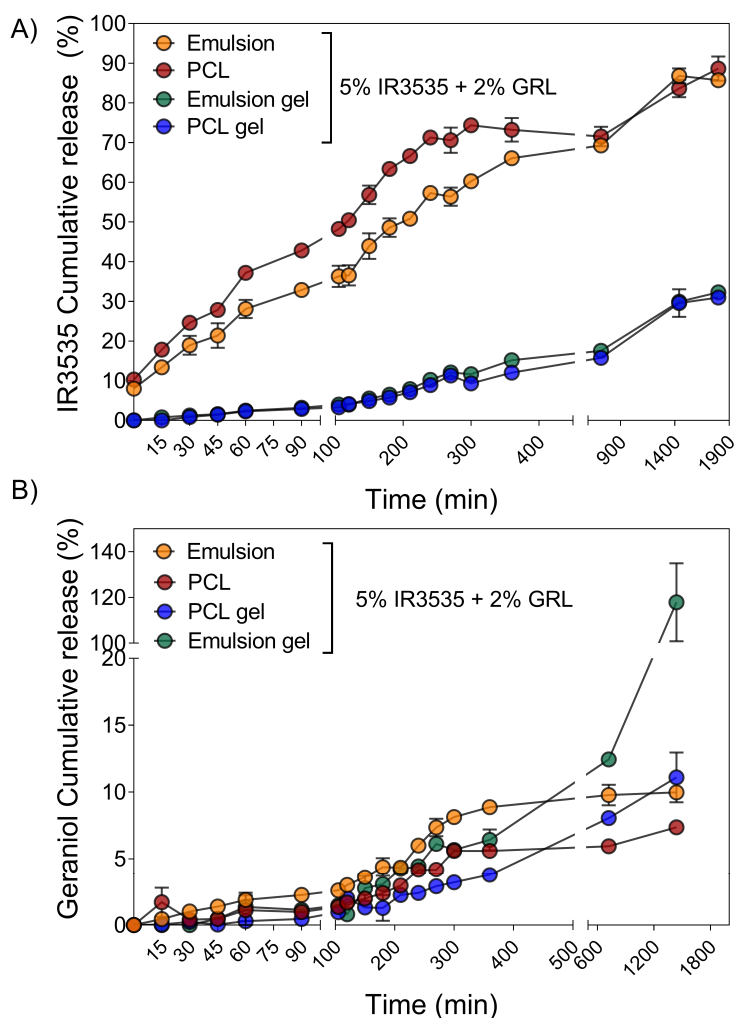


Figure 3. *In vitro* release assay of IR3535 and GRL from PCL nanoparticles and PCL nanoparticles incorporated into hydrogels. The release assay was performed using the vertical diffusion cell system at 32.5 °C, in aqueous media containing 3% of PVA as co-solvent over 30 hours. Values represent the mean $\pm$ SD (n=3).

The release kinetics allowed examination of formulation performance as drug release matrices. According to the models, different release profiles between the GRL and IR3535 were observed. This combination is interesting and open perspectives for

developing a repellent formulation with prolonged action and a possible synergistic effect promoted by the mixed mechanisms of the components.

In vitro permeation assays were also performed to predict bioactives permeation profiles from the nanoparticles and hydrogels as well as their performance when in contact with the skin surface (Figure 4).

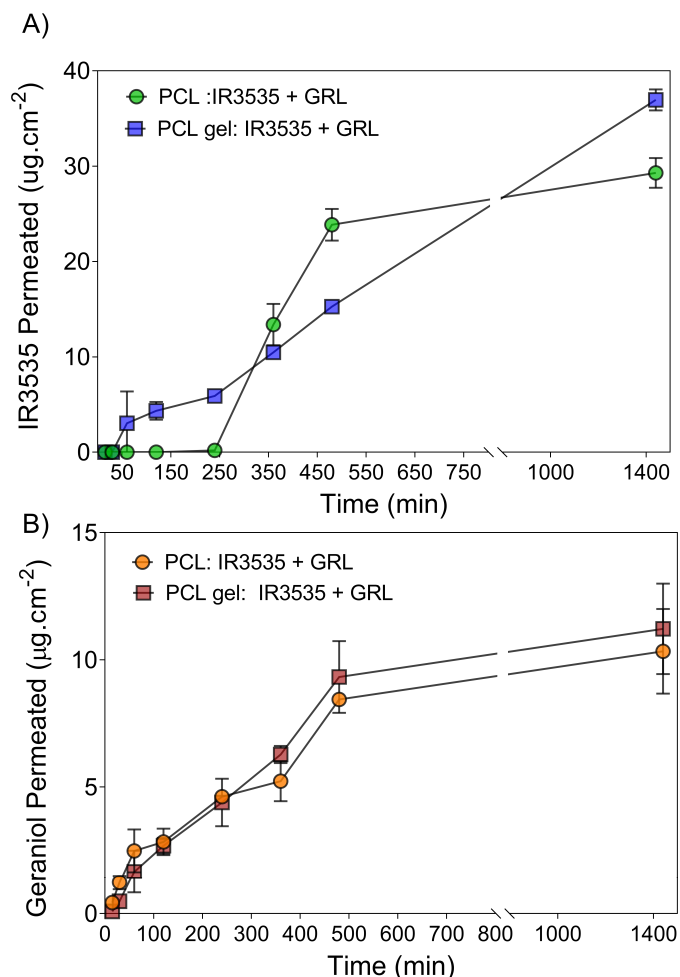


Figure 4 – *In vitro* permeation profile of PCL nanoparticles suspension and PCL nanoparticles incorporated into hydrogels. The permeation study was performed using vertical diffusion cell at 32.5 °C, in phosphate buffer pH 7.4. (A) IR3535 permeation profile and (B) GRL permeation profile. The values represent the mean $\pm$ SD ($n = 3$).

According to analyzed data, for all formulations the repellent concentration released as a function of time showed a linear relationship, as confirmed by the correlation coefficients ($R^2 = 0.926\text{--}0.981$), except for the IR3535 released from the liquid PCL formulation, which showed a burst release after 6 h. Both GRL and IR3535

permeation results showed differential effects depending on formulation type, nanoparticle suspension or hydrogel. GRL permeation from PCL nanoparticles and PCL nanoparticles in the hydrogel showed similar profiles, where high GRL permeated concentrations were observed after 8 and 24 h. The GRL loading into the PCL and PCL-hydrogel was 20 mg/g, indicating that <1% permeated after 24 h. This showed that both formulations (PCL nanoparticles and PCL in the hydrogel) controlled bioactive percutaneous absorption. GRL flux values were 0.015 and 0.019 $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for the PCL nanoparticles and PCL in hydrogels, respectively (Table 2). In contrast, IR3535 permeation was modulated by the formulations, where permeated amounts were higher for the PCL nanoparticles in hydrogels ($36.9 \pm 1.1 \mu\text{g}/\text{cm}^2$) compared to IR3535-PCL nanoparticles ($29.2 \pm 1.5 \mu\text{g}/\text{cm}^2$). These profiles exhibited flux values of 0.023 and 0.026 $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for the PCL nanoparticles and PCL in hydrogels, respectively. However, the most pronounced difference was observed during initial permeation time, with values of ~250 and 50 min observed for PCL nanoparticles and PCL nanoparticles in hydrogels, respectively (Table 2).

Table 2 – Permeation parameters of IR3535 and GRL across Strat-M® artificial membranes from PCL nanoparticles loading both compounds (GRL + IR3535) and PCL nanoparticles loading both compounds (GRL + IR3535) incorporated into hydrogel.

Formulations	Flux ($10^{-4}\cdot\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$)	Permeability coefficient ($10^{-4}\cdot\text{cm}\cdot\text{min}^{-1}$)	Lag time (min)
PCL suspension			
GRL	148.2	74.1	57.2
IR3535	227.0	45.4	5.62
PCL gel			
GRL	185.3	92.6	24.2
IR3535	257.3	51.4	26.1

Similar results were reported in other studies regarding the permeation of ICA and GRL from NLC gel formulations. A 14-fold increase in permeation of ICA and 40-fold increase of GRL from NLC incorporated in the HPMC gel were reported [57]. Comparing NLC and PCL formulations, the permeation rate of synthetic actives (ICA

and IR3535) through the Strat-M membrane was higher for IR3535 compared to the liquid formulations. However, comparing NLC and PCL in gels, the permeation rate of ICA was 6 times higher. The lag times (T_{lag}) of synthetic actives were similar (23.8–26.1 min), but the natural active GRL showed a significant difference in NLC and PCL suspension at 591.4 and 57.2 min, respectively. Similarly, comparing the NLC and PCL gels, significant differences were observed at 43.9 and 5.6 min, respectively. Briefly, the NLC suspension showed a 10-fold increased T_{lag} compared to PCL in the suspension formulation and the NLC gel exhibited an 8-fold increased T_{lag} compared to the PCL gel.

3.4 Permeation mechanisms: epidermis structural analysis

The literature reports a natural repellent effect in human skin originating from the unsaturated fatty acids present in stratum corneum (SC) lipid matrix [58]. For repellent topical applications, protection depends mainly on formulation retention on the skin surface, avoiding permeation of the bioactive [59]. Thus, preservation of the stratum corneum barrier integrity after using repellent formulations is necessary. FTIR was used to evaluate the interaction between the gels and SC after 4 h of contact.

According to Boncheva et al. (2008), CH_2 stretching vibrations are powerful markers to indicate structural changes in the SC lipid matrix. Parameters including the shape of the CH_2 stretching bands and its precise wavenumber are related to lipid supramolecular organization in the SC. When the $-CH_2$ symmetric stretching band is centered at a wavenumber of $<2850\text{ cm}^{-1}$ the lipids are organized in an orthorhombic (OR) structure. At $>2850\text{ cm}^{-1}$ the lipids transition from OR to hexagonal (HEX) or liquid-crystalline (LIQ) phases [46]. In porcine ear, the SC lipid organization is characterized by mixed OR and HEX phases.

Herein, symmetric CH_2 stretching for the control sample was observed at 2851 cm^{-1} , confirming a mixture of OR and HEX phases. The treated samples showed lower CH_2 stretching band resolution due to overlap of the CH_2 symmetric and asymmetric stretching bands. Consequently, enlargement of the CH_2 stretching symmetric band reflected structural changes in the SC lipid matrix organization.

