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SÍNTESE, CARACTERIZAÇÃO E ATIVIDADE ANTIMICROBIANA DE SISTEMAS ENCAPSULADOS DE ÓLEO DE CANELA E CINAMALDEÍDO ASSOCIADOS AO POLÍMERO POLI(Ɛ-CAPROLACTONA) (PCL)

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A Deus.

Porque dele, por ele e para ele, são todas as coisas.

Aos meus pais.

Pelo amor, dedicação e por não medirem esforços em meu favor.

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NOTAS PRELIMINARES

A presente dissertação foi redigida segundo o Manual para Normatização da Defesa do Trabalho Final proposto pelo Programa de Pós-Graduação em Odontologia da Universidade Federal da Paraíba, adotando o formato alternativo (Anexo A). Apenas um artigo científico compõe o presente trabalho de defesa, o qual foi redigido de acordo com as exigências da revista a qual será enviado (Biosystems Engineering).

RESUMO

Diante da resistência microbiana, dos efeitos tóxicos e das reações adversas frente à terapêutica antibiótica tradicional, a busca por novos agentes antimicrobianos a partir das plantas e seus fitoconstituintes tem se intensificado. Nesta perspectiva, realizou-se a síntese, caracterização físico-química e avaliação da atividade antimicrobiana de sistemas encapsulados de óleo de canela e cinamaldeído associados ao polímero policaprolactona (PCL). Para tanto, procedeu-se com a síntese de partículas de PCL contendo os óleos, mediante a técnica de emulsificação/evaporação do solvente. Posteriormente, foram realizadas a análise da morfologia e das características físico-químicas dos sistemas encapsulados através de Microscopia Eletrônica de Varredura (MEV) e de Transmissão (MET); Difração de Raios X (DRX) e análise de Espectroscopia na Região do Infravermelho com Transformada de Fourier (FTIR). Além disso, determinou-se a Concentração Inibitória Mínima (CIM), através da técnica da microdiluição, e Concentração Fungicida e Bactericida Mínima (CFM/ CBM) dos óleos livres e encapsulados sobre *Candida albicans* (ATCC 11006), *Staphylococcus aureus* ATCC (15656) e *Enterococcus faecalis* ATCC (14506). A análise morfológica pelo MEV e MET revelou que ocorreu a formação dos sistemas encapsulados, dotados de micropartículas com superfície rugosa e com presença de orifícios. Os resultados da DRX e da FTIR sugerem que os sistemas encapsulados dos óleos conservaram as características do seu polímero estruturante. Além disso, observou-se que tanto o óleo de canela livre como encapsulado apresentaram CIM de 892 µg/mL, sobre *C. albicans*. Para o cinamaldeído verificou-se que o óleo livre, bem como encapsulado apresentaram CIM de 446 µg/mL sobre *C. albicans*. Com relação às cepas bacterianas pode-se verificar que o óleo de canela livre e encapsulado frente ao *E. faecalis* apresentaram CIM de 1.784 µg/mL. O cinamaldeído livre e encapsulado em PCL sobre o *E. faecalis* obteve uma CIM igual a 446 µg/mL. Frente ao *S. aureus*, o óleo de canela livre e encapsulado apresentou CIM de 1.784 µg/mL; já o cinamaldeído livre obteve CIM de 892 µg/mL e CBM de 1.784 µg/mL quando encapsulado CIM e CBM de 892. Verificou-se que o PCL livre dos óleos não apresentou atividade antimicrobiana sobre as cepas estudadas. Além disso, pode-se observar que

os óleos, tanto livres como encapsulados, apresentaram apenas ação fungiostática sobre a *C. albicans e* bacteriostática sobre *E. faecalis*. As micropartículas contendo óleo de canela e cinamaldeído apresentam adequadas características físico-químicas, bem como apresentam atividade antimicrobiana.

Palavras-chave: Nanotecnologia, Óleo de Canela, Cinamaldeído, *Candida albicans*, *Staphylococcus aureus, Enterococcus faecalis.*

Abstract

Microbial resistance, toxic effects and adverse reactions front of the traditional antibiotic therapy intensify the search for new antimicrobial agents from plants and their phytochemicals. In this perspective, there was the synthesis, physicochemical characterization and evaluation of the antimicrobial activity of cinnamon oil and cinnamaldehyde encapsulated systems associated with the polymer polycaprolactone (PCL). Therefore, it was proceeded to the synthesis of PCL particles containing the oil, through the technique of emulsification / solvent evaporation. Later, there were the analysis of the morphology and the physical and chemical characteristics of encapsulated systems using Scanning Electron Microscopy (SEM) and transmission (TEM); X-ray diffraction (XRD) and Infrared Spectroscopy analysis in the Region Fourier Transform (FTIR). In addition, the Minimum Inhibitory Concentration (MIC) was determined by the microdilution technique and concentration Fungicide and Minimum Bactericidal (MFC/MBC) of free oil and encapsulated on *Candida albicans* (ATCC 11006), *Staphylococcus aureus* (ATCC 15656) and *Enterococcus faecalis* (ATCC 14506). The morphological analysis by SEM and TEM revealed that there was the formation of encapsulated systems, equipped with microparticles with rough surface and the presence of holes. The results of XRD and FTIR systems suggest that encapsulated oil retained the characteristics of the structuring polymer. In addition, it was observed that both free and encapsulated cinnamon oil showed a MIC of 892 µg/mL, against *C. albicans*. For cinnamaldehyde it was found that free oil, and encapsulated showed MIC of 446 µg/mL of *C. albicans*. The free cinnamon oil and encapsulated against *E. faecalis* showed MIC of 1.784 µg/mL. Free cinnamaldehyde and encapsulated in PCL on *E. faecalis* obtained a CIM equal to 446 µg/mL. Against *S. aureus*, the free and encapsulated cinnamon oil showed MIC of 1.784 µg/mL; already free cinnamaldehyde obtained MIC 892 µg/mL and MBC 1,784 µg/mL when encapsulated MIC and MBC of 892 µg/mL. It was found that the PCL free of oils showed no antimicrobial activity against the strains studied. Furthermore, it can be seen that the oils, both free and encapsulated showed only fungistatic action on bacteriostatic *C. albicans* and *E. faecalis*. The microparticles containing cinnamon oil and cinnamaldehyde have appropriate physical and chemical characteristics and have antimicrobial activity.

Key words: Nanotechnology, Cinnamon oil, Cinnamaldehyde, *Candida albicans*, *Staphylococcus aureus, Enterococcus faecalis.*

LISTA DE ABREVIATURAS E SIGLAS

ATCC – *American Type Culture Collection*

CIM – Concentração Inibitória Mínima

CBM – Concentração Bactericida Mínima

CFM – Concentração Fungicida Mínima

CRF – Code of Federal Regulations

DRX - Espectroscopia de difração de raios X

FTIR - Espectroscopia na Região do Infravermelho com Transformada de Fourier

GRAS – Generally Recognized as Safe

MEV – Microscopia Eletrônica de Varredura

MET – Microscopia Eletrônica de Transmissão

PCL - Poli (ε-caprolactona)

UFC – Unidades Formadoras de Colônias

SUMÁRIO

INTRODUÇÃO

A cavidade oral possui uma complexa microbiota, a qual pode estar aderida à mucosa epitelial ou formando biofilme. A maioria desses microrganismos são comensais, com uma minoria causando infecções oportunistas. Dentre esses microrganismos oportunistas, podem-se citar o fungo *Candida albicans* e espécies bacterianas como o *Enterococcus faecalis e* o *Staphylococcus aureus.* As leveduras de *C. albicans* podem desenvolver a candidose oral, principalmente em indivíduos imunocomprometidos (RORIG; COLACITE; ABEGG, 2009); o *Enterococcus faecalis* está relacionado com a colonização de canais radiculares de dentes e são considerados os patógenos mais resistentes encontrados nesses sítios, sendo responsáveis pelo insucesso dos tratamentos endodônticos (STUART et al., 2006; BALLAL et al., 2011;). Já o *S. aureus* é responsável por uma ampla incidência de infecções, desde moderadas infecções de pele até as mais agressivas, bacteremias e septicemias (HARDY et al., 2004; KIM et al., 2004), assim como pode estar envolvida em casos de endorcadite bacteriana, infecções endodônticas, osteomielites da mandíbula, infecção da glândula parótida e em uma forma de mucosite oral em idosos, principalmente em pacientes recebendo nutrição parenteral (SMITH *et al.*, 2001; MURDOCH et al., 2004).

A infecção oral causada por *C. albicans* é comumente tratada com agentes polienos (nistatina e anfotericina B) e com triazóis, como o fluconazol (POWDERLY et al., 1999). Estudos demonstraram que o desenvolvimento de resistência ao fluconazol em cepas de *Candida*, ocorre durante o tratamento (REDDING et al., 1994; JOHNSON et al., 1995;), onde são administradas altas doses, com o uso prolongado desses antifúngicos (SULLIVAN et al., 1995; PFALLER et al., 1999; RUHNKE et al., 2000). Além disso, há relatos de cepas bacterianas que desenvolveram resistência intermediária ou alta à penicilina (TENG et al., 1998). Porém essa resistência não é somente aos b-lactâmicos, mas também a muitos outros agentes antimicrobianos, como a vancomicina (FRIDKIN et al.,1999; KIM et al., 2004; TSUDA et al., 2002).

Além da resistência microbiana, a literatura científica evidencia outras limitações relacionadas ao tratamento das infecções bacterianas e fúngicas que acometem a cavidade oral, dentre essas limitações pode-se citar: o relato

de efeitos tóxicos e reações adversas desenvolvidas pelos recursos terapêuticos convencionais. Nesse sentindo, estudos relacionados às plantas e seus derivados, visando investigar suas propriedades terapêuticas e antimicrobianas tem crescido consideravelmente (BARKER, ROGERS, 2006; SCHELZ, HOHMANN, 2006; FONTENELLE et al., 2011), tais estudos visam verificar a atividade biológica das plantas e seus fitoconstituintes possibilitando, assim; a descoberta e desenvolvimento de novos agentes antimicrobianos (AQUINO, 2003; MOREIRA et al., 2007; AHMAD et al., 2010).

Desde a antiguidade, as plantas atuam como fonte de medicamentos para o tratamento das enfermidades que acometem a humanidade, uma vez que produzem metabólitos secundários, os quais consistem em moléculas de baixo peso molecular que são indispensáveis para a sobrevivência do organismo produtor, atuando como um mecanismo de defesa das espécies vegetais contra insetos, vírus, fungos fitogênicos, entre outras situações adversas (LIMA et al., 2003; SANTOS et al., 2004; SARTORATTO et al., 2004; BAKKALI et al., 2008).

Dentre esses metabólitos secundários podem-se citar os óleos essenciais, os quais se caracterizam por serem compostos naturais complexos, lipofílicos, voláteis e por apresentarem forte aroma. Os óleos essenciais apresentam em sua composição diferentes substâncias químicas, entre elas: terpenos, alcalóides, taninos, flavonoides e saponinas que lhe conferem reconhecida atividade terapêutica, agindo sobre infecções bacterianas, fúngicas, apresentando potencial antiviral, analgésico, sedativo, anti-inflamatório, antioxidante e anestésico (CHAMI et al., 2004; SANTOS et al., 2004; DUARTE et al., 2005; GOBBO-NETO et al., 2007; MIGLIATO et al., 2007; NASCIMENTO et al., 2007; CASTRO; LIMA., 2010).

Os óleos essenciais se caracterizam por apresentarem uma estrutura de hidrocarbonetos, a qual lhe confere um caráter lipofílico, e por apresentarem grupos funcionais hidrofílicos. O óleo extraído da folha da *Cinnamomun zeylanicum* (canela) pode ser citado como um exemplo de óleo essencial, o qual possui em sua composição o cinamaldeído como um de seus componentes (KNOBLOCH et al., 1989; KALEMBA; KUNICKA, 2003). A *C. zeylanicum* é uma das espécies de plantas que apresenta reconhecida atividade biológica (RAO; GAN, 2014). Vários estudos evidenciam suas

propriedades analgésica, anti-séptica e antimicrobiana (GAYOSO et al, 2005; LIMA et al., 2006; MATAN et al., 2006; CASTRO e LIMA, 2013; RANASINGHE et al, 2013; OLIVEIRA et al, 2014,)

O cinamaldeído (aldeído cinâmico ou 3-fenil-2-propenal) é um álcool terpênico cíclico, com baixa solubilidade em água e alta volatilidade (GOMES; MOREIRA; CASTELL-PEREZ, 2011), sendo o principal componente do óleo de cássia e do óleo da folha da canela; apontado como um dos componentes responsáveis pela atividade antimicrobiana desses óleos. Além de atividade antibacteriana, o cinamaldeído apresenta atividade anticancerígena, antifúngica e inibe a produção de micotoxinas (BEUCHAT, 1994; GARG; SINGH, 2011; WANI et al., 2014). A possível aplicabilidade farmacêutica do cinamaldeído torna-se viável devido ao fato desse composto ser, segundo a Food and Drug Administration (FDA), reconhecido com seguro para uso alimentar (GRAS), baseado no código CFR 21(Código de Regulamentação Federal) parte 172, 515 (CFR 2009). O óleo da canela é utilizado como flavorizante, aromatizante e conservante natural de alimentos (ZAGO et al., 2009). As características supracitadas possibilitam a utilização do cinamaldeído como potencial agente antimicrobiano para o controle e tratamento das principais afecções de origem bacteriana e fúngica que acometem a cavidade oral (GOMES; MOREIRA; CASTELL-PEREZ, 2011).

Nesse sentido o desenvolvimento de sistemas de partículas de polímero com dimensões submicrométricas que promovam o encapsulamento do cinamaldeído e do óleo de canela torna-se uma alternativa viável, uma vez que tais sistemas aumentam a biodisponibilidade desses compostos, bem como podem potencializar sua atividade biológica (WANI et al., 2014).

Os dispositivos micrométricos e nanométricos podem ser sintetizados a partir de diversas matérias primas, bem como podem apresentar arranjos estruturais variados. Dentre esse arranjos estruturais destacam-se as micropartículas e nanopartículas com composição polimérica. A seleção do dispositivo em escala submicrométrica a ser desenvolvido para o carreamento dos óleos está relacionada à ação desejada e a compatibilidade físico-química com a substância em questão. Os sistemas carreadores de fármacos podem ser classificados em microparticulados ou nanoparticulados. Os sistemas microparticulados apresentam partículas cujo diâmetro é superior a 1 μm,

enquanto que os sistemas nanoencapsulados possuem diâmetro na ordem de nanômetros, geralmente compreendido na faixa de 1 a 1000nm (COUVREUR et al., 2002; PANYAM; LABHASETWAR, 2003).

A expressão micro/nanopartículas inclui micro/nanocápsulas e as micro/nanoesferas, as quais diferem entre si com relação a sua composição e organização estrutural. As micro/nanocápsulas são constituídas por um invólucro polimérico no qual em seu interior há um núcleo oleoso, podendo a droga estar dissolvido neste núcleo e/ou adsorvido à parede polimérica. Já as micro/nanoesferas não apresentam uma fase oleosa em sua composição, são apenas formadas por uma matriz polimérica, na qual a droga pode ficar retida ou adsorvida (SCHAFFAZICK et al., 2003).

A síntese de sistemas micro e nanoparticulados é alvo de diversos estudos nas últimas décadas, uma vez que o encapsulamento de substâncias oferecem os seguintes benefícios: protege o princípio ativo das agressões ambientais, bem como de reações de deterioração, permite o mascaramento do odor e do sabor e permite a liberação controlada da substância encapsulada (GOSH, 2006). Além disso, a nanotecnologia possibilita a encapsulação de substâncias hidrófobas em polímeros com superfície hidrófila, os quais servem de arcabouço para a administração e liberação controlada da substância aprisionada (LOQUERCIO et al., 2015).

A aplicabilidade dos sistemas encapsulados apresenta limitações fisicoquímicas e fisiológicas, uma vez que os polímeros usados devem ser biocompativeis e biodegradáveis a metabólitos inócuos ao organismo. Os polímeros caracterizam-se por serem macromoléculas de elevada massa molecular, as quais são constituídas a partir de estruturas menores (monômeros) mediante reações químicas de polimerização (SCHAFFAZICK et al., 2003; REIS; NEUFELD; VEIGA, 2006).

Dentre esses polímeros, destaca-se o poli(ε-caprolactona) (PCL), o qual tem sido extensivamente utilizado na síntese de nanocarreadores de fármacos, devido à sua biodegrabilidade, biocompatibilidade e ausência de toxicidade (LEMOINE et al., 2004; WOODRUFF; SINHA et al., 2004). O PCL é um poliéster alifático que se destaca por ser objeto de estudo de diversas pesquisas devido ao fato de apresentar degradação lenta e não liberar resíduos ácidos (WOODRUFF;HUTMACHER, 2010; DASH; KONKIMALLA,

2012). Tais características são importantes porque a degradação lenta permite a liberação controlada do fármaco, aumentando, assim; a sua meia-vida, reduzindo a frequência de administração e, consequentemente, motivando a adesão do paciente à terapia medicamentosa (ORIVE et al., 2003; SINHA et al., 2004). As vantagens da utilização das micropartículas e nanopartículas como carreadores de drogas devem-se à sua dimensão sub-celular, a biocompatibilidade com tecidos e células e à versatilidade de formulações (SCHAFFAZICK et al., 2003; CUI et al., 2006; DURÁN; MATTOSO; MORAIS, 2008).

Diante do fato dos produtos naturais constituírem uma fonte incomparável de moléculas bioativas que podem atuar como antimicrobianos (ROEMER; BOONE, 2011) surge à necessidade de estudos que investiguem seu potencial terapêutico quando incorporado a sistemas de liberação controlada. Nesse sentido, o presente estudo objetiva sintetizar, caracterizar e verificar a atividade antimicrobiana de sistemas encapsulados de óleo de canela e cinamaldeído associado ao polímero policaprolactona (PCL).

2. CAPÍTULO 1

O manuscrito a seguir será submetido para publicação no periódico "Biosystems Engineering".

Synthesis, characterization and antimicrobial activity of cinnamon oil and cinnamaldehyde encapsulated systems associated with polymer poly(ε-caprolactone) (PCL).

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Abstract

This work evaluated the physical and chemical characterization of *Cinnamon zeylanicum* leaves oil (CO), cinnamaldehyde (CN) and theirs microparticles, MCO and MCN, respectively, whose encapsulation was with poli(□-caprolactone). Thus, the synthesis were performed by emulsification and solvent evaporation, the tests were conducted through Scanning Electron and Scanning Transmition Microscope (SEM and TEM, in this order), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Minimum Inhibitory Concentration (MIC) by microdilution method and Minimum Fungicide and Bactericidal Concentrations (MFC and MBC, respectively) against *Candida albicans*, *Staphylococcus aureus* and *Enterococcus faecalis*. MEV and MET showed that the microparticles' surfaces are roughened and with holes. DRX and FTIR indicate that probably their surfaces are made only by PCL. For *C. albicans*, the MIC were 892 µg/mL, 892 µg/mL, 446 µg/mL and 446 µg/mL to CO, MCO, CN and MCN. The free CO and MCO against *E. faecalis* showed MIC of 1.784 µg/mL. Free cinnamaldehyde and encapsulated on *E. faecalis* obtained a CIM equal to 446 µg/mL. Against *S. aureus,* the MIC of CN was 892 µg/mL and its MBC was 1784 µg/mL, while for MCN the MIC and MBC were 892 µg/mL. All samples showed only fungistatic and bacteriostatic actions.