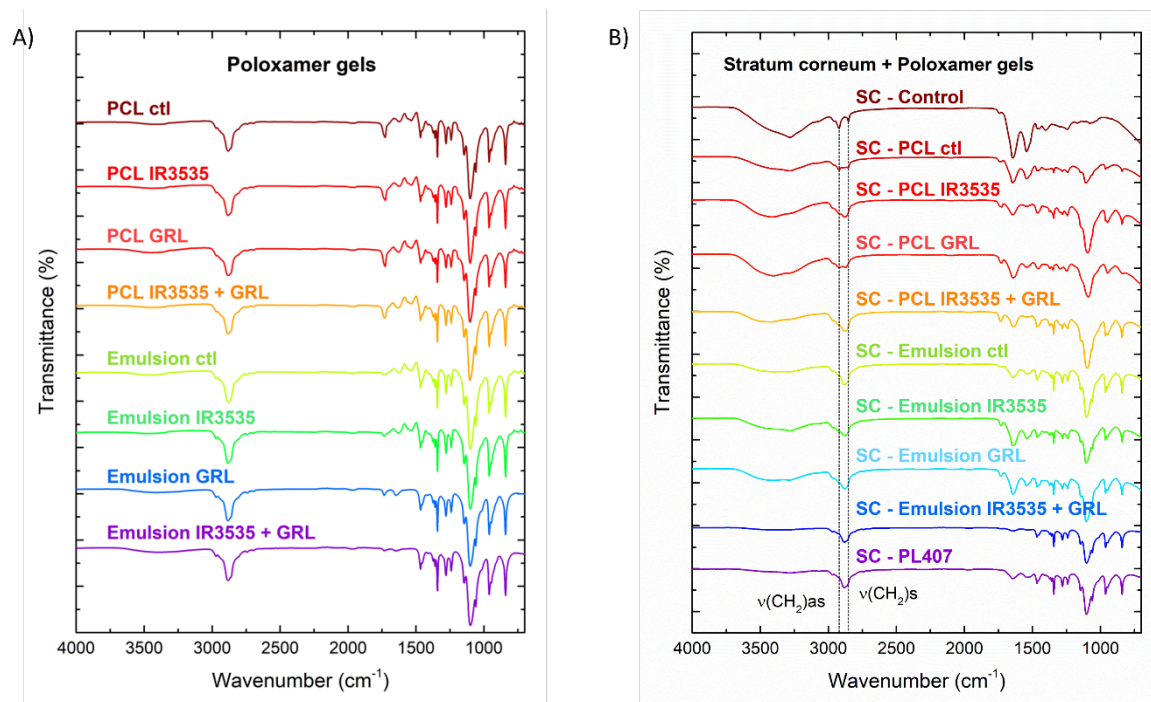


Figure 5. Infrared characterization of (A) poloxamer gels and additives, and (B) stratum corneum samples after treatment with the gels per 4 hours. FTIR spectra were recorded in the 4000–650 cm⁻¹ range with 1 cm⁻¹ resolution. The treatments used were: PCL nanoparticles (PCL ctl), PCL nanoparticles loading IR3535 (PCL IR3535), PCL nanoparticles loading GRL (PCL GRL), PCL nanoparticles loading both compounds (PCL IR3535 + GRL), control emulsion, non-encapsulated IR3535 (Emulsion IR3535), non-encapsulated GRL (emulsion GRL) and mixture of non-encapsulated compounds (Emulsion IR3535 + GRL).

Previous studies showed a high permeation capacity for repellent molecules regularly used in commercial formulations [60], e.g., DEET. Besides being considered safe for dermal applications, extensive literature discussions regarding the correlation between exposure to DEET and negative health effects are ongoing [61]. In contrast, GRL is a natural control agent exhibiting low toxicity in mammals [19,62] and IR3535 is a derivative of β -alanine with very low toxicity and similar efficacy as DEET [63].

As a monoterpene, GRL has been applied as a natural additive to improve drug skin penetration in formulations containing caffeine and diclofenac sodium. Thus, interactions between the formulations and SC were expected for samples containing GRL [19]. Permeation analysis (Figure 5) showed limited skin permeation behavior for gel formulations compared to the synthetic molecules [60]. Therefore, SC changes caused by interactions with formulations may not represent a safety risk to consumers, but the product efficacy can be impaired due to the repellent permeation [60].

3.5 *In vitro* cell viability assay

The formulation with IR3535 + GRL encapsulated in PCL nanoparticles at 500 mg/mL concentrations of IR3535 showed the most significant cytotoxic effects after 24 h with cell viability of $74 \pm 2\%$ and $46 \pm 1\%$ for HaCaT and 3T3 cells, respectively (Figure 6A). GRL at 200 mg/mL showed cell viability of $65 \pm 3\%$ and $59 \pm 6\%$ for HaCaT and 3T3 cells, respectively (Figure 6B).

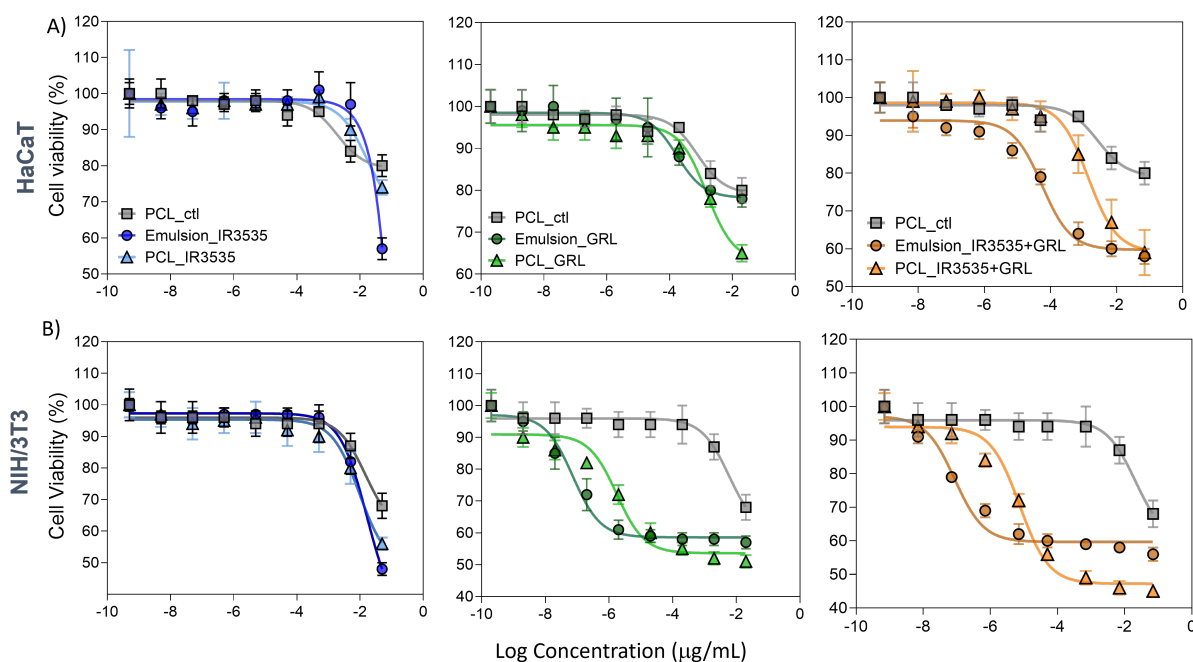


Figure 6. Cytotoxicity assay of non-encapsulated IR3535 (emulsion_IR3535), non-encapsulated GRL (emulsions_GRL), mixture of both compounds non-encapsulated (emulsion_IR3535 + GRL), PCL nanoparticles (PCL_ctl), PCL nanoparticles loading IR3535 (PCL_IR3535), PCL nanoparticles loading GRL (PCL_GRL), PCL nanoparticles loading both compounds (PCL_IR3535 + GRL) using (A) HaCaT cell line and (B) NIH-3T3 cell line after 24 h. Cell viability was evaluated by the MTT assay. The values represent the mean $\pm$ SD of three independent experiments.

The reduced cell viability may be related to the nanoparticle composition and presence of the repellent active since reduced toxicity was observed for the control nanoparticles. Ridolfi et al., (2011) studied the cytotoxic potential of solid lipid nanoparticles (SLNs) prepared with different lipids using the MTT method with the same cell lines, and verified the dose-dependent cytotoxicity of solid lipids used in nanoparticle synthesis. Mendes et al., (2015) evaluated the MTT cytotoxicity of 3 nanostructured systems and observed that the number of nanoparticles in the dispersion negatively influenced cell viability, especially NLCs.

Previous studies also evaluated GRL cytotoxicity in skin tumor cells from Swiss albino mice, showing suppression of the Ras/Raf/ERK1/2 signaling pathway inducing apoptosis [66]. Another study evaluated GRL cytotoxicity in human tumor cell lines (HepG2 and A549) using MTT methods, demonstrating dose-dependent decreased viability [67]. Corroborating the results presented herein, Hazarika et al. (2022) evaluated the cytotoxicity of a formulation containing clove oil, citronella, and lemongrass, showing decreased viability of human lung epithelial cells (L-132) with increasing concentrations of essential oils, especially between 500 and 1000 $\mu\text{g/mL}$. [69] also reported dose-dependent GRL cytotoxicity in human lymphocyte cells, with 80% viable cells at a 2000 $\mu\text{g/mL}$ concentration. Although the formulations showed more significant cytotoxicity towards the NIH-3T3 line, it should be noted that these repellent formulations focus on topical use. Therefore, the main objective is active volatilization, forming a vapor barrier on the skin due to a super concentrated local formulation. In addition, screening studies of polymeric and lipid nanoparticles on the skin found that PCL nanoparticles remained localized in lipid domains between the SC and corneocytes [70,71].

The cytotoxicity results presented herein show that carrier systems based on PCL showed reduced toxic effects when compared to the emulsions, highlighting the potential of these systems as a tool to prolong protection times with lower actives concentrations. In addition, the gel formulations tested did not show significant cytotoxicity, without indication of a toxicity halo around or under the test substance, and with cells remaining intact without any morphological changes for both cell lines (Figure 7). However, in this formulation the nanoparticles were incorporated or dispersed in a polymeric matrix (hydrogel), which likely reduced direct contact with the cells and, consequently, presented nontoxicity.

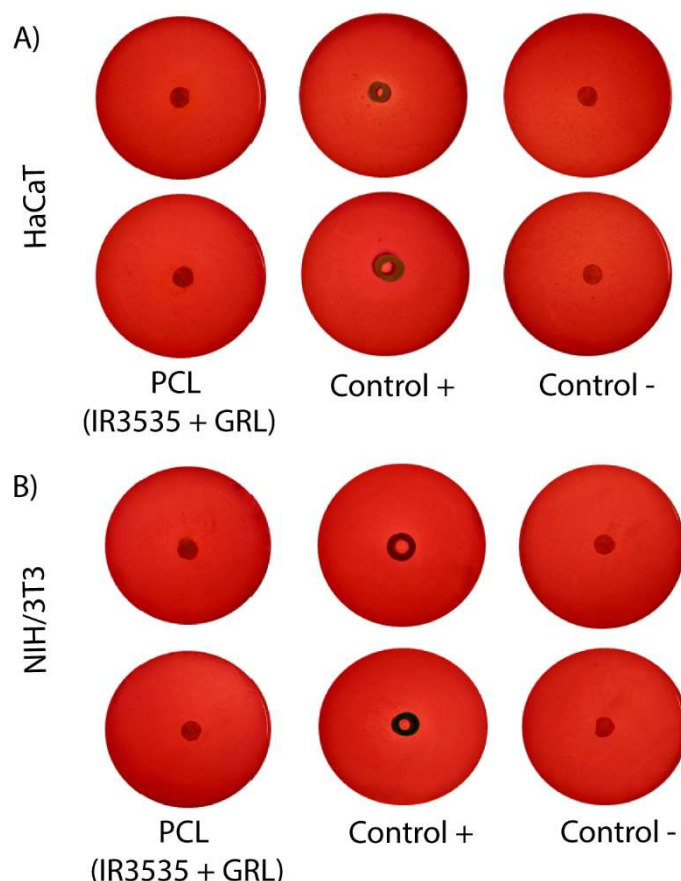


Figure 7. Cytotoxic assay of hydrogel containing PCL nanoparticles loading both compounds (PCL IR3535 + GRL) using (A) HaCaT cell line and (B) NIH-3T3 cell line after 24 h. Cell viability was evaluated by the halo formation. The values represent the mean $\pm$ SD of three independent experiments.

Conclusions

This study developed a novel generation of repellent formulations containing PCL nanoparticle encapsulated IR3535 + GRL. Stability was achieved over 120 days with EE values of 60% and 99% for IR3535 and GRL, respectively, with a spherical morphology. PCL hydrogels showed greater stability over the frequency range tested when compared to the other prepared systems. The release profiles of IR3535 were more evident in the PCL formulation incorporated in poloxamer 407 hydrogel compared to GRL. However, both actives exhibited low permeation after 24 h, indicating that the formulations (PCL nanoparticles and PCL in hydrogel) controlled the bioactive percutaneous absorption. Minor changes in the SC caused by interactions with formulations pose a small possible safety risk to the consumer. The cytotoxicity results show that the carrier systems based on PCL exhibited reduced toxicity when compared to emulsions, with potential for these systems to be used as a tool to prolong the protection time with reduced concentrations of repellent actives.

Acknowledgements

The authors are grateful for financial support provided by MCTIC-CNPq/MEC-CAPES/MS-Decit/FNDCT No 14/2016—Prevenção e Combate ao Vírus Zika (process #440350/2016-6). The authors thank the São Paulo State Research Foundation (FAPESP) for scholarships awarded to DA (#2018/14734-0), CR (#2018/02404-5), EC (2017/24402-1), AAV (2019/14773-8) and AFS (CAPES grant code #01). Also, authors would like to thank National Council for Scientific and Technological Development (CNPq).

References

1. Lequime S, Lambrechts L. Vertical transmission of arboviruses in mosquitoes: a historical perspective. *Infect Genet Evol.* 2014;28:681–90.
2. Miller BR. Arboviruses. In: Mahy BWJ, Van Regenmortel MHV, editors. *Encyclopedia of Virology (Third Edition)* [Internet]. Oxford: Academic Press; 2008: p. 170–6.
3. Huang Y-JS, Higgs S, Vanlandingham DL. Arbovirus-Mosquito Vector-Host Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses. *Front Microbiol.* 2019;10:22.
4. Mayer SV, Tesh RB, Vasilakis N. The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. *Acta Tropica.* 2017;166:155–63.
5. Artsob H, Lindsay R. Arboviruses. *International Encyclopedia of Public Health.* 2008;219–26.
6. Diaz JH. Chemical and Plant-Based Insect Repellents: Efficacy, Safety, and Toxicity. *Wilderness Environ Med.* 2016;27:153–63.
7. Tavares M, da Silva MRM, de Oliveira de Siqueira LB, Rodrigues RAS, Bodjolle-d'Almeida L, Dos Santos EP, et al. Trends in insect repellent formulations: A review. *Int J Pharm.* 2018;539:190–209.
8. EPA. United States Environmental Protection (2021). DEET [Internet]. 2021. Available from: <https://www.epa.gov/insect-repellents/deet>
9. Frances SP, Debboun M. CHAPTER 16 - The role of arthropod repellents in the control of vector-borne diseases. In: Corona C, Debboun M, Coats J, editors. *Advances in Arthropod Repellents* [Internet]. Academic Press; 2022; 323–36.
10. Alfihili MA, Nkany MB, Weidner DA, Lee M-H. Stimulation of eryptosis by broad-spectrum insect repellent N,N-Diethyl-3-methylbenzamide (DEET). *Toxicol Appl Pharmacol.* 2019;370:36–43.

11. Osimitz TG, Murphy JV, Fell LA, Page B. Adverse events associated with the use of insect repellents containing N,N-diethyl-m-toluamide (DEET). *Regul Toxicol Pharmacol.* 2010;56:93–9.
12. Katz TM, Miller JH, Hebert AA. Insect repellents: historical perspectives and new developments. *J Am Acad Dermatol.* 2008;58:865–71.
13. Nguyen Q-BD, Vu M-AN, Hebert AA. Insect Repellents: An Updated Review for the Clinician. *J Am Acad Dermatol.* 2018;S0190-9622(18)32824-X.
14. Alzogaray RA. Behavioral and Toxicological Responses of *Rhodnius prolixus* (Hemiptera: Reduviidae) to the Insect Repellents DEET and IR3535. *J Med Entomol.* 2016;53:387–93.
15. Fradin MS. 6 - Insect Protection. In: Keystone JS, Kozarsky PE, Connor BA, Nothdurft HD, Mendelson M, Leder K, editors. *Travel Medicine (Fourth Edition)* [Internet]. London: Elsevier; 2019 [cited 2022 May 19]. p. 43–52.
16. Kim IS, Ren X, Chang JS, Lee JW, Yu HW, Kim SJ, et al. The Effect of Environmental Micropollutant (DEET) on the Expression of Cell Cycle and Apoptosis Regulatory Proteins in Human Cells. *Biotechnology and Bioprocess Engineering* [Internet]. 2011 [cited 2022 May 19];
17. Antwi FB, Shama LM, Peterson RKD. Risk assessments for the insect repellents DEET and picaridin. *Regul Toxicol Pharmacol.* 2008;51:31–6.
18. Gou Y, Li Z, Fan R, Guo C, Wang L, Sun H, et al. Ethnobotanical survey and evaluation of traditional mosquito repellent plants of Dai people in Xishuangbanna, Yunnan Province, China. *J Ethnopharmacol.* 2020;262:113124.
19. Chen W, Viljoen AM. Geraniol — A review of a commercially important fragrance material. *South African Journal of Botany.* 2010;76:643–51.
20. King SB, Kaplinsky CH, Washington R, Carroll SP. CHAPTER12 - Human subject studies of arthropod repellent efficacy, at the interface of science, ethics, and regulatory oversight. In: Corona C, Debboun M, Coats J, editors. *Advances in Arthropod Repellents* [Internet]. Academic Press; 2022; p. 237–58.
21. Mappin F, DeGennaro M. CHAPTER 7 - Multimodal mechanisms of repellency in arthropods. In: Corona C, Debboun M, Coats J, editors. *Advances in Arthropod Repellents* [Internet]. Academic Press; 2022; p. 113–30.
22. Pluym N, Stöckelhuber M, Weber T, Scherer G, Scherer M, Kolossa-Gehring M. Time trend of the exposure to geraniol in 24-h urine samples derived from the German Environmental Specimen Bank from 2004 to 2018. *Int J Hyg Environ Health.* 2022;239:113880.
23. Alavez-Rosas D, Socorro-Benitez C, Cruz-Esteban S. Repellent and adulticidal effect of essential oils mixtures on *Aedes aegypti* females. 2022 [cited 2022 May 18];
24. Noosidum A, Chareonviriyaphap T, Chandrapatya A. Synergistic repellent and irritant effect of combined essential oils on *Aedes aegypti* (L.) mosquitoes. *J Vector Ecol.* 2014;39:298–305.

25. da Silva MRM, Ricci-Júnior E. An approach to natural insect repellent formulations: from basic research to technological development. *Acta Trop.* 2020;212:105419.
26. Ortiz Zamora L, Eliza Maciel de Faria Mota Oliveira A, Carlos Tavares Carvalho J, Pinho Fernandes C. Chapter 22 - Development of eco-friendly nanomoscitocides against arboviruses vectors. In: Formiga FR, Inamuddin, Severino P, editors. *Applications of Nanobiotechnology for Neglected Tropical Diseases* [Internet]. Academic Press; 2021; p. 493–507.
27. Zielińska A, Carreiró F, Oliveira AM, Neves A, Pires B, Venkatesh DN, et al. Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. *Molecules.* 2020;25:E3731.
28. Abdel-Rashid RS, Helal DA, Alaa-Eldin AA, Abdel-Monem R. Polymeric versus lipid nanocapsules for miconazole nitrate enhanced topical delivery: in vitro and ex vivo evaluation. *Drug Deliv.* 2022;29:294–304.
29. Madkour L. Nanoparticle and polymeric nanoparticle-based targeted drug delivery systems. 2019. p. 191–240.
30. Werdin González JO, Gutiérrez MM, Ferrero AA, Fernández Band B. Essential oils nanoformulations for stored-product pest control - characterization and biological properties. *Chemosphere.* 2014;100:130–8.
31. Gao W, Zhang Y, Zhang Q, Zhang L. Nanoparticle-Hydrogel: A Hybrid Biomaterial System for Localized Drug Delivery. *Ann Biomed Eng.* 2016;44:2049–61.
32. Dannert C, Stokke BT, Dias RS. Nanoparticle-Hydrogel Composites: From Molecular Interactions to Macroscopic Behavior. *Polymers.* Multidisciplinary Digital Publishing Institute; 2019;11:275.
33. Hoffman AS. Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews.* 2012;64:18–23.
34. Thoniyot P, Tan MJ, Karim AA, Young DJ, Loh XJ. Nanoparticle–Hydrogel Composites: Concept, Design, and Applications of These Promising, Multi-Functional Materials. *Advanced Science.* 2015;2:1400010.
35. Liu J, Zheng H, Poh PSP, Machens H-G, Schilling AF. Hydrogels for Engineering of Perfusable Vascular Networks. *Int J Mol Sci.* 2015;16:15997–6016.
36. Buntum T, Kongprayoon A, Mungyai W, Charoenram P, Kiti K, Thanomsilp C, et al. Wound-aided semi-solid poly(vinyl alcohol) hydrogels incorporating essential oil-loaded chitosan nanoparticles. *Int J Biol Macromol.* 2021;189:135–41.
37. Ngampunwetchakul L, Toonkaew S, Supaphol P, Suwantong O. Semi-solid poly(vinyl alcohol) hydrogels containing ginger essential oil encapsulated in chitosan nanoparticles for use in wound management. *J Polym Res.* 2019;26:224.
38. Vitorino C, Carvalho FA, Almeida AJ, Sousa JJ, Pais AACC. The size of solid lipid nanoparticles: an interpretation from experimental design. *Colloids Surf B Biointerfaces.* 2011;84:117–30.