Therefore, MCO and MCN are microparticles coated with PCL, filled their respective oils and they have antimicrobial activity.

Key words: Nanotechnology, Cinnamon oil, Cinnamaldehyde, *Candida albicans*, *Staphylococcus aureus, Enterococcus faecalis.*

1. Introduction

The scientific literature evidences limitations on the treatment of bacterial and fungal infections that affect the oral cavity, of these limitations can be mentioned: the emergence of bacterial resistance and some fungal strains to conventional antibiotics and antifungals, as well as reporting toxic effects and adverse reactions of these therapeutic resources. In this sense, studies related to plants and its derivatives in order to investigate its therapeutic and antimicrobial properties has grown considerably [1-3].

The essential oils extracted from plants can be cited as one of derivatives which have in their composition different chemicals, including: alkaloids, tannins, flavonoids and saponins which give recognized therapeutic activity by acting on bacterial, fungal, showing antiviral potential, analgesic, sedative, anti-inflammatory, antioxidant and anesthetic [4-10].

Such substances are characterized by presenting a hydrocarbon structure which gives it a lipophilic nature and hydrophilic functional groups present. The oil extracted from cinnamon bark can be cited as an example of essential oil, which has in its composition the cinnamaldehyde as its major component [11,12].

The cinnamaldehyde (cinnamic aldehyde or 3-phenyl-2-propene) is a cyclic terpenic alcohol with low solubility in water and high volatility [13]. The main cassia oil component and oil of cinnamon bark is pointed as the component responsible for antimicrobial activity cinnamon. In addition to antibacterial activity, cinnamaldehyde has anticancer, antifungal activity and inhibits production of mycotoxins [14-16]. Possible pharmaceutical applicability of cinnamaldehyde becomes feasible due to the fact that this compound is generally recognized as safe for food use (GRAS), based on code 21 CFR (Code of Federal Regulations) Part 172, 515 (CFR 2009).

However, the hydrophobic nature of oils hinders the use of these substances in research to verify its actions against microorganisms and tumor cells. The development of nanoparticle systems that promote encapsulation of cinnamaldehyde and cinnamon oil becomes a viable alternative since those systems increase the bioavailability of hydrophobic compounds and potentiate its biological activity [13, 16].

The synthesis of micro systems and nanoparticles are targets of many studies in the past decades, since the encapsulating substances offer the following benefits: it protects the active ingredient from environmental stressors, as well as degradation reactions, allows for the masking of odor and flavor and allows the controlled release of the encapsulated substance [17, 18]. Furthermore, nanotechnology enables the encapsulation of substances in hydrophobic polymers with hydrophilic surface, which serve as a framework for the delivery and controlled release of the entrapped substance [19].

The applicability of encapsulated systems (microspheres, microcapsules, nanocapsules, nanospheres) exhibits physicochemical and physiological limitations, since the polymers used must be biocompatible and biodegradable to harmless metabolites by the body. Among these polymers, there is poly (ε-caprolactone) (PCL), which has been extensively used in the nanocarriers synthesis of drugs, owing to their biodegradability, biocompatibility and absence of toxicity [20, 21]. The PCL is an aliphatic polyester which stands out as an object of study of several studies due to the fact that it presents slow degradation and not release acid residues [22-24]. These characteristics are important because the slow decay allows the controlled release of the drug, increasing the half-life by reducing the frequency of administration, encouraging patient adherence to drug therapy [25, 21]. The advantages of using the microparticles and nanoparticles as drug carriers are due to their size sub-cellular biocompatibility with tissues and cells and the versatility formulations [26-28].

Faced with the fact that natural products constitute an unrivaled source of bioactive molecules that can act as antimicrobials [29], comes the need of studies that investigate their therapeutic potential when incorporated into nanosystems controlled release. In this sense, this study aims to synthesize,

characterize and verify the antimicrobial activity of cinnamon oil and cinnamaldehyde nanocoated systems associated with the PCL polymer.

2. Experiment

2.1 Synthesis of PCL particles

The polymeric nanoparticles are obtained by emulsification technique / evaporation of the solvent, with some modifications [30-32]. For this purpose there was prepared a PCL-acetone solution in which 140 mg of PCL polymer were added to 27 ml of acetone, subjected to magnetic stirring at 37 ° C until complete solubilization of the polymer. Then it was added 72μL of surfactant polysorbate 80. This solution remained under magnetic stirring at room temperature for 15 minutes to homogenization. Subsequently, with the aid of an injection pump solution PCL / acetone / polysorbate 80 was dropped at a speed rate of 330 μL / min in 53 ml of water under magnetic stirring. After dripping, the solution composed of PCL / acetone / polysorbate 80 / distilled water remained under stirring at room temperature until complete evaporation of the solvent (acetone). The volume solution of acetone lost by evaporation was taken up by adding distilled water to the solution.

The PCL used in this work was obtained from Perstorp (Warrington, UK), Capa® 6500 code, and has weight average molar mass (Mw) to 50,000 g / mol.

2.2 Encapsulation of cinnamon oil and cinnamaldehyde in PCL particles

The oils encapsulated synthesis system in PCL was performed similarly to above PCL synthesis of particles. Differing, however, in the fact that after the solubilisation of the polymer (PCL) in the solvent (acetone) was added 1mL of cinnamon oil (Sigma-Aldrich, SAFC, Lot # MKBB5045V) (Annex B) or cinnamaldehyde (Sigma-Aldrich, SAFC, Lot # MKBN5878V) (Annex C), addition of 500μL of polysorbate 80 for the preparation of encapsulated respective systems.

Thus, the following solutions were obtained: Particle PCL (MPCL), PCL particles containing encapsulated cinnamon oil (MCO), cinnamaldehyde PCL particles containing encapsulated (MCN).

2.3 Physico-chemical characterization

2.3.1 Morphological analysis

The analysis of the morphology and distribution of particle size of PCL and systems encapsulated cinnamon oil and cinnamaldehyde in PCL were performed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

For SEM analysis the particles have been previously lyophilized and then metallised with a layer of gold, and the equipment carried FEI Quanta model 450. As for the TEM (Morgagni G20-FEI) analysis 10 µL of the suspension of encapsulated systems were distributed uniformly on a clean coverslip, dried and spotless and placed on a suitable support and taken to the equipment.

2.3.2 Spectroscopy X-ray diffraction (XRD):

Information on the crystallography of materials present in encapsulated cinnamon oil systems, cinnamaldehyde and PCL were obtained by XRD analysis. Data were acquired through a diffractometer X Lab, XRD-6000, Shimadzu, which conducted the scan x-ray in open-angle 2θ between 5 ° and 30 ° (1° / min).

2.3.2 Chemical analysis: Spectroscopy in the Infrared Fourier Transform (FTIR).

For identification of the main groups of chemical compounds present in the particles of encapsulated systems and cinnamaldehyde, cinnamon oil PCL, applied to FTIR analysis. This analysis was performed in equipment Shimadzu, IRAffinity model - 1 / FTIR - 8000 series, with accessory Miracle, which replaces the use of potassium bromide in reflectance mode. Samples were analyzed in

the range of 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1} and number of scans equal to 64 which analyzed the interaction of electromagnetic radiation with the functional groups of the molecules under analysis, taking into consideration deformation or stretching movements of their respective chemical bonds by adsorption of a number of specific wave starting from the principle of energy quantization. This chemical analysis allows the identification, determination of functional groups, the structure and conformation study of molecules and macromolecules, generally organic, enabling the identification of components of a sample.

2.4 Evaluation of the antimicrobial activity of encapsulated systems - in vitro assay

2.4.1 Determination of Minimum Inhibitory Concentration (MIC) of the systems encapsulated cinnamon oil and cinnamaldehyde on *Candida albicans, Staphylococcus aureus* and *Enterococcus faecalis.*

For the evaluation of the antifungal activity compared to cinnamon oil and cinnamaldehyde, free associated with PCL particles used to *Candida albicans* strain ATCC 11006 (American Type Culture Collection) grown on Sabouraud dextrose agar. The tests were based on the broth microdilution technique according to the Clinical and Laboratory Standards Institute - CLSI, standard M27 - A2 (NCCLS, 2002), volume 22, No. 15; with minor modifications [33].

Initially, they distributed 100 µL broth Sabouraud Dextrose (CSD) (HIMEDIA®) into the holes of microdilution plates containing 96 wells. Then 100 µL were distributed testing of substances, which was serially diluted from the removal of an aliquot of 100 µL of the most concentrated well to the successor cavity. The holes of each column were dispensed aliquots of 100 µL of inoculum corresponding to the tested strain. For inoculum preparation the turbidity of the microbial suspensions were adjusted using a spectrophotometer and compared, adding sufficient brine to achieve the equivalent transmittance of a standard solution of the McFarland scale (0.5) at a wavelength of 530 nm. This procedure

provides a standard yeast suspension containing 1.0 x 10^6 at 5.0 x 10^6 cells per ml. The working slurry is produced by making a 1: 100 dilution followed by a 1:20 dilution of the suspension with standard liquid culture medium, resulting in concentration of 5.0 x 10^2 at 2.5 x 10^3 cells per mL.

At the same time, they were carried out controlling the viability of the yeast strain of the test and control sterility of the culture medium. Yet they were conducted sensitivity control front strain to antifungal action of nystatin (MICOSTALAB; 100,000 UI/mL), considered standard clinical use; and investigated the possible antimicrobial activity of the PCL and polysorbate 80.

Assays were performed in triplicate and incubated at 37 ° C for 48 hours. The reading for determining the MIC of the test substances on the yeast strains was performed, first, from the visual method, which took into account the formation or absence of cell clusters ("button") in the cavity bottom of board. Thus, it was regarded as MIC, the lowest concentration of the test product capable of producing visible inhibition on the growth of yeast strain used in the microbiological assay.