39. Vigato AA, Machado IP, Del Valle M, da Ana PA, Sepulveda AF, Yokaichiya F, et al. Monoketonic Curcuminoid-Lidocaine Co-Deliver Using Thermosensitive Organogels: From Drug Synthesis to Epidermis Structural Studies. *Pharmaceutics*. 2022;14:293.
40. De Souza Ferreira SB, Moço TD, Borghi-Pangoni FB, Junqueira MV, Bruschi ML. Rheological, mucoadhesive and textural properties of thermoresponsive polymer blends for biomedical applications. *J Mech Behav Biomed Mater*. 2015;55:164–78.
41. Shriky B, Kelly A, Isreb M, Babenko M, Mahmoudi N, Rogers S, et al. Pluronic F127 thermosensitive injectable smart hydrogels for controlled drug delivery system development. *J Colloid Interface Sci*. 2020;565:119–30.
42. Ikura H, Uchida K, Ogawa-Fuse C, Bito K, Naitou S, Hosokawa M, et al. Effects of Temperature and Humidity on the Skin Permeation of Hydrophilic and Hydrophobic Drugs. *AAPS PharmSciTech*. 2019;20:264.
43. Uchida T, Kadhum WR, Kanai S, Todo H, Oshizaka T, Sugibayashi K. Prediction of skin permeation by chemical compounds using the artificial membrane, Strat-M™. *Eur J Pharm Sci*. 2015;67:113–8.
44. Kaur L, Singh K, Paul S, Singh S, Singh S, Jain SK. A Mechanistic Study to Determine the Structural Similarities Between Artificial Membrane Strat-M™ and Biological Membranes and Its Application to Carry Out Skin Permeation Study of Amphotericin B Nanoformulations. *AAPS PharmSciTech*. 2018;19:1606–24.
45. de Araujo DR, Padula C, Cereda CMS, Tófoli GR, Brito RB, de Paula E, et al. Bioadhesive films containing benzocaine: correlation between in vitro permeation and in vivo local anesthetic effect. *Pharm Res*. 2010;27:1677–86.
46. Boncheva M, Damien F, Normand V. Molecular organization of the lipid matrix in intact Stratum corneum using ATR-FTIR spectroscopy. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2008;1778:1344–55.
47. Caussin J, Gooris GS, Janssens M, Bouwstra JA. Lipid organization in human and porcine stratum corneum differs widely, while lipid mixtures with porcine ceramides model human stratum corneum lipid organization very closely. *Biochim Biophys Acta*. 2008;1778:1472–82.
48. Hasanovic A, Winkler R, Resch GP, Valenta C. Modification of the conformational skin structure by treatment with liposomal formulations and its correlation to the penetration depth of aciclovir. *European Journal of Pharmaceutics and Biopharmaceutics*. 2011;79:76–81.
49. Barbosa TC, Nascimento LÉD, Bani C, Almeida T, Nery M, Santos RS, et al. Development, Cytotoxicity and Eye Irritation Profile of a New Sunscreen Formulation Based on Benzophenone-3-poly(ε-caprolactone) Nanocapsules. *Toxics*. 2019;7.
50. Główna E, Wosicka-Frąckowiak H, Hyla K, Stefanowska J, Jastrzębska K, Kłapiszewski L, Jesionowski T, Cal K. Polymeric nanoparticles-embedded organogel for roxithromycin delivery to hair follicles. *European Journal of Pharmaceutics and Biopharmaceutics*. 2014;88:75-84.

51. Ashour AE, Badran M, Kumar A, Hussain T, Alsarra IA, Yassin AEB. Physical PEGylation Enhances The Cytotoxicity Of 5-Fluorouracil-Loaded PLGA And PCL Nanoparticles. *Int J Nanomedicine*. 2019;14:9259–73.
52. de Oliveira ÉL, Ferreira SBS, de Castro-Hoshino LV, Campanholi K da SS, Calori IR, de Moraes FAP, et al. Thermoresponsive Hydrogel-Loading Aluminum Chloride Phthalocyanine as a Drug Release Platform for Topical Administration in Photodynamic Therapy. *Langmuir*. 2021;37:3202–13.
53. Victorelli FD, Calixto GMF, Ramos MADS, Bauab TM, Chorilli M. Metronidazole-Loaded Polyethyleneimine and Chitosan-Based Liquid Crystalline System for Treatment of Staphylococcal Skin Infections. *J Biomed Nanotechnol*. 2018;14:227–37.
54. Nnamani PO, Hansen S, Windbergs M, Lehr C-M. Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application. *Int J Pharm*. 2014;477:208–17.
55. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol Pharm*. 2010;67:217–23.
56. Fu Y, Kao WJ. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. *Expert Opin Drug Deliv*. 2010;7:429–44.
57. Abrantes DC, Rogerio CB, de Oliveira JL, Campos EVR, de Araújo DR, Pampana LC, et al. Development of a Mosquito Repellent Formulation Based on Nanostructured Lipid Carriers. *Front Pharmacol*. 2021;12:760682.
58. Skinner WA, Tong HC, Maibach HI, Skidmore D. Human skin-surface lipid fatty acids--mosquito repellents. *Experientia*. 1970;26:728–30.
59. Fediuk DJ, Wang T, Raizman JE, Parkinson FE, Gu X. Tissue deposition of the insect repellent DEET and the sunscreen oxybenzone from repeated topical skin applications in rats. *Int J Toxicol*. 2010;29:594–603.
60. Kasichayanula S, House JD, Wang T, Gu X. Percutaneous characterization of the insect repellent DEET and the sunscreen oxybenzone from topical skin application. *Toxicol Appl Pharmacol*. 2007;223:187–94.
61. Swale DR, Bloomquist JR. Is DEET a dangerous neurotoxicant? *Pest Manag Sci*. 2019;75:2068–70.
62. Schmitt S, Schaefer UF, Doeblner L, Reichling J. Cooperative interaction of monoterpenes and phenylpropanoids on the in vitro human skin permeation of complex composed essential oils. *Planta Med*. 2009;75:1381–5.
63. Shrestha B, Nhuchhen Pradhan R, Nath DK, Lee Y. Cellular and molecular basis of IR3535 perception in *Drosophila*. *Pest Manag Sci*. 2022;78:793–802.
64. Ridolfi DM, Marcato PD, Machado D, Silva RA, Justo GZ, Durán N. In vitro cytotoxicity assays of solid lipid nanoparticles in epithelial and dermal cells. *J Phys: Conf Ser*. IOP Publishing; 2011;304:012032.

65. Mendes LP, Delgado JMF, Costa ADA, Vieira MS, Benfica PL, Lima EM, et al. Biodegradable nanoparticles designed for drug delivery: The number of nanoparticles impacts on cytotoxicity. *Toxicology in Vitro*. 2015;29:1268–74.
66. Chaudhary SC, Siddiqui MS, Athar M, Alam MS. Geraniol inhibits murine skin tumorigenesis by modulating COX-2 expression, Ras-ERK1/2 signaling pathway and apoptosis. *Journal of Applied Toxicology*. 2013;33:828–37.
67. Crespo R, Rodenak-Kladniew BE, Castro MA, Soberón MV, Lavarías SML. Induction of oxidative stress as a possible mechanism by which geraniol affects the proliferation of human A549 and HepG2 tumor cells. *Chem Biol Interact*. 2020;320:109029.
68. Hazarika H, Krishnatreyya H, Tyagi V, Islam J, Gogoi N, Goyary D, et al. The fabrication and assessment of mosquito repellent cream for outdoor protection. *Sci Rep*. Nature Publishing Group; 2022;12:2180.
69. Sinha S, Jothiramajayam M, Ghosh M, Mukherjee A. Evaluation of toxicity of essential oils palmarosa, citronella, lemongrass and vetiver in human lymphocytes. *Food Chem Toxicol*. 2014;68:71–7.
70. Jain AK, Jain A, Garg NK, Agarwal A, Atul Jain, Jain SA, Tyagi RK, Jain RK, Agrawal H, Agrawal GP. Adapalene loaded solid lipid nanoparticles gel: An effective approach for acne treatment. *Colloids and Surfaces B: Biointerfaces*. 2014;121: 222-229.
71. Kahraman E, Güngör S, Özsoy Y. Potential enhancement and targeting strategies of polymeric and lipid-based nanocarriers in dermal drug delivery. *Therapeutic Delivery*. 2017; 11:967-985.

SUPPLEMENTARY INFORMATION

REPELLENT ACTIVE INGREDIENTS ENCAPSULATED IN POLYMERIC NANOPARTICLES: POTENTIAL ALTERNATIVE FORMULATIONS TO CONTROL ARBOVIRUSES

Daniele Carvalho Abrantes¹, Carolina Barbara Rogerio¹, Estefânia V. R Campos¹, Tais Germano-Costa², Aryane Alves Vigato³, Ian Pompermeyer Machado³, Anderson Ferreira Sepulveda³, Renata Lima², Daniele Ribeiro de Araújo³, and Leonardo Fernandes Fraceto^{1*}

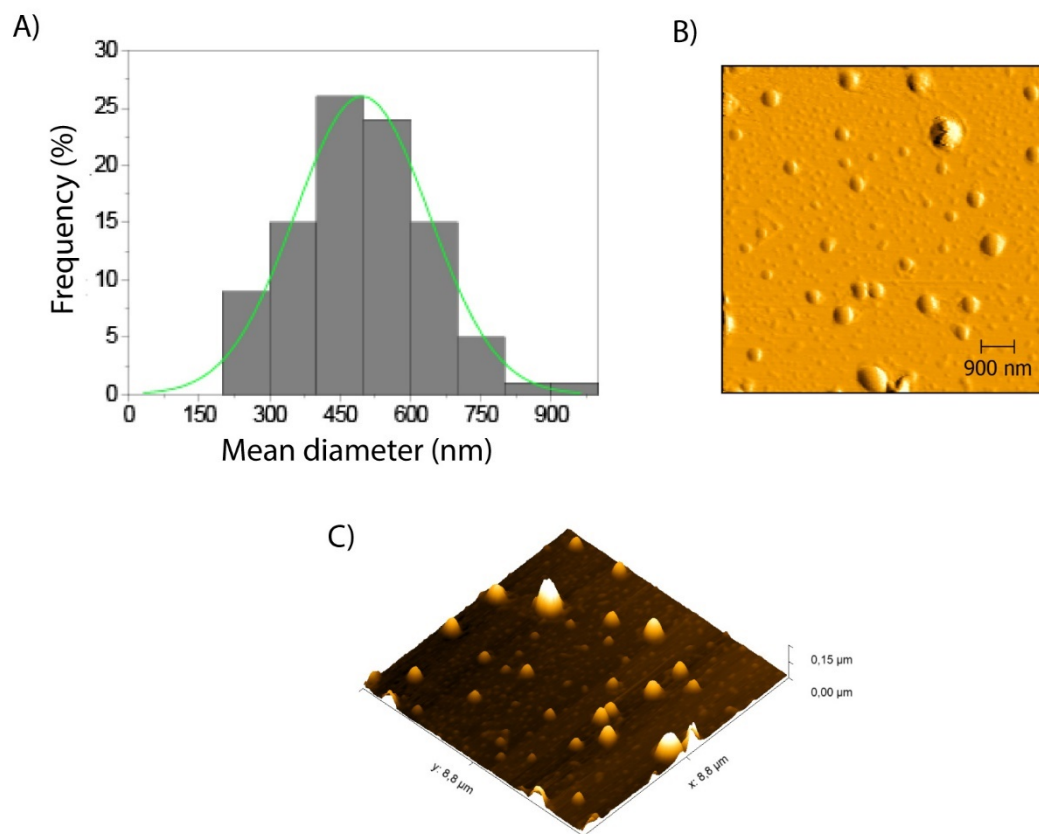
¹ São Paulo State University (UNESP), Institute of Science and Technology, Avenida Três de Março 511, Alto da Boa Vista, Sorocaba, São Paulo, 18087-180, Brazil
Jaboticabal, São Paulo, 14884-900, Brazil

² Laboratory of Bioactivity Assessment and Toxicology of Nanomaterials, University of Sorocaba, Sorocaba, São Paulo, Brazil

³ Human and Natural Sciences Center, Federal University of ABC, Santo André, São Paulo, 09210-580, Brazil

Figures

Figure 1S. Images obtained from AFM, with a scanning window of 8 mm x 8 mm. A) Surface topography of PCLs (IR3535 5% + GRL 2%) and average-sized frequency distribution histograms; B) 3D topography of the PCLs (IR3535 5% + GRL 2%).



Table

Table 1S – Mathematical model parameters, such as release constant (k_2); correlation coefficient (r^2), and exponent of the release mechanism (n) after application of zero order, first order, Higuchi and Korsmeyer - Peppas models to the release curve of IR3535 and GRL from the PCL nanoparticles formulation and hydrogel containing PCL nanoparticles formulation.

Release Kinetics	Zero Order		First Order		Higuchi		Korsmeyer-Peppas		
Formulations	K_2	r^2	K_2	r^2	K_2	r^2	K_2	n	r^2
PCL_Suspension									
IR3535	1.081	0.9415	0.00251	0.7849	23.90	0.9896	0.6513	1.53	0.9889
GRL	0.0020	0.9473	0.0005	0.7295	0.0476	0.9328	0.8467	1.18	0.8467
PCL_Gel									
GRL	0.0197	0.9051	0.0050	0.6808	0.4549	0.8947	0.6858	1.45	0.8453
IR3535	0.1590	0.9346	0.00316	0.8520	3.697	0.9400	0.8737	1.14	0.9508

CONCLUSÕES GERAIS E PERSPECTIVAS

Existe uma diversidade enorme de mosquitos presentes no ecossistema, sendo que algumas espécies são vetores de doenças que afligem milhares de pessoas. A expansão geográfica destes vetores vem sendo uma constante e está relacionada com o processo desenfreado de urbanização, fatores climáticos e a globalização. Atualmente, os relatórios mundiais estão notificando casos crescentes que incluem múltiplas doenças causadas por arboviroses, que tem como principal vetor o *Aedes aegypti*. Dentre as estratégias de controle adotadas pelos órgãos de saúde, os repelentes de contato são amplamente usados para evitar a exposição a doenças. As novas tecnologias representam um aspecto fundamental no design de formulações menos tóxica, mais segura e características sensoriais agradáveis, além de ter potencial de reduzir os mecanismos de resistências ao repelente. Desta forma, é essencial ressaltar a importância deste estudo onde além do desenvolvimento e caracterização de um novo sistema repelente baseado em nanopartículas e incorporado em gel, também foi avaliado o perfil de liberação, permeação e análise estrutural da epiderme através de sistemas *in vitro*, bem como analisadas as características reológicas, controle microbiológico e estudo de estabilidade.

A formulação de carreadores lipídicos nanoestruturados contendo icaridina e geraniol desenvolvidas neste trabalho apresentaram formato esférico, como diâmetro médio de 252 ± 5 nm, índice de polidispersão característicos de formulações estáveis (abaixo de 0,2), alta eficiência de encapsulação ($> 90\%$), além disso uma boa estabilidade nas condições experimentais e em função do tempo de armazenamento em condições controladas de temperatura e umidade. O estudo de estabilidade acelerado demonstrou que as características organolépticas, como a cor, o odor, a aparência e a homogeneidade dos NLCs não apresentaram alterações significativas. Quanto ao controle microbiológico, as análises apresentaram resultados da contagem microbiana e pesquisa de patógenos dentro do esperado, atendendo às especificações de garantia da qualidade.

O perfil reológico da formulação em gel apresentou duas características essenciais como a pseudoplasticidade e tixotropia, permitindo uma boa espalhabilidade do repelente na pele, bem como maior vida de prateleira. O perfil de liberação da icaridina nos NLC em suspensão ($79 \pm 4\%$) foi significativamente maior quando comparado ao gel ($66 \pm 3\%$). A liberação do geraniol não apresentou diferença significativa entre a suspensão ($17 \pm 2\%$) e o gel ($18 \pm 1\%$). Embora a diferença na porcentagem liberada entre os dois ativos, ambos mostraram uma liberação sustentada, fato este que, possivelmente, irá proporcionar a repelência prolongada. A permeação *in vitro* da icaridina foi menor do que a formulação em

gel, pressupondo uma menor penetração transdérmica e possivelmente uma menor toxicidade.

A formulação contendo sistema repelente de nanopartículas poliméricas à base de PCL e encapsuladas com uma mistura de IR3535 + geraniol em suspensão e incorporadas em hidrogel apresentou-se estável por 120 dias com eficiência de encapsulamento (EE) 60% IR3535 e 99% geraniol e morfologia esférica. GRL e IR3535 encapsulado em PCL liberaram $7 \pm 0,1$ e $83 \pm 2\%$ em 24h, respectivamente. Enquanto a mesma formulação incorporada em hidrogel de poloxâmero 407 liberou $11 \pm 0,9\%$ de GRL e $29 \pm 3\%$ de IR3535. A permeação de GRL de nanopartículas de PCL e nanopartículas de PCL em hidrogel apresentaram perfis semelhantes, enquanto a permeação de IR3535 também foi modulada, onde as diferenças nas quantidades permeadas de IR3535 foram maiores para nanopartículas de PCL em hidrogéis ($36,9 \pm 1,1 \text{ mg/cm}^2$) quando comparadas a IR3535- Nanopartículas de PCL ($29,2 \pm 1,5 \text{ mg/cm}^2$). No entanto, ambos os ativos apresentam baixa permeabilidade após 24 h, indicando que as formulações (nanopartículas de PCL e PCL em hidrogel) foram capazes de controlar a absorção percutânea do bioativo. Houve pequenas alterações no estrato córneo (SC) causadas por interações com formulações que podem não representar um risco de segurança para o consumidor comum. Os resultados de citotoxicidade apresentados mostram que os sistemas carreadores à base de poli-épsilon caprolactona (PCL) tiveram um efeito tóxico reduzido quando comparados às emulsões, abrindo perspectivas para o uso desses sistemas como ferramenta para prolongar o tempo de proteção com menor concentração de repelente ativos.

Sendo assim, os dois sistemas repelentes baseados em nanopartículas (NLC e PCL) mostraram potencial para a integrar a nova geração de repelentes contra mosquitos. Baseado nas análises de liberação e permeação *in vitro*, ambos os sistemas apresentaram uma performance mais adequada na formulação líquida devido ao melhor perfil de liberação em 24h e menor concentração de ativos permeados através da membrana Strat-M. No entanto, estes parâmetros podem ser otimizados na formulação e ambas (líquidas e gel) possuem potencial de serem comercializadas.

REFERENCIAS

- ANVISA. **Guia de Estabilidade de Produtos Cosméticos**, 2004. Disponível em: <<http://www.anvisa.gov.br/divulga/public/series/cosmeticos.pdf>>
- ANVISA. **Manual de protocolos para testes de produtos desinfetantes**, 2007. Disponível em: <<http://www.crq4.org.br/downloads/desinfestantes.pdf>>. Acesso em: 10 set. 2017
- ANVISA. Farmacopeia Brasileira. **Agência Nacional de Vigilância Sanitária** 6ª ed., 2019. Disponível em: <<https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/farmacopeia-brasileira/brasileira>>. Acesso em : 21 jan, 2021.
- ALFHILI, M. A. et al. Stimulation of eryptosis by broad-spectrum insect repellent N,N-Diethyl-3-methylbenzamide (DEET). **Toxicology and Applied Pharmacology**, v. 370, p. 36–43, 2019.
- ALZOGARAY, R. A. Behavioral and Toxicological Responses of *Rhodnius prolixus* (Hemiptera: Reduviidae) to the Insect Repellents DEET and IR3535. **Journal of Medical Entomology**, v. 53, p. 287–393, 2016.
- ANTWI, F. B.; SHAMA, L. M.; PETERSON, R. K. D. Risk assessments for the insect repellents DEET and picaridin. **Regulatory Toxicology and Pharmacology**, v. 51, n. 1, p. 31–36, 2008.
- APPEL, E. A. et al. Self-assembled hydrogels utilizing polymer–nanoparticle interactions. **Nature Communications**, v. 6, n. 1, p. 6295, 19 fev. 2015. AFIFY, A. et al. Commonly Used Insect Repellents Hide Human Odors from *Anopheles* Mosquitoes. *Current Biology*, v. 29, n. 21, p. 3669–3680, 2019.
- ARTSOB, H.; LINDSAY, R. Arboviruses. *International Encyclopedia of Public Health*, p. 219–226, 2008.
- BINDER, L. et al. The role of viscosity on skin penetration from cellulose ether-based hydrogels. **Skin research and technology: official journal of International Society for Bioengineering and the Skin (ISBS)**, v. 25, n. 5, p. 725–734, 2019.
- BELOQUI, A. et al. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. **Nanomedicine: Nanotechnology, Biology and Medicine**, v. 12, n. 1, p. 143–161, 2016.
- BROSCHARD, T. H. et al. Biotransformation and toxicokinetics of the insect repellent IR3535® in male and female human subjects after dermal exposure. *Toxicology Letters*, v. 218, n. 3, p. 246–252, 2013.
- BRIASSOULIS, G.; NARLIOGLOU, M.; HATZIS, T. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. **Human & Experimental Toxicology**, v. 20, n. 1, p. 8–14, 2001.

- BUNTUM, T. et al. Wound-aided semi-solid poly(vinyl alcohol) hydrogels incorporating essential oil-loaded chitosan nanoparticles. **International Journal of Biological Macromolecules**, v. 189, p. 135–141, 2021.
- CACCAVO, D. An overview on the mathematical modeling of hydrogels' behavior for drug delivery systems. **International Journal of Pharmaceutics**, v. 560, p. 175–190, 2019.
- CAMPOS, D. et al. Responses of the aquatic midge *Chironomus riparius* to DEET exposure. **Aquatic Toxicology**, v. 172, p. 80–85, 2016.
- CARISSIMO, G. et al. Antiviral immunity of *Anopheles gambiae* is highly compartmentalized, with distinct roles for RNA interference and gut microbiota. **PNAS**, n. 29, p. 176–185, 2014.
- CARROLL, S. P.; LOYE, J. PMD, a registered botanical mosquito repellent with deet-like efficacy. **Journal of the American Mosquito Control Association**, v. 22, n. 3, p. 507–514, 2006.
- CHATTERJEE, S. et al. Dual-responsive (pH/temperature) Pluronic F-127 hydrogel drug delivery system for textile-based transdermal therapy. **Scientific Reports**, v. 9, p. 11658, 2019.
- CHEN, W.; VILJOEN, A. M. Geraniol — A review of a commercially important fragrance material. **South African Journal of Botany**, v. 76, n. 4, p. 643–651, 2010.
- CONWAY, M. J.; COLPITTS, T. M.; FIKRIG, E. Role of the Vector in Arbovirus Transmission. **Annual Review of Virology**, v. 1, p. 71-88, 2014.
- CHANG, C.; DOBASHI, T. Preparation of alginate complex capsules containing eucalyptus essential oil and its controlled release. **Colloids and Surfaces B: Biointerfaces**, v. 32, p. 257–262, 1 nov. 2003.
- DAO, H. et al. Microbial Stability of Pharmaceutical and Cosmetic Products. **AAPS PharmSciTech**, v. 19, n. 1, p. 60–78, jan. 2018. DA SILVA, M. R. M.; RICCI-JÚNIOR, E. An approach to natural insect repellent formulations: from basic research to technological development. *Acta Tropica*, v. 212, p. 105419, 2020.
- DA SILVA, N. P. et al. Improved anti-Cutibacterium acnes activity of tea tree oil-loaded chitosan-poly(ϵ -caprolactone) core-shell nanocapsules. **Colloids and Surfaces B: Biointerfaces**, v. 196, p. 111371, 2020.
- DANNERT, C.; STOKKE, B. T.; DIAS, R. S. Nanoparticle-Hydrogel Composites: From Molecular Interactions to Macroscopic Behavior. **Polymers**, v. 11, p. 275, 2019.
- DAS, N. G. et al. Synergistic mosquito-repellent activity of *Curcuma longa*, *Pogostemon heyneanus* and *Zanthoxylum limonella* essential oils. **Journal of Infection and Public Health**, v. 8, n. 4, p. 323–328, 2015.