In order to confirm the presence of viable microorganisms in non-inhibitory concentrations, 35 µL was used TCT dye (2, 3, 5 triphenyl tetrazolium chloride), which reflected the activity of dehydrogenase enzymes involved in cellular respiration process. This fact made it possible to distinguish the living samples, colored red, of those killed, which maintained their color [34].

The evaluation of the antibacterial activity of cinnamon oil and cinnamaldehyde, and free associated with PCL nanoparticles on bacterial strains of *S. aureus* ATCC 15656 and *E. faecalis* ATCC 14506 were performed by broth microdilution technique according to the Clinical and Laboratory Standards Institute - CLSI, standard M27 - A6 (NCCLS, 2002), Volume 23, No. 2; with minor modifications.

The microdilution technique was carried out similarly to the aforementioned for the fungal strain, differing in the culture medium used, which was BHI (Brain Heart Infunsion Broth) and the dye used for reading results, which is resazurin. The reading was the interpretation of microorganisms sensitive substances, based on the color changes of the evaluated wells. When the resazurin sodium maintained its blue coloration visually suggested a medium free of viable microorganisms and in that occur appearance of viable

microorganisms, the wells had a pink-purplish color. The CIM consisted of the last well of bluish color.

Assays were performed in triplicate and incubated at 37 ° C for 24 hours. Was also performed control of viability of the bacterial strains and the control sensitivity of the strain across the antibacterial activity of chlorhexidine (Clorhexidina 2%, FGM), which was diluted to a concentration of 0, 12%. The chlorhexidine is considered standard clinical use. Still it was performed sterility control of the medium and positive control to check if the PCL has antimicrobial activity.

2.4.2 Determination of Fungicide Concentration and Minimum Bactericidal (MFC/MBC)

After determining the MIC, the inhibitory concentration corresponding to the two concentrations immediately and more concentrated, and the positive control were subcultured on sabouraud dextrose agar plates to the fungal strains and BHI agar for bacterial strains. After 48 hours incubation at 37 ° C (fungal strain) and 24 hours (bacterial strains) in bacteriology furnace, readings were taken of the MFC/MBC based on the growth of the controls being considered MFC/MBC the lowest drug concentration that prevented visible growth the subculture.

3. Results

3.1 Obtaining particles PCL and systems encapsulated cinnamon oil and cinnamaldehyde in PCL:

The particles were successfully obtained by synthesis technique employed by emulsification / solvent evaporation, as shown by photomicrographs (Figure 1, Figure 2) obtained by Transmission Electron Microscopy (TEM).

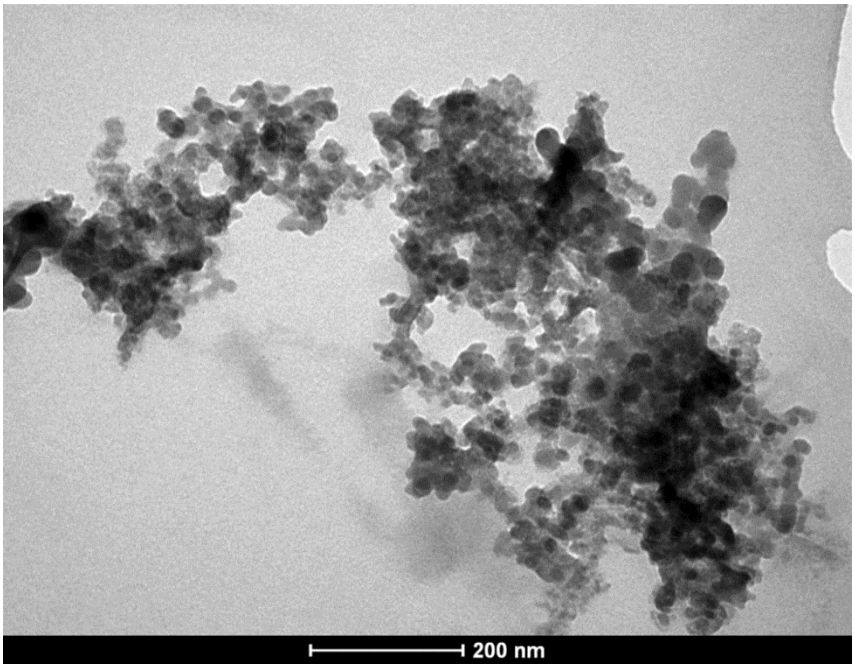

Figure 1 - Image obtained by Transmission Electron Microscopy System Encapsulated cinnamon oil in PCL.

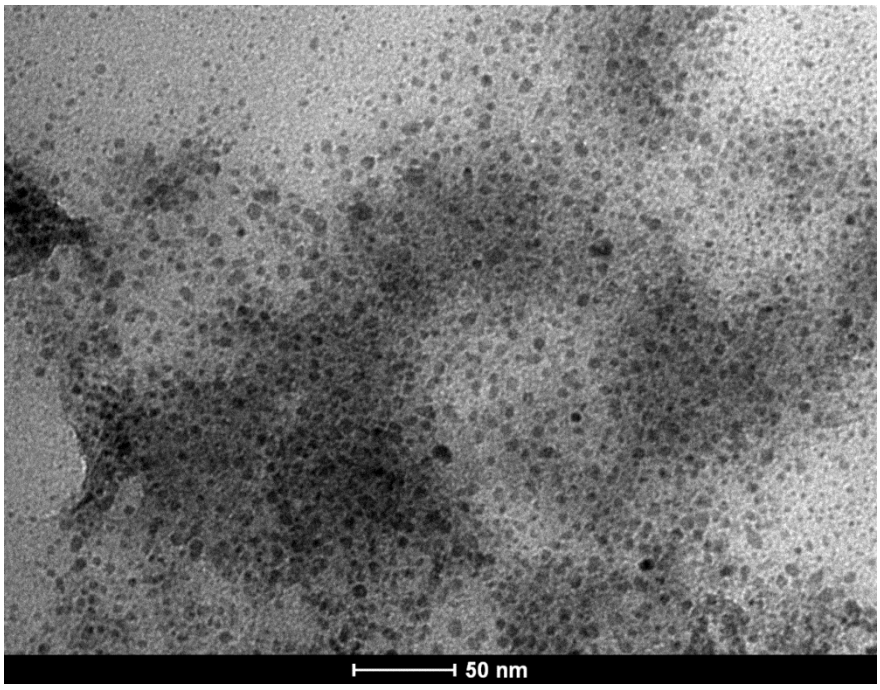

Figure 2 - Image obtained by Transmission Electron Microscopy System Encapsulated cinnamaldehyde in PCL.

3.3 Morphological analysis:

The PCL polymer particles devoid of oils have spherical shape and smooth surface as can be seen on the images obtained by SEM (Figure 3, Figure 4). Since the PCL particles containing cinnamon oil and cinnamaldehyde showed irregular shape and surface, as illustrated suffering coalescing the images obtained by SEM (Figure 5, Figure 6).

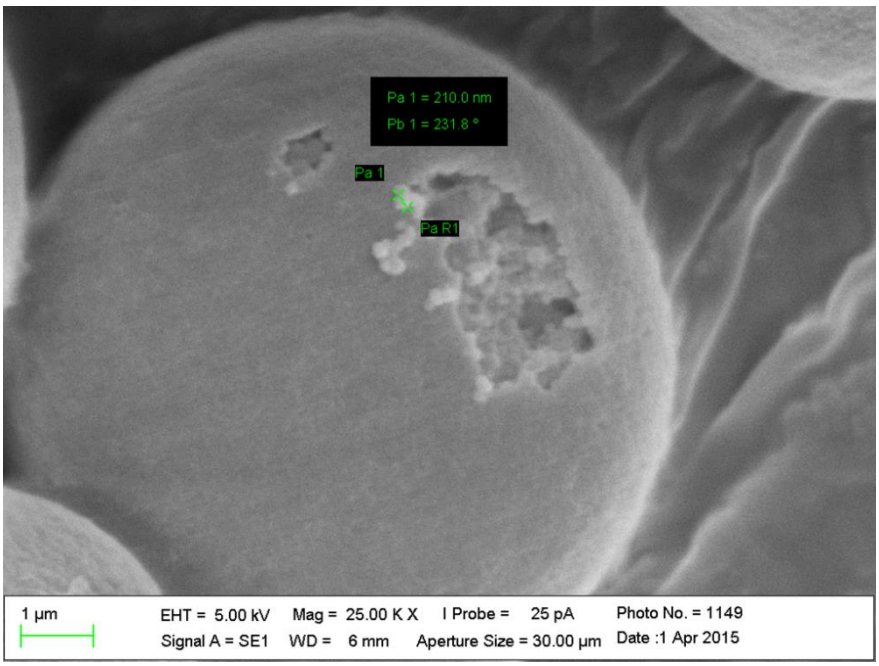

Figure 3 - Image obtained by Scanning Electron Microscopy of PCL particles.

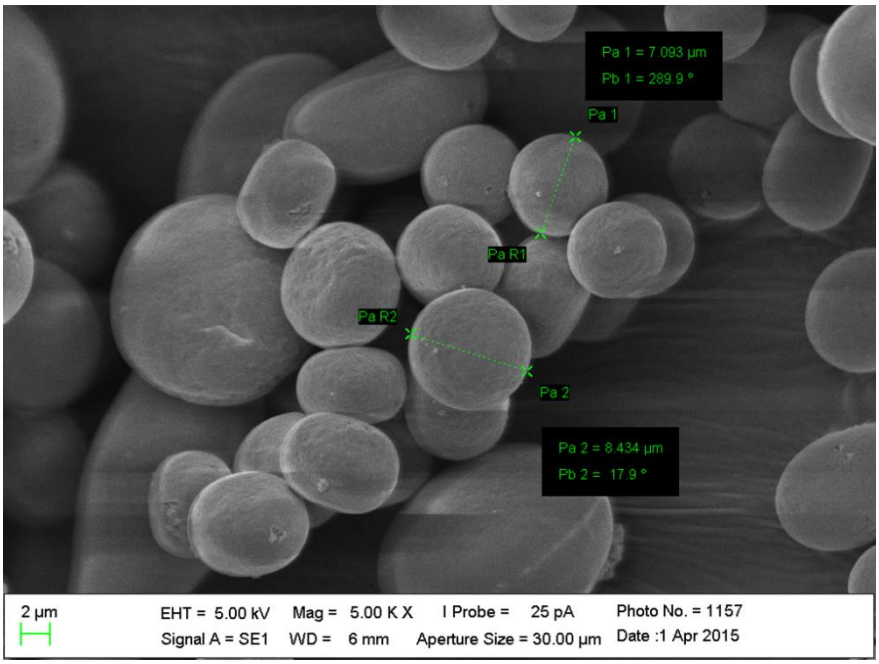

Figure 4 - Image obtained by Scanning Electron Microscopy of PCL particles. Note the presence of spherical microparticles having a smooth surface and dimensions ranging from 7,093 µm to 8,434 µm.

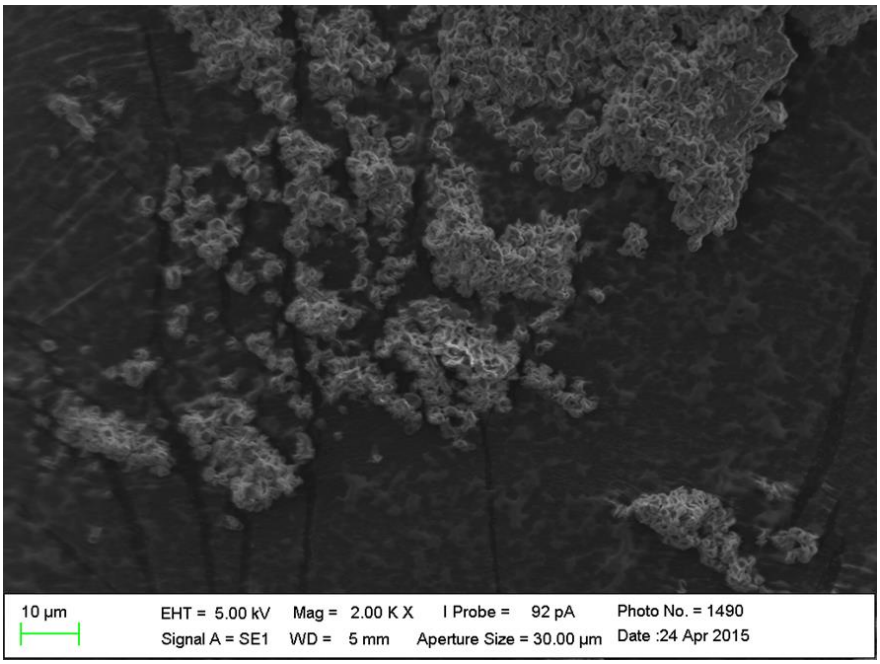

Figure 5 - Image obtained by scanning electron microscopy of PCL particles containing cinnamon oil. Note the presence of microparticles coalescent.

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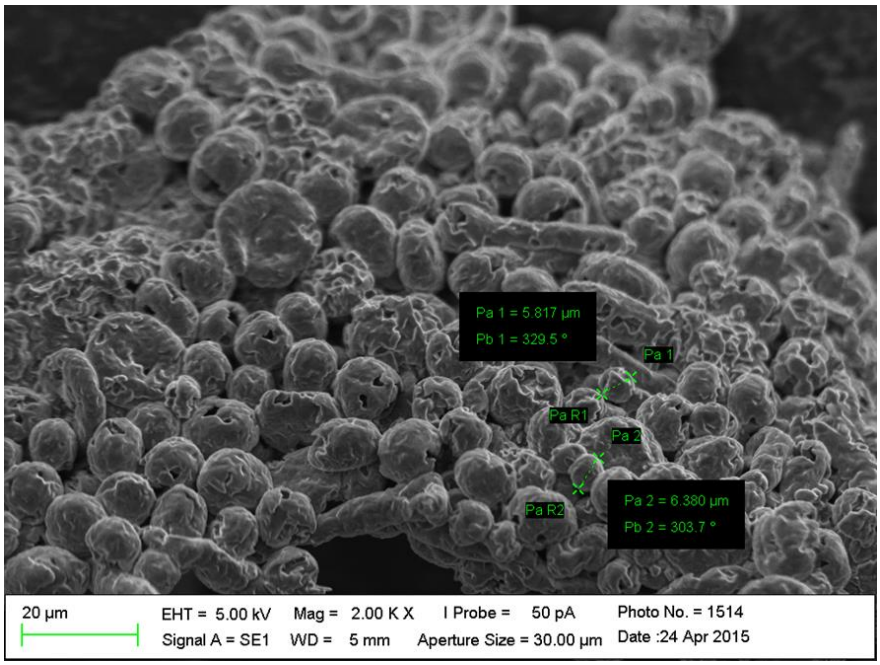

Figure 6 - Image obtained by PCL particles of Scanning Electron Microscopy containing cinnamaldehyde. Note the presence of spherical microparticles, coalescents, rough surface with presence of holes; such particles have dimensions ranging between 5,817µm to 6,380 µm.

3.4 Spectroscopy X-ray diffraction (XRD)

According to the obtained diffraction patterns (Figure 7, Figure 8, Figure 9) there is the presence of well-developed peaks at 2θ ~ 21.3, ~ 21.8 and ~ 23.7 , which are characteristic of the semi-crystalline polymer PCL.

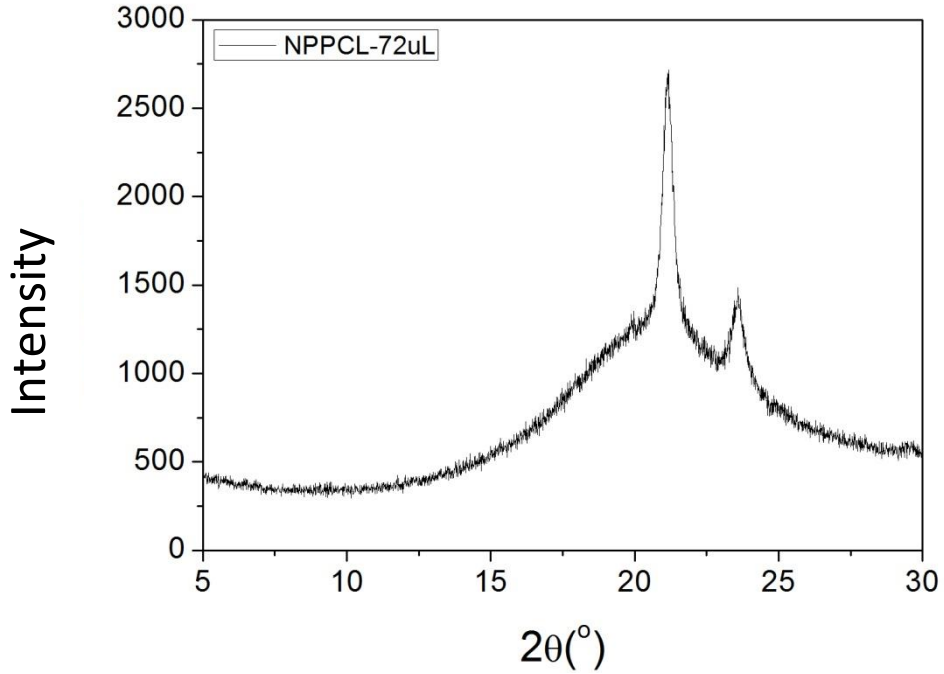

Figure 7 - XRD spectra of the particles of the PCL oil free polymer.