- DE ARAUJO, D. R. et al. Bioadhesive films containing benzocaine: correlation between in vitro permeation and in vivo local anesthetic effect. **Pharmaceutical Research**, v. 27, n. 8, p. 1677–1686, 2010.
- DE OLIVEIRA, J. L. et al. Geraniol Encapsulated in Chitosan/Gum Arabic Nanoparticles: A Promising System for Pest Management in Sustainable Agriculture. **Journal of Agricultural and Food Chemistry**, v. 66, n. 21, p. 5325–5334, 30 maio 2018.
- DESFRAÇOIS, C.; AUZÉLY, R.; TEXIER, I. Lipid Nanoparticles and Their Hydrogel Composites for Drug Delivery: A Review. **Pharmaceuticals**, v. 11, n. 4, 1 nov. 2018.
- DHARMALINGAM, K.; ANANDALAKSHMI, R. Fabrication, characterization and drug loading efficiency of citric acid crosslinked NaCMC-HPMC hydrogel films for wound healing drug delivery applications. **International Journal of Biological Macromolecules**, v. 134, p. 815–829, 2019.
- DIAZ, J. H. Chemical and Plant-Based Insect Repellents: Efficacy, Safety, and Toxicity. **Wilderness & Environmental Medicine**, v. 27, n. 1, p. 153–163, 2016.
- DICKENS, J. C.; BOHBOT, J. D. Mini review: Mode of action of mosquito repellents. Pesticide Biochemistry and Physiology, Special Issue: Advances in Vector and Urban **Pest Management and Resistance**. v. 106, n. 3, p. 149–155, 1 jul. 2013.
- DINIZ, I. M. A. et al. Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. **Journal of Materials Science: Materials in Medicine**, v. 26, p. 153, 2015.
- DUARTE, J. L. et al. Novel bioadhesive polycarbophil-based liquid crystal systems containing *Melaleuca alternifolia* oil as potential repellents against *Aedes aegypti*. **Journal of Molecular Liquids**, v. 314, p. 113626, 2020.
- DOKTOROVOVA, S.; SOUTO, E. B. Nanostructured lipid carrier-based hydrogel formulations for drug delivery: a comprehensive review. **Expert Opinion on Drug Delivery**, v. 6, n. 2, p. 165–176, 2009.
- EPA - UNITED STATES ENVIRONMENTAL PROTECTION. **DEET**. Disponível em: <<https://www.epa.gov/insect-repellents/deet>>.
- EL-MEKAWY, R. E.; JASSAS, R. S. Recent trends in smart and flexible three-dimensional cross-linked polymers: synthesis of chitosan-ZnO nanocomposite hydrogels for insulin drug delivery. **MedChemComm**, v. 8, n. 5, p. 897–906, 2017.
- FONTENILLE, D.; POWELL, J. R. Opinion From Anonymous to Public Enemy: How Does a Mosquito Become a Feared Arbovirus Vector? **Pathogens**, v. 9, p. 265, 2020.
- FRADIN, M. S. Insect Protection. *Travel Medicine*, p. 43–52, 2019.
- FRANCES, S. P.; DEBBOUN, M. The role of arthropod repellents in the control of vector-borne diseases. **Advances in Arthropod Repellents**, p. 323–336, 2022.

- FROILLO, F. et al. Essential Oils-Loaded Polymer Particles: Preparation, Characterization and Antimicrobial Property. **Polymers**, v. 11, p. 1017, 2019.
- FANG, J.-Y. et al. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). **European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V.**, v. 70, n. 2, p. 633–640, out. 2008.
- FERREIRA, A. O. **Guia Prático da Farmácia Magistral**. 4^a ed. São Paulo: Pharmabooks, 2010.
- GASPAR, L. R.; MAIA CAMPOS, P. M. B. G. Rheological behavior and the SPF of sunscreens. **International Journal of Pharmaceutics**, v. 250, n. 1, p. 35–44, 2 jan. 2003. GAO, W. et al. Nanoparticle-Hydrogel: A Hybrid Biomaterial System for Localized Drug Delivery. **Annals of Biomedical Engineering**, v. 44, n. 6, p. 2049–2061, 2016.
- GARCÊS, A. et al. Formulations based on solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for cutaneous use: A review. **European Journal of Pharmaceutical Sciences**, v. 112, p. 159–167, 2018.
- GHODRATI, M.; FARAHPOUR, M. R.; HAMISHEHKAR, H. Encapsulation of Peppermint essential oil in nanostructured lipid carriers: In-vitro antibacterial activity and accelerative effect on infected wound healing. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 564, p. 161–169, 2019.
- GONZÁLEZ, J. O. W. et al. Polymer nanoparticles containing essential oils: new options for mosquito control. **Environmental Science and Pollution Research**, v. 24, p. 17006–17015, 2017.
- GOU, Y. et al. Ethnobotanical survey and evaluation of traditional mosquito repellent plants of Dai people in Xishuangbanna, Yunnan Province, China. **Journal of Ethnopharmacology**, v. 262, p. 113124, 2020.
- GOULD, E. et al. Emerging arboviruses: Why today? **One Health (Amsterdam, Netherlands)**, v. 4, p. 1–13, dez. 2017.
- GRILLO, R. et al. Influence of hybrid polymeric nanoparticle/thermosensitive hydrogels systems on formulation tracking and in vitro artificial membrane permeation: A promising system for skin drug-delivery. **Colloids and Surfaces. B, Biointerfaces**, v. 174, p. 56–62, 1 fev. 2019.
- GUARNER, J.; HALE, G. L. Four human diseases with significant public health impact caused by mosquito-borne flaviviruses: West Nile, Zika, dengue and yellow fever. **Seminars in Diagnostic Pathology**, v. 36, n. 3, p. 170–176, 2019.
- HALEEM, Z. M. et al. Exposure to N,N-Diethyl-Meta-Toluamide Insect Repellent and Human Health Markers: Population Based Estimates from the National Health and Nutrition Examination Survey. **Am. J. Trop. Med. Hyg.**, v. 103, n. 2, p. 812–814, 2020.

- HASHEM, F. M. et al. Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. **AAPS PharmSciTech**, v. 12, n. 3, p. 879–886, 2011.
- HAZARIKA, H. et al. The fabrication and assessment of mosquito repellent cream for outdoor protection. **Scientific Reports**, v. 12, p. 2180, 2022.
- HAY, W. T. et al. Improved hydroxypropyl methylcellulose (HPMC) films through incorporation of amylose-sodium palmitate inclusion complexes. **Carbohydrate Polymers**, v. 188, p. 76–84, 15 maio 2018.
- HELFRICHT, N. et al. Writing with Fluid: Structuring Hydrogels with Micrometer Precision by AFM in Combination with Nanofluidics. **Small** (Weinheim an Der Bergstrasse, Germany), v. 13, n. 31, 2017.
- HOFFMAN, A. S. Hydrogels for biomedical applications. **Advanced Drug Delivery Reviews**, v. 64, p. 18–23, 1 dez. 2012.
- HUANG, Y.-J.; HIGGS, S.; VANLANDINGHAM, D. L. Arbovirus-Mosquito Vector-Host Interactions and the Impact on Transmission and Disease Pathogenesis of Arboviruses. **Frontiers in Microbiology**, v. 10, p. 1–14, 2019.
- HUANG, Y.-J. S.; HIGGS, S.; VANLANDINGHAM, D. L. Biological Control Strategies for Mosquito Vectors of Arboviruses. **Insects**, v. 8, p. 21, 2017.
- HUBÁLEK, Z.; RUDOLF, I.; NOWOTNY, N. Arboviruses Pathogenic for Domestic and Wild Animals. **Advances in Virus Research**, v. 89, p. 201–275, 2014.
- IIKURA, H. et al. Effects of Temperature and Humidity on the Skin Permeation of Hydrophilic and Hydrophobic Drugs. **AAPS PharmSciTech**, v. 20, n. 7, p. 264, 2019.
- ISAAC, V. et al. **Protocolo para ensaios físico-químicos de estabilidade de fitocosméticos**. Disponível em: <https://www.repositorio.ufpa.br/bitstream/handle/2011-6/10000/1/Protocolo-para-ensaios-f%C3%ADsico-qu%C3%ADmicos-de-de-Isaac-Cefali/fa0ba805b342e0aaae6b4db73c30c08c4a1e033f>. Acesso em: 21 jan. 2021.
- ISLAM, J. et al. Mosquito repellents: An insight into the chronological perspectives and novel discoveries. **Acta Tropica**, v. 167, p. 216–230, 2017.
- JUNYAPRASERT, V. B. et al. Q10-loaded NLC versus nanoemulsions: stability, rheology and in vitro skin permeation. **International Journal of Pharmaceutics**, v. 377, n. 1–2, p. 207–214, 2009.
- KATZ, T. M.; MILLER, J. H.; HEBERT, A. A. Insect repellents: Historical perspectives and new developments. **Journal of the American Academy of Dermatology**, v. 58, n. 5, p. 865–871, 2008.
- KAUR, L. et al. A Mechanistic Study to Determine the Structural Similarities Between Artificial Membrane Strat-M™ and Biological Membranes and Its Application to Carry Out Skin