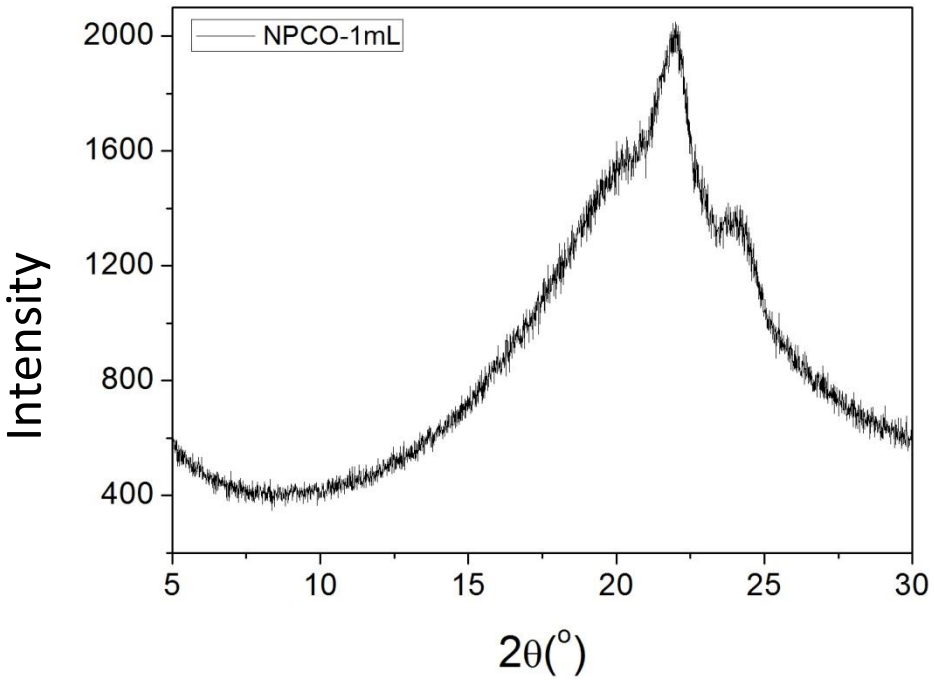

Figure 8 - XRD spectra of PCL particles containing cinnamon oil

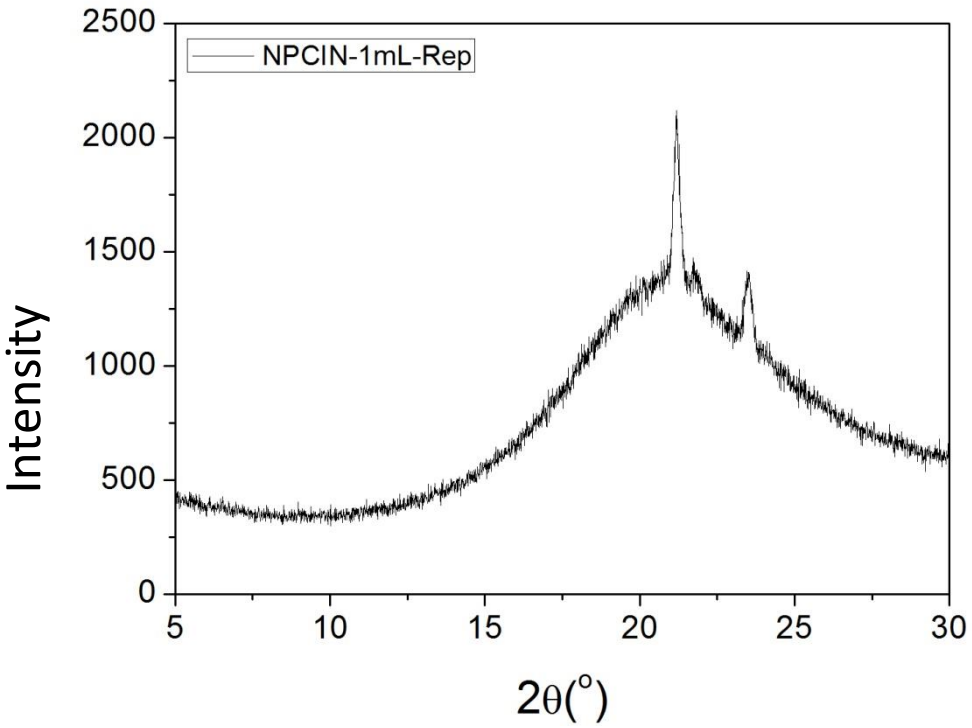

Figure 9 - XRD spectra of PCL particles containing cinnamaldehyde.

3.5 Chemical analysis: Infrared Spectroscopy (FTIR)

The FTIR spectra of PCL particles with or without the oils showed similar peaks with predominance of precursor polymer characteristics (PCL) and are presented in the charts below (Figure 10, Figure 11).

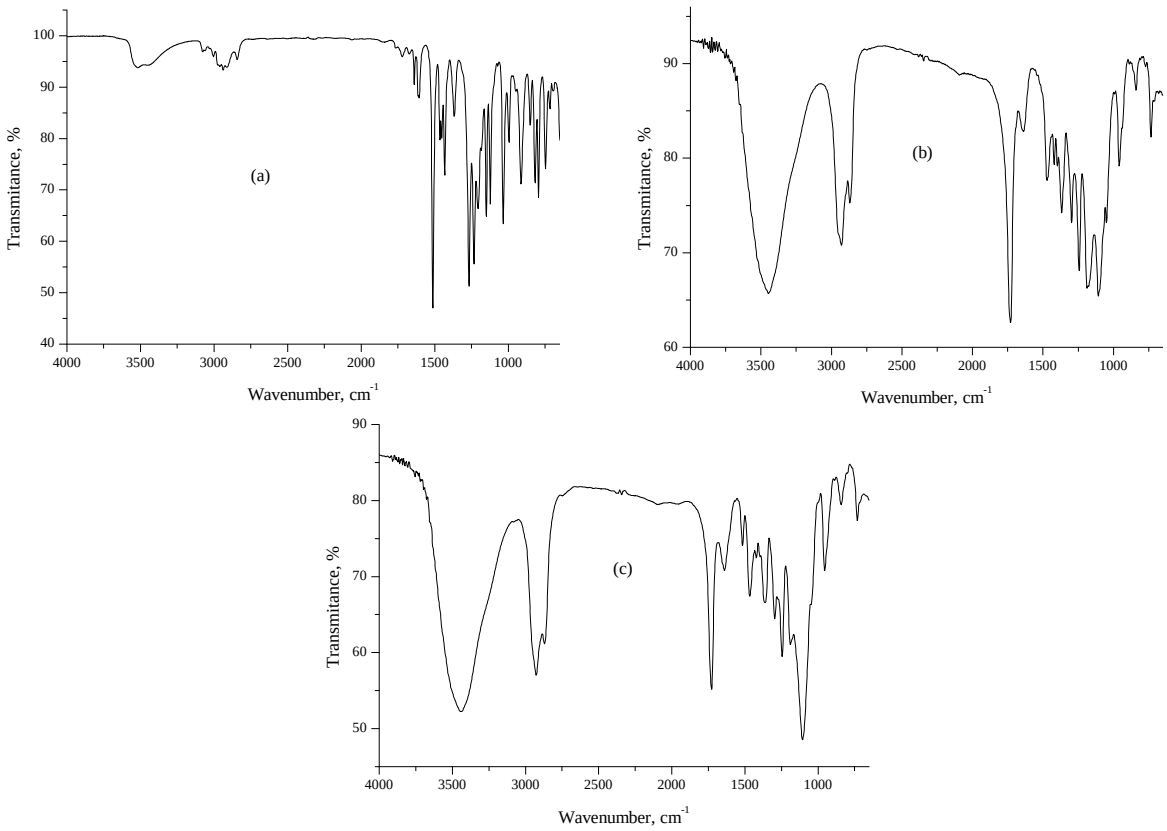

Figure 10 - FTIR profiles of free PCL oil particles (NP PCL), cinnamon oil (Cinnamon) and PCL particles containing the cinnamon oil (NP Cinnamon).

It is observed in the IV spectrum of PCL absorptions at 1728 cm^{-1} related to stretching C = O ester; the range of 1384 to 1228 cm^{-1} CO relates stretch to the alcoholic part 1190 and the 1040 cm^{-1} to the alcohol portion of the ester. The stretching CH alkanes appears in the range 2926 cm^{-1} and 2868 cm^{-1}. It is observed in 1472 cm^{-1} CH shear deformation methylenes (CH2) and 731 cm^{-1} strain "rocking" CH2 long chain band (when four or more CH2 groups are present in the aliphatic chain.

The oil from the *Cinnamomum zeylanicum* kind of cinnamon leaves have up to 79% of eugenol concentrations in its composition. Thus, in 1512cm^{-1} is observed C=O stretch of ester, 1034 cm^{-1} C-O stretch refers to the alcohol moiety of the ester at 1265 cm^{-1} C-O stretch has portion of the acidic ester. In 1637 cm^{-1} and 1605cm^{-1}, observed stretch C=C aromatic ring, as in 1462 cm^{-1} and 1450 cm^{-1}; 1232 cm^{-1} is the stretching C-O phenol; at 995 cm^{-1} there is a deformation out of the plane =C-H monosubstituted alkene; and 912 cm^{-1} and

851 cm^{-1} shows =C- H deformation trisubstituted aromatic ring out of plane at positions 1, 2 and 4 superimposed with stretch =C-H monosubstituted alkene.

It can be seen that for NP cinnamon oil, that is, the absorptions related to the oil does not appear, however the PCL are disclosed, suggesting that the surfaces of these particles are coated PCL only, while the oil he was inside. Thus, it was found that the absorptions are: 1726 cm^{-1} related to C=O stretching ester; in 1384 the 1228 cm^{-1}, has C=O stretch of the ester of the acid; in 1213 the 1031 cm^{-1}, C-O we stretch the alcohol moiety of the ester; 2926 in the 2868 cm^{-1}, has the C-H stretching alkanes; 1465 cm^{-1}, there is C-H shear deformation methylenes; and 731 cm^{-1} absorption is related to the deformation out of the plane type "rocking" CH2.

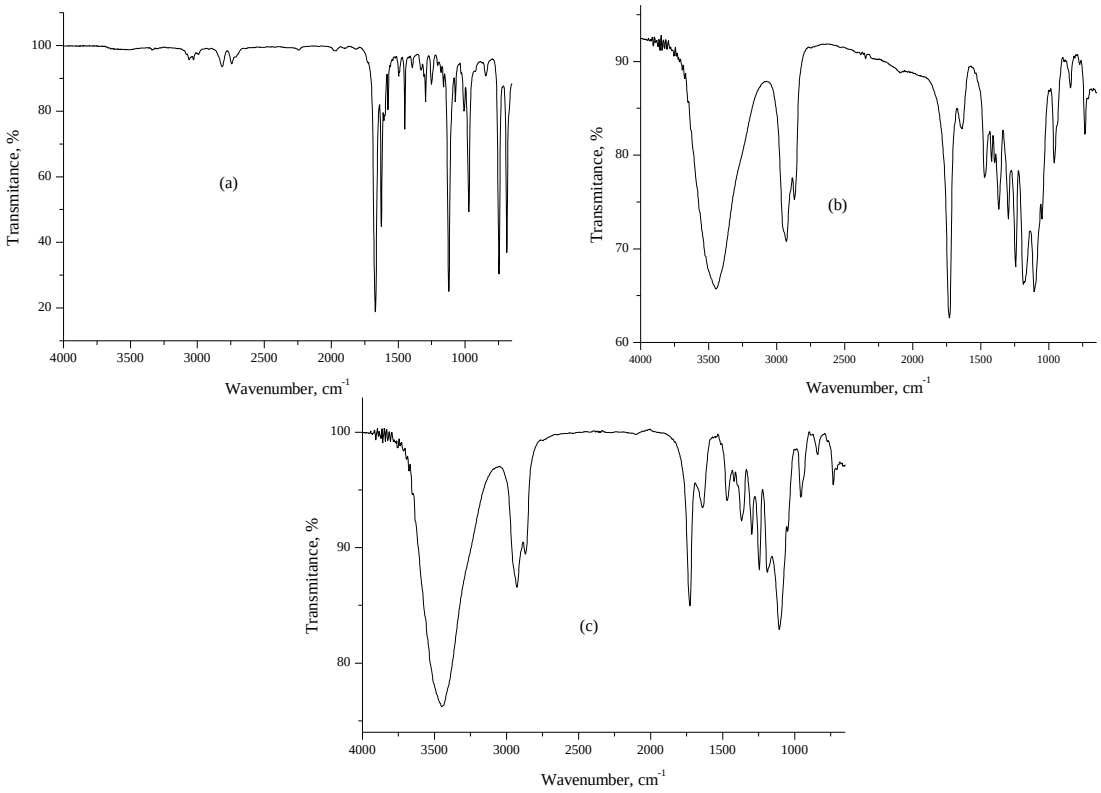

Figure 11 - FTIR profiles of free PCL oil particles (NP PCL) of cinnamaldehyde and PCL particles containing cinnamon oil (NP PCL Cinnamaldehyde).