- Permeation Study of Amphotericin B Nanoformulations. **AAPS PharmSciTech**, v. 19, n. 4, p. 1606–1624, 2018.
- KHANUM, H. et al. Fabrication and in vitro characterization of HPMC-g-poly(AMPS) hydrogels loaded with loxoprofen sodium. **International Journal of Biological Macromolecules**, v. 120, p. 1624–1631, 2018.
- KHOSA, A.; REDDI, S.; SAHA, R. N. Nanostructured lipid carriers for site-specific drug delivery. **Biomedicine & Pharmacotherapy**, v. 103, p. 598–613, 2018.
- KUDARHA, R. et al. Box-Behnken study design for optimization of bicalutamide-loaded nanostructured lipid carrier: stability assessment. **Pharmaceutical Development and Technology**, v. 20, n. 5, p. 608–618, 2015.
- KURI-MORALES, P. A. et al. Repellency of 29 Synthetic and Natural Commercial Topical Insect Repellents Against *Aedes aegypti* (Diptera: Culicidae) in Central Mexico. **Journal of Medical Entomology**, v. 54, n. 5, p. 1305–1311, 2017a.
- KURI-MORALES, P. A. et al. Repellency of 29 Synthetic and Natural Commercial Topical Insect Repellents Against *Aedes aegypti* (Diptera: Culicidae) in Central Mexico. **Journal of Medical Entomology**, v. 54, n. 5, p. 1305–1311, 2017b.
- KWAN, M. W. C. et al. A Low-Cost, Passive Release Device for the Surveillance and Control of Mosquitoes. **International Journal of Environmental Research and Public Health**, v. 16, n. 9, 2019.
- KWON, Y. et al. Drosophila TRPA1 channel is required to avoid the naturally occurring insect repellent citronellal. **Current biology : CB**, v. 20, n. 18, p. 1672–1678, 28 set. 2010.
- LAMMARI, N. et al. Encapsulation of Essential Oils via Nanoprecipitation Process: Overview, Progress, Challenges and Prospects. **Pharmaceutics**, v. 12, n. 5, 7 maio 2020.
- LANE, M. E. Nanoparticles and the skin--applications and limitations. **Journal of Microencapsulation**, v. 28, n. 8, p. 709–716, 2011.
- LANG, C. et al. Nanoparticle tools to improve and advance precision practices in the Agrifoods Sector towards sustainability - A review. **Journal of Cleaner Production**, v. 293, p. 126063, 15 abr. 2021.
- LEGEAY, S. et al. Unusual modes of action of the repellent DEET in insects highlight some human side effects. **European Journal of Pharmacology**, v. 825, p. 92–98, 2018.
- LEQUIME, S.; LAMBRECHTS, L. Vertical transmission of arboviruses in mosquitoes: A historical perspective. **Infection, Genetics and Evolution**, v. 28, p. 681–690, 2014.
- LI, M. X. et al. Repellent, larvicidal and adulticidal activities of essential oil from Dai medicinal plant *Zingiber cassumunar* against *Aedes albopictus*. **Plant Diversity**, v. 43, n. 4, p. 317–323, 2021.

- LIANG, G. et al. Arboviruses and their related infections in China: A comprehensive field and laboratory investigation over the last 3 decades. **Reviews in Medical virology**, v. 28, p. 1959, 2017.
- LEE, C. H.; MOTURI, V.; LEE, Y. Thixotropic property in pharmaceutical formulations. **Journal of Controlled Release: Official Journal of the Controlled Release Society**, v. 136, n. 2, p. 88–98, 5 jun. 2009.
- LETA, S. et al. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. **International Journal of Infectious Diseases**, v. 67, p. 25–35, 2018.
- LIPPACHER, A.; MÜLLER, R. H.; MÄDER, K. Liquid and semisolid SLN dispersions for topical application: rheological characterization. **European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V.**, v. 58, n. 3, p. 561–567, 2004.
- LIU, J. et al. Hydrogels for Engineering of Perfusable Vascular Networks. **International Journal of Molecular Sciences**, v. 16, p. 15997-16016, 2015.
- LIU, Y. et al. Reviewing estimates of the basic reproduction number for dengue, Zika and chikungunya across global climate zones. **Environmental Research**, v. 182, p. 109114, mar. 2020.
- LOMBARDO, D.; KISELEV, M. A.; CACCAMO, M. T. Smart Nanoparticles for Drug Delivery Application: Development of Versatile Nanocarrier Platforms in Biotechnology and Nanomedicine. **Journal of Nanomaterials**, v. 2019, p. e3702518, 27 fev. 2019.
- LUPI, E.; HATZ, C.; SCHLAGENHAUF, P. The efficacy of repellents against *Aedes*, *Anopheles*, *Culex* and *Ixodes* spp. - a literature review. **Travel Medicine and Infectious Disease**, v. 11, n. 6, p. 374–411, 2013.
- MADKOUR, L. H. Nanoparticle and polymeric nanoparticle-based targeted drug delivery systems. In: **Nucleic Acids as Gene Anticancer Drug Delivery Therapy**. [s.l.] Academic Press, 2019. p. 15.
- MARTINET, J.-P. et al. Mosquitoes of North-Western Europe as Potential Vectors of Arboviruses: A Review. **Viruses**, v. 11, p. 1059, 2019.
- MAYER, S. V.; TESH, R. B.; VASILAKIS, N. The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. **Acta Tropica**, v. 166, p. 155–163, 2017.
- MAIA, M. F.; MOORE, S. J. Plant-based insect repellents: a review of their efficacy, development and testing. **Malaria Journal**, v. 10 Suppl 1, p. S11, 2011.
- MARKLEWITZ, M.; JUNGLEN, S. Evolutionary and ecological insights into the emergence of arthropod-borne viruses. **Acta Tropica**, v. 190, p. 52–58, fev. 2019.

- MERCK. **IR3535 Technical Material. Interin specification WHO/IS/667/2001**, 2001.
Disponível em: <<http://www.who.int/whopes/quality/en/IR3535Interim.pdf>>. Acesso em: 18 jun. 2018
- MESSINA, J. P. et al. The current and future global distribution and population at risk of dengue. **Nature Microbiology**, v. 4, n. 9, p. 1508–1515, set. 2019.
- MILLER, B. R. Arboviruses. **Encyclopedia of Virology**, p. 170–176, 2008.
- MINISTÉRIO DA SAÚDE. Monitoramento dos casos de Arboviroses até a semana 18 de 2022. **Boletim epidemiológico**. v 18, 2022.
- MISNI, N.; NOR, Z. M.; AHMAD, R. New Candidates for Plant-Based Repellents Against *Aedes aegypti*. **Journal of the American Mosquito Control Association**, v. 32, n. 2, p. 117–123, 2016.
- MOTAWEA, A.; BORG, T.; ABD EL-GAWAD, A. E.-G. H. Topical phenytoin nanostructured lipid carriers: design and development. **Drug Development and Industrial Pharmacy**, v. 44, n. 1, p. 144–157, 2018.
- MÜLLER, R. H.; RADTKE, M.; WISSING, S. A. Nanostructured lipid matrices for improved microencapsulation of drugs. **International Journal of Pharmaceutics**, v. 242, n. 1–2, p. 121–128, 2002.
- NA, Y.-G. et al. Development and evaluation of a film-forming system hybridized with econazole-loaded nanostructured lipid carriers for enhanced antifungal activity against dermatophytes. **Acta Biomaterialia**, v. 101, p. 507–518, 2020.
- NANOSCOOPING. Nano Neem, 2020. Disponível em: <<https://www.nanoscopying.com.br/nano-neem>>. Acesso em: 6 nov 2021.
- NANOVETORE. Body Care / Nanolcaridina. Disponível em: <<https://www.nanovetores.com.br/icaridina>>. Acesso em: 23 fev. 2021.
- NARARAK, J. et al. Excito-repellent activity of β -caryophyllene oxide against *Aedes aegypti* and *Anopheles minimus*. **Acta Tropica**, v. 197, p. 105030, set. 2019.
- NAUCKE, T. J. et al. Field evaluation of the efficacy of proprietary repellent formulations with IR3535 and picaridin against *Aedes aegypti*. **Parasitology Research**, v. 101, n. 1, p. 169–177, 2007.
- NASCIMENTO, M. H. M. et al. Hyaluronic acid in Pluronic F-127/F-108 hydrogels for postoperative pain in arthroplasties: Influence on physico-chemical properties and structural requirements for sustained drug-release. **International Journal of Biological Macromolecules**, v. 111, p. 1245–1254, 2018.
- NDIAYE, E. H. et al. Arboviruses isolated from the Barkedji mosquito-based surveillance system, 2012-2013. **BMC Infectious Diseases**, v. 18, n. 1, p. 1–14, 2018.

- NELMS, B. M. et al. Overwintering biology of culex (diptera: Culicidae) mosquitoes in the sacramento Valley of California. **Journal of Medical Entomology**, v. 50, n. 4, p. 773–790, 2013.
- NERIO, L. S.; OLIVERO-VERBEL, J.; STASHENKO, E. Repellent activity of essential oils: a review. **Bioresource Technology**, v. 101, n. 1, p. 372–378, 2010.
- NGAMPUNWETCHAKUL, L. et al. Semi-solid poly(vinyl alcohol) hydrogels containing ginger essential oil encapsulated in chitosan nanoparticles for use in wound management. **Journal of Polymer Research**, v. 26, p. 224, 2019.
- NGUYEN, Q.-B. D.; VU, M.-A. N.; HEBERT, A. A. Insect Repellents: An Updated Review for the Clinician. **Journal of the American Academy of Dermatology**, 2018.
- NORRIS, E. J.; COATS, J. R. Current and Future Repellent Technologies: The Potential of Spatial Repellents and Their Place in Mosquito-Borne Disease Control. **International Journal of Environmental Research and Public Health**, v. 14, n. 2, fev. 2017.
- ODALO, J. O. et al. Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*. **Acta Tropica**, v. 95, n. 3, p. 210–218, 2005.
- OHRT, C.; NGO, T. D.; NGUYEN, T. Q. Preparing for the Next Global Threat: A Call for Targeted, Immediate Decisive Action in Southeast Asia to Prevent the Next Pandemic in Africa. **Towards Malaria Elimination - A Leap Forward**, 18 jul. 2018.
- ORTIZ ZAMORA, L. et al. Development of eco-friendly nano-mosquitocides against arboviruses vectors. **Applications of Nanobiotechnology for Neglected Tropical Diseases**, p. 493–507, 2021.
- OLIVEIRA, J. L. DE et al. Association of zein nanoparticles with botanical compounds for effective pest control systems. **Pest Management Science**, v. 75, n. 7, p. 1855–1865, 2019.
- PALMESE, L. L. et al. Hybrid hydrogels for biomedical applications. **Current Opinion in Chemical Engineering**, v. 24, p. 143–157, 2019.
- PELTONEN, L.; SINGHAL, M.; HIRVONEN, J. Principles of nanosized drug delivery systems. In: **Nanoengineered Biomaterials for Advanced Drug Delivery**. [s.l.] Elsevier, 2020. p. 23.
- PIVETTA, T. P. et al. Development of nanoparticles from natural lipids for topical delivery of thymol: Investigation of its anti-inflammatory properties. **Colloids and Surfaces B: Biointerfaces**, v. 164, p. 281–290, 2018.
- PINTO, M. F. et al. A new topical formulation for psoriasis: development of methotrexate-loaded nanostructured lipid carriers. **International Journal of Pharmaceutics**, v. 477, n. 1–2, p. 519–526, 2014.