The IV spectrum for the cinnamaldehyde shows overlap in 1668 cm^{-1} and 1624 cm^{-1} of stretching absorption bands C=C aromatic ring that appears in pairs with the stretching of the carbonyl (C=O) relative to the carboxylic acid. Still for the carboxylic acid stretches C-O are observed at 1121 cm^{-1}; O-H stretch at 3136-2957 cm^{-1} and O-H deformation outside the plane at 840 cm^{-1}. For the aromatic ring, we have in 2898 cm^{-1} to 2610 cm^{-1} has stretch =C-H; in 1489 cm^{-1} and 1448 cm^{-1} is observed stretch C=C; and the absorptions at 744 cm^{-1} and 689 cm^{-1} are relative to deformation outside the plane =C-H monosubstituted aromatic ring. It is observed in the 966 cm^{-1} band that refers to deformation outside the plane =C-H disubstituted alkene in the trans position.

For the IV Cinnamaldehyde MP, it is observed that the absorption bands are very similar to those obtained for MP PCL, suggesting that the particles are obtained capsules whose filling would cinnamaldehyde and wall material PCL. Thus, the absorption of MP Cinnamaldehyde be characteristic in 1726 cm^{-1} stretching C = O ester 1213 cm^{-1} to 1040 cm^{-1} representing the alcohol part of the ester C-O stretch and 1398 to 1222 cm^{-1} C-O acidic stretch of the ester. In 2928 to 2866 cm^{-1}, has the CH stretching alkanes, 1466 cm^{-1} refers to C-H shear deformation methylenes (CH2) and 732 cm^{-1} strain "rocking" CH2.

3.2 Evaluation of the antimicrobial activity of encapsulated systems - *in vitro* assay:

By analyzing the tables (Table 1, Table 2, Table 3, Table 4) you can see that for yeast *C. albicans* that both free and encapsulated cinnamon oil showed a MIC of 892 µg/mL. For cinnamaldehyde it was found that free oil, and encapsulated showed MIC of 446 µg/mL of *C. albicans*. The free cinnamon oil and encapsulated against *E. faecalis* showed MIC of 1.784 µg/mL. Free cinnamaldehyde and encapsulated in PCL on *E. faecalis* obtained a CIM equal to 446 µg/mL. Against *S. aureus*, the free and encapsulated cinnamon oil showed MIC of 1.784 µg/mL; already free cinnamaldehyde obtained MIC 892 µg/mL and MBC 1,784 µg/mL when encapsulated MIC and MBC of 892 µg/mL. Moreover, it was found that free oils PCL showed no antimicrobial activity on the investigated strains.

Furthermore, it can be seen that the oils, both free and encapsulated, show only fungistatic and bacteriostatic action and does not exhibit fungicidal action on C. albicans and bactericidal for *E. faecalis*. However, the cinnamaldehyde both free (CBM = 1,784 µg/mL) as encapsulated in PCL (CBM = 892 µg/mL) showed bactericidal activity against the strain *S. aureus*.

The positive control of Nystatin action on C. albicans showed MIC less than 50 µg/mL. While the positive control of bacterial strains performed with chlorhexidine presented MIC less than 1.92 µg/mL.

Table 1 - Minimum Inhibitory Concentration (MIC), Fungicide Minimum Concentration (MFC) and Minimum Bactericidal Concentration (MBC) of free oil cinnamon on *C. albicans*, *E. faecalis* and *S. aureus.*

Cinnamon oil free (CO)	**MIC**	**MFC/MBC**
C. albicans	892 µg/mL	-------
E. faecalis	1.784 µg/mL	-------
S. aureus	1.784 µg/mL	-------

Table 2 - Minimum Inhibitory Concentration (MIC), Fungicide Minimum Concentration (MFC) and Minimum Bactericidal Concentration (MBC) of cinnamon oil encapsulated on *C. albicans*, *E. faecalis* and *S. aureus.*

Cinnamon oil encapsulated (MCO)	**MIC**	**MFC/MBC**
C. albicans	892 µg/mL	-------
E. faecalis	1.784 µg/mL	-------
S. aureus	1.784 µg/mL	-------

Table 3 - Minimum Inhibitory Concentration (MIC), Fungicide Minimum Concentration (MFC) and Minimum Bactericidal Concentration (MBC) free cinnamaldehyde on *C. albicans*, *E. faecalis* and *S. aureus*.

Cinnamaldehyde free (CN)	**MIC**	**MFC/MBC**
C. albicans	446 µg/mL	-------
E. faecalis	446 µg/mL	-------
S. aureus	892 µg/mL	1.784 µg/mL

Table 4 - Minimum Inhibitory Concentration (MIC), Fungicide Minimum Concentration (CFM) and Minimum Bactericidal Concentration (MBC) of cinnamaldehyde encapsulated on *C. albicans*, *E. faecalis* and *S. aureus*.

Cinnamaldehyde Encapsulated (MCN)	**MIC**	**MFC/MBC**
C. albicans	446 µg/mL	-------
E. faecalis	446 µg/mL	-------
S. aureus	892 µg/mL	892 µg/mL

4. Discussion

The analysis of the results of this study shows that the technique of emulsification/evaporation of the solvent employed for the synthesis of encapsulated systems allowed obtaining PCL microparticles containing cinnamaldehyde, and cinnamon oil [30]. Among the advantages offered by this technique can mention the possibility of developing structures carried hydrophilic or hydrophobic activity by the formation of simple oil-in-water (O / W) [35]. Another advantage of this technique lies in the fact of providing a final product free from traces of solvent (acetone) to avoid possible toxic effects on tissues in which the encapsulated systems will be employed [36-38].

The relative simplicity of execution and reproduction method, the characteristics of the substances which have been encapsulated, the indication

for the synthesis of particles submicron dimensions from biodegradable polymers, as well as the possibility of controlling certain parameters involved in preparing - such as speed homogenization, drip speed of the polymer solution, volume of aqueous and organic phases, among other factors caused this technique was voted the ideal for this study.

The polymers directly influence the characteristics of the microparticles, since they interfere in stabilizing the drug in the body and toxicity. Thus, the polymers used in the synthesis of encapsulated systems should be biocompatible and biodegradable to harmless metabolites the body [20, 21]. Thus, the PCL was the polymer selected in order that it presents characteristics suitable for carrying substances with potential biological applicability, besides having the advantage of being a polymer approved by the Food and Drug Administration (FDA) and the European Commission (EC Mark) for SLC [39,40]. Furthermore, PC is more resistant to chemical hydrolysis and degrades more slowly than PLGA and PLA polymers are more suitable for long-term controlled release [41]. It is noteworthy that the PLC is a synthetic polymer, a fact which makes its advantageous use in view of versatility as to the manipulation of its monomeric organization, which results in properties such as biodegradability and compatibility in a biological environment, besides having high purity as compared the natural polymers [20, 21, 41].

The surface morphology of the microparticles of polymers is an important factor because it is directly involved in the control of drug release kinetics [48]. Furthermore, there is a relationship between the state of the particle surface and particle size [49]. Studies suggest that the PCL particles resulting from the process of evaporation of the solvent from the oil / water emulsion present in the majority of cases, rough morphology, coreless, large holes and deep on its surface, a fact that justifies the presented morphology by particle systems encapsulated cinnamon oil and cinnamaldehyde obtained in this study [49-51]. This research found that the surface state of the microparticles was changed to spherical, smooth damaged, the presence of holes and crevices, which can be related to the parameters adopted during the preparation of such encapsulated systems [52,53].

Some factors involved in obtaining encapsulated systems that may impact on damage the morphology of the particles, being them can be

mentioned: the molecular weight and concentration of polymer, the oil / polymer ratio, type and amount of surfactant, as well as speed of microencapsulated system unrest are factors that influence the particle size, the concentration of the drug as well as the release of this substance [54-56]. However, studies on the influence that each of these factors has on the conformation of the surface of the microparticles are scarce [60, 48, 61, 62]. It is known that faults in the morphology of the microparticles occurs where there evaporation of the solvent under reduced pressure conditions, due to the amorphous state of the polymer matrix [63,64]. Although the simultaneous control of the amount of oil to be encapsulated, as well as the morphology of the microparticles are complex; the scientific literature indicates the importance of adopting standard conditions in particle synthesis process in order to obtain a suitable surface morphology of the drug release parameters [38]. With respect to the classification of the particles of encapsulated systems as its size, it is observed that the diameter of the found value, the obtained beads can be classified as microparticles [21, 24].

The interactions between the substance and the polymer in an encapsulated system, as well as the physical state of the formulation, whether crystalline or amorphous, play an important role in controlling the drug release [65]. According to the obtained XRD patterns verifies the presence of well-developed peaks at 2θ ~ 21.3, 21.8 ~ 23.7 and ~, which are characteristic of the semi-crystalline polymer PCL. Suggesting, as well; the addition of cinnamon oil and cinnamaldehyde did not alter the crystallinity of the PCL pattern.