- PRIDGEON, J. W.; BERNIER, U. R.; BECNEL, J. J. Toxicity comparison of eight repellents against four species of female mosquitoes. **Journal of the American Mosquito Control Association**, v. 25, n. 2, p. 168–173, 2009.
- PORTILLA-PULIDO, J. S. et al. Design of a Repellent Against *Aedes aegypti* (Diptera: Culicidae) Using in silico Simulations With AaegOBP1 Protein. **Journal of Medical Entomology**, v. 57, n. 2, p. 463–476, 27 fev. 2020.
- PUBCHEM. **Geraniol**. Disponível em: <<https://pubchem.ncbi.nlm.nih.gov/compound/637566>>. Acesso em: 7 out. 2019a.
- PUBCHEM. **Icaridin**. Disponível em: <<https://pubchem.ncbi.nlm.nih.gov/compound/125098>>. Acesso em: 7 out. 2019b.
- RADWAN, I. T. et al. Nanostructured Lipid Carriers (NLC) for Biologically Active Green Tea and Fennel Natural Oils Delivery: Larvicidal and Adulticidal Activities against *Culex pipiens*. *Molecules*, v. 27, p. 1939, 2022.
- RAGHAVENDRA, B. S.; PRATHIBHA, K. P.; VIJAYAN, V. A. Synergistic effect of *Eugenia jambolana* Linn. and *Solidago canadensis* Linn. leaf extracts with deltamethrin against the dengue vector *Aedes aegypti* Linn. at Mysore. **Environmental Science and Pollution Research International**, v. 20, n. 6, p. 3830–3835, jun. 2013a.
- RAGHAVENDRA, B. S.; PRATHIBHA, K. P.; VIJAYAN, V. A. Synergistic effect of *Eugenia jambolana* Linn. and *Solidago canadensis* Linn. leaf extracts with deltamethrin against the dengue vector *Aedes aegypti* Linn. at Mysore. **Environmental Science and Pollution Research International**, v. 20, n. 6, p. 3830–3835, jun. 2013b.
- RAWAT, S. et al. Molecular mechanism of poly(vinyl alcohol) mediated prevention of aggregation and stabilization of insulin in nanoparticles. **Molecular Pharmaceutics**, v. 12, n. 4, p. 1018–1030, 2015.
- REHMAN, J. U.; ALI, A.; KHAN, I. A. Plant based products: Use and development as repellents against mosquitoes: A review. **Fitoterapia**, v. 95, p. 65–74, 2014.
- REHMAN, J. U.; ALI, A.; KHAN, I. A. Plant based products: use and development as repellents against mosquitoes: A review. **Fitoterapia**, v. 95, p. 65–74, 2014.
- REINHOLD, J. M.; LAZZARI, C. R.; LAHONDÈRE, C. Effects of the Environmental Temperature on *Aedes aegypti* and *Aedes albopictus* Mosquitoes: A Review. **Insects**, v. 9, p. 158, 2018.
- RODRIGUEZ, S. D. et al. The Efficacy of Some Commercially Available Insect Repellents for *Aedes aegypti* (Diptera: Culicidae) and *Aedes albopictus* (Diptera: Culicidae). **Journal of Insect Science (Online)**, v. 15, p. 140, 2015.
- RÜCKERT, C.; EBEL, G. D. How Do Virus–Mosquito Interactions Lead to Viral Emergence? **Trends in Parasitology**, v. 34, n. 4, p. 310–321, 2018.

- SALVI, V. R.; PAWAR, P. Nanostructured lipid carriers (NLC) system: A novel drug targeting carrier. **Journal of Drug Delivery Science and Technology**, v. 51, p. 255–267, 2019.
- SANGHA, G. K. G. Toxicology and Safety Evaluation of the New Insect Repellent Picaridin (Saltidin). **Hayes' Handbook of Pesticide Toxicology**, p. 2219–2230, 2010.
- SAPORITO, F. et al. Essential oil-loaded lipid nanoparticles for wound healing. **International Journal of Nanomedicine**, v. 13, p. 175–186, 2018.
- SCHRAMM, G. **Reologia e Reometria: fundamentos teóricos e práticos**. 2. ed. São Paulo: Altiber, 2006.
- SETHIL-NATHAN, S. A Review of Resistance Mechanisms of Synthetic Insecticides and Botanicals, Phytochemicals, and Essential Oils as Alternative Larvicidal Agents Against Mosquitoes. **Frontiers in Physiology**, v. 10, 25 fev. 2020.
- SHAALAN, E. A.-S. et al. Synergistic efficacy of botanical blends with and without synthetic insecticides against *Aedes aegypti* and *Culex annulirostris* mosquitoes. **Journal of Vector Ecology: Journal of the Society for Vector Ecology**, v. 30, n. 2, p. 284–288, dez. 2005.
- SHARMA BORA, N. et al. Formulation development and accelerated stability testing of a novel sunscreen cream for ultraviolet radiation protection in high altitude areas. **Drug Development and Industrial Pharmacy**, v. 45, n. 8, p. 1332–1341, ago. 2019.
- SHARMA, G. et al. Nanostructured Lipid Carriers: A New Paradigm in Topical Delivery for Dermal and Transdermal Applications. **Critical Reviews in Therapeutic Drug Carrier Systems**, v. 34, n. 4, p. 355–386, 2017.
- SHARMA, P. K. et al. Biochemical characteristics of a novel vegetative tissue geraniol acetyltransferase from a monoterpene oil grass (*Palmarosa*, *Cymbopogon martinii* var. *Motia*) leaf. **Plant Science: An International Journal of Experimental Plant Biology**, v. 203–204, p. 63–73, 2013.
- SHOPE, R. E.; MEEGAN, J. M. Arboviruses. In: *Viral Infections of Humans: Epidemiology and Control*. 4. ed. **Plenum Publishing Corporation**, p. 1091, 1997.
- STATNANO, Nanotechnology Products Database, **Cosmetic**. Disponível em: <https://product.statnano.com/industry/cosmetics>. Acesso em: 5 de agosto de 2022.
- SWALE, D. R. et al. Neurotoxicity and Mode of Action of N, N-Diethyl-Meta-Toluamide (DEET). **PLoS ONE**, v. 9, n. 8, p. 103713, 2014.
- SARANGI, B. et al. Systematic approach for the formulation and optimization of atorvastatin loaded solid lipid NANOARTICLES using response surface methodology. **Biomedical Microdevices**, v. 20, n. 3, p. 53, 2018.
- SITTERBERG, J. et al. Utilising atomic force microscopy for the characterisation of nanoscale drug delivery systems. **European Journal of Pharmaceutics and**

- Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V**, v. 74, n. 1, p. 2–13, 2010.
- SOLOMON, B. et al. Microencapsulation of citronella oil for mosquito-repellent application: Formulation and in vitro permeation studies. **European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V**, v. 80, p. 61–6, 8 set. 2011.
- SONG, A. et al. Effect of liquid-to-solid lipid ratio on characterizations of flurbiprofen-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) for transdermal administration. **Drug Development and Industrial Pharmacy**, v. 42, n. 8, p. 1308–1314, 2016.
- SONG, S. et al. Simultaneous determination of neonicotinoid insecticides and insect growth regulators residues in honey using LC-MS/MS with anion exchanger-disposable pipette extraction. **Journal of Chromatography. A**, v. 1557, p. 51–61, 2018.
- SOUTO, E. B. et al. SLN and NLC for topical, dermal, and transdermal drug delivery. **Expert Opinion on Drug Delivery**, v. 17, n. 3, p. 357–377, mar. 2020.
- STANAWAY, J. D. et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. **The Lancet. Infectious Diseases**, v. 16, n. 6, p. 712–723, jun. 2016.
- TAMBWE, M. M.; SWAI, J. K.; MOORE, S. J. Semifield system and experimental huts bioassays for the evaluation of spatial (and topical) repellents for indoor and outdoor use. **Advances in Arthropod Repellents**, p. 163–192, 2022.
- TAMJIDI, F. et al. Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. **Innovative Food Science & Emerging Technologies**, v. 19, p. 29–43, 2013.
- TAVARES, M. et al. Trends in insect repellent formulations: A review. **International Journal of Pharmaceutics**, v. 539, n. 1–2, p. 190–209, 25 mar. 2018.
- TEERANACHAIDEEKUL, V. et al. Cetyl palmitate-based NLC for topical delivery of Coenzyme Q(10) - development, physicochemical characterization and in vitro release studies. **European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik e.V**, v. 67, n. 1, p. 141–148, ago. 2007.
- TEICH, V.; ARINELLI, R.; FAHHAM, L. Aedes aegypti e sociedade: o impacto econômico das arboviroses no Brasil. **J. bras. econ. saúde (Impr.)**, p. 267–276, 2017.
- THANGAMANI, S. et al. Vertical transmission of zika virus in aedes aegypti mosquitoes. **American Journal of Tropical Medicine and Hygiene**, v. 95, n. 5, p. 1169–1173, 2016.

- THONIYOT, P. et al. Nanoparticle-Hydrogel Composites: Concept, Design, and Applications of These Promising, Multi-Functional Materials. **Advanced Science**, v. 2, p. 1400010, 2015.
- TORTORICI, S. et al. Nanostructured lipid carriers of essential oils as potential tools for the sustainable control of insect pests. **Industrial Crops and Products**, v. 181, p. 114766, 2022.
- UCHIDA, T. et al. Prediction of skin permeation by chemical compounds using the artificial membrane, Strat-M™. **European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences**, v. 67, p. 113–118, 25 jan. 2015.
- UNIYAL, A. et al. Behavioral Response of Aedes aegypti Mosquito towards Essential Oils Using Olfactometer. **Journal of Arthropod-Borne Diseases**, v. 10, n. 3, p. 370–380, 2016.
- VITORINO, C. et al. The size of solid lipid nanoparticles: an interpretation from experimental design. **Colloids and Surfaces. B, Biointerfaces**, v. 84, n. 1, p. 117–130, 1 maio 2011.
- VONTAS, J. et al. Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. **Pesticide Biochemistry and Physiology**, v. 104, n. 2, p. 126–131, 2012.
- WEAVER, S. C.; REISEN, W. K. Present and future arboviral threats. *Antiviral Research*, v. 85, n. 2, p. 328–345, 2010.
- WEBB, C. E.; HESS, I. M. A review of recommendations on the safe and effective use of topical mosquito repellents. **Public Health Research & Practice**, v. 26, n. 5, 2016.
- WILDER-SMITH, A. et al. Epidemic arboviral diseases: priorities for research and public health. **The Lancet Infectious Diseases**, v. 17, n. 3, p. e101–e106, 2017.
- WILKE, A. B. B. et al. Community Composition and Year-round Abundance of Vector species of Mosquitoes make Miami-Dade County, Florida a Receptive Gateway for Arbovirus entry to the United states. **Scientific Reports**, v. 9, p. 8732, 2019.
- WISSING, S. A.; MÜLLER, R. H. A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. **International Journal of Cosmetic Science**, v. 23, n. 4, p. 233–243, 2001.
- XU, P. et al. DEET and other repellents are inhibitors of mosquito odorant receptors for oviposition attractants. **Insect Biochemistry and Molecular Biology**, v. 113, p. 103224, 2019.
- YEGUERMAN, C. et al. Insecticidal application of essential oils loaded polymeric nanoparticles to control German cockroach: Design, characterization and lethal/sublethal effects. **Ecotoxicology and Environmental Safety**, v. 189, p. 110047, 2020.

- YOUNG, P. R. et al. Arbovirus Infections. **Manson's Tropical Diseases: Twenty-Third Edition**, p. 129- 161, 2014.
- ZARA, A. L. DE S. A. et al. [Aedes aegypti control strategies: a review]. **Epidemiologia E Servicos De Saude: Revista Do Sistema Unico De Saude Do Brasil**, v. 25, n. 2, p. 391–404, jun. 2016.
- ZIELÍ NSKA, A. et al. Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. **Molecules**, v. 25, p. 3731, 2020.