With respect to the chemical characterization of the samples by FTIR, it can be seen that the oils derived from *Cinnamomum zeylanicum* species of cinnamon leaves had more than 79% eugenol concentrations in the composition, confirming the IR absorptions for cinnamon oil in this task [66 - 68]. Furthermore, by FTIR analysis of the microparticles cinnamaldehyde, it is observed that the absorption bands are very similar to those obtained for the microparticles PCL, suggesting that the obtained particles are capsules containing cinnamaldehyde inside and as a wall material PCL. This was also observed for the system encapsulated cinnamon oil, since through the analysis of the graph it is apparent that the absorptions related to the oil does not appear, however the PCL are disclosed, suggesting that the surfaces of these particles are coated only PCL, while the oil was inside.

Despite the existence of various antibiotic agents, the search for new antimicrobial therapies is target of biomedical sciences, in view of the existence of bacterial and fungal strains resistant to conventional therapeutic methods. It is against this table studies involving the investigation of the antimicrobial properties of the natural products emerge, since such products have reduced toxicity and better biodegradability compared to antibiotics available on the market [12]. In this context, the encapsulated polymer systems loaded with essential oils that exhibit antimicrobial activity arise as a subject of studies in order that enable controlled release of substances, reducing the concentration and dose frequency, besides having bioactivity, providing stability to the oils encapsulated, preventing loss of volatile compounds, as well as improving the solubility of the oil in the hydrophilic medium [42-44].

Accordingly antifungal and antibacterial activity of formulations containing encapsulated systems cinnamon oil or cinnamaldehyde; were evaluated against the strains of *C. albicans* ATCC 11006, *S. aureus* ATCC 15656 and *E. faecalis* ATCC 14506. The antimicrobial tests were conducted in order to determine the activity of the oils encapsulated in PCL compared to oils free, by observing the growth of the strains in contact with the agents, and establishment of the CIM and CFM CBM.

The hypothesis of this study is to investigate whether the oils encapsulated in microparticles as a carrier base PCL could improve the bioavailability and antimicrobial activity of cinnamon oil and cinnamaldehyde due to the small size of the particles in which they are contained. However, it can be observed in this study that there was no difference in activity between the antimicrobriana free and encapsulated PCL oils. Suggesting that by reducing the micrometric dimensions, particles containing cinnamon oil and encapsulated cinnamaldehyde had no better biological properties, a fact that may be related to the methodology used during the synthesis of the encapsulated systems.

Furthermore, it can be seen that the oils, both free and encapsulated, show fungiostática and bacteriostatic action and does not exhibit fungicidal action of the bactericide on *C. albicans* and *E. faecalis*. However, both the free cinnamaldehyde (CBM = 1.784 mg / mL) as encapsulated in PCL (CBM = 892 mg / mL) showed bactericidal activity against the strain of *S. aureus*.

It is important to clarify that it is difficult to establish a relationship between the values of the CIM and CFM / CBM of the present study with values obtained in other studies, since there is a scarcity of studies addressing methodology similar to the present research, which makes such compared problematic [45]. Since this is the first study showing PCL systems containing the encapsulated cinnamon oil and cinnamaldehyde, as a future alternative for treatment of oral infections caused by *C. albicans*, *S. aureus* and *E. faecalis*. However, there are reports that show that cinnamaldehyde particles encapsulated in polymers exhibit antimicrobial activity against pathogenic fungi, gram-positive and gram-negative bacteria [46, 47].

One of the advantages of encapsulation of oils is that it promotes a controlled release of the antimicrobial substance. Further, these systems enable the synthesis of structures with small dimensions makes it feasible to access the antimicrobial substance encapsulated in the cytoplasmic membrane of the microorganisms [13]. The microparticles containing cinnamon oil and cinnamaldehyde, which were developed in this study have appropriate physical and chemical characteristics and have antimicrobial activity. These facts make the power delivery systems of the test substances, which can be an alternative for treatment of infections that affect major oral cavity, given that the free encapsulated antimicrobial oils and presentation on the studied strains. Suggesting thus the need for the implementation of future studies that verify the encapsulation efficiency of oil, strengthening their encapsulation technique to obtain particles smaller studies to verify the kinetics and profile of the release of oils, in addition to checking the toxicity of these systems. Among the suggestions for applicability of encapsulated systems developed in this research in the dental clinic, we can mention: use as irrigating solution for endodontic treatment in view of its effect on *E. faecalis*; and the possibility to associate such systems to dental materials and medicines in order to verify that it potentializes antimicrobial action of these resources.

5. Conclusion

The microparticles containing cinnamon oil and cinnamaldehyde developed in this study have appropriate physical and chemical characteristics and have antimicrobial activity. These facts make the potential release test

systems of substances, which can be an alternative for treatment of infections that affect major oral cavity.

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3. Considerações gerais

A nanotecnologia consiste em uma ciência multidisciplinar, que surgiu em meados da década de 80, a qual objetiva o desenvolvimento e aplicação de estruturas, dispositivos e materiais em escala manométrica (HÖLAND et al., 2009). Ao reduzir a dimensão dos materiais a nanoescala, verifica-se que sua superfície de contato aumenta consideravelmente e esse fato possibilita que esse material apresente melhores propriedades mecânicas, elétricas, ópticas, catalíticas, magnéticas, química, bem como relevante intensificação de sua atividade biológica (ZHANG et al.,2012).

Nesse sentido, as propriedades dessas nanopartículas tem sido alvo de investigação em diversas áreas de estudo. Dentre essas áreas de estudo, destaca-se a saúde, na qual o emprego dessas nanoestruturas aponta para uma promissora oportunidade para o desenvolvimento das ciências médicas no tratamento das doenças e atenção à saúde humana, possibilitando transpor as limitações impostas ao diagnóstico molecular, através da investigação da fisiopatologia das doenças, bem como permitir o desenvolvimento de uma prática terapêutica personalizada ao perfil do paciente (BYSTRZEJEWSKA-PIOTROWSKA; GOLIMOWSKI; URBAN, 2009; JAIN et al., 2013).

Associado às vantagens do emprego da nanotecnologia, observa-se que o tratamento das infecções fúngicas e bacterianas são comumente realizados mediante a administração tópica ou sistêmica de antimicrobianos disponíveis no mercado, os quais se caracterizam por ocasionarem reações adversas associadas à citoxicidade, fatos esses que limitam seu uso e eficácia terapêutica. O presente estudo objetivou sintetizar, caracterizar e verificar a atividade antimicrobiana de sistemas nanoencapsulados de cinnamon oil (óleo de canela) e cinamaldeído associado ao polímero policaprolactona. Verificando que as partículas contendo óleo de canela e cinamaldeído apresentam adequadas características físico-químicas, bem como apresentam atividade antimicrobiana. Tais fatos as tornam potenciais sistemas de liberação das substâncias testes, as quais podem constituir uma alternativa para o tratamento das principais infecções que acometem a cavidade oral. Contudo, se faz necessário a realização de estudos prévios que objetivem investigar a melhor via de síntese a fim de aperfeiçoar a eficiência de retenção do óleo, bem como

o tamanho das partículas. Pois ao modificar e aprimorar os parâmetros de processamento das formulações contendo sistemas encapsulado torna-se possível controlar a quantidade do composto ativo aprisionado no polímero, bem como o tamanho das partículas, promovendo sistemas que apresentem permeabilidade adequada, boa estabilidade química, resistência mecânica (SINJAIN; ROBINSON, 2003). Além disso, vale salientar a necessidade de se realizar estudos de cunho toxicológico e clínico a fim de conferir a segurança da aplicabilidade desses fitoconstituintes como fármaco.

4. Conclusão

- Os sistemas encapsulado de óleo de canela e de cinamaldeído em PCL foram obtidos com êxitos através do método de emulsificação/evaporação do solvente. Esse método permitiu encapsular os óleos, comprovando que a emulsão simples foi adequada para encapsulação de substâncias hidrofóbicas.

- A análise da atividade antimicrobiana, *in vitro*, demonstrou que o óleo de canela e o cinamaldeído tanto livres como encapsulados em PCL foram eficazes na inibição do crescimento de *C. albicans*, *E. faecalis* e *S. aureus*.

- A morfologia da superfície das micropartículas foi alterado de esférica e lisa para danificada, com a presença de orifícios e rugosidades os quais podem estar relacionados aos parâmetros adotados durante a formulação desses sistemas encapsulados.

- As caracterizações físico-químicas demonstraram os sistemas encapsulados de óleo de canela e de cinamaldeído conservaram as características do seu polímero estruturante, o PCL.

- As micropartículas contendo óleo de canela e cinamaldeído, as quais foram desenvolvidas no presente estudo apresentam adequadas características físico-químicas, bem como apresentam atividade antimicrobiana. Tais fatos as tornam potenciais sistemas de liberação das substâncias testes, as quais podem constituir uma alternativa para o tratamento das principais infecções que acometem a cavidade oral e infecções sistêmicas causadas por *S. aureus*.

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ANEXOS

A – Normas para a Defesa da Dissertação do Programa de Pós-graduação em Odontologia da UFPB (modelo alternativo):

- Capa
- Folha de rosto (primeira folha interna)
- Folha de aprovação
- Dedicatória (Opcional)
- Agradecimentos (Opcional)
- Epígrafe (Opcional)
- Resumo (com no máximo quinhentas palavras)
- Abstract (com no máximo quinhentas palavras)
- Lista de Abreviaturas e Siglas (Opcional)
- Sumário

1. Introdução (trata-se da formulação clara e simples do tema investigado, constando a delimitação do assunto tratado, justificativa e objetivos do estudo).
2. Capítulos (cópias de artigos de autoria ou co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a análise).
3. Considerações gerais (de caráter opcional, poderá conter argumentos para estabelecer relações entre os artigos apresentados nos capítulos).
4. Conclusão

- Referências
- Anexo (Opcional)
- Apêndices (Opcional)

B – Especificações do óleo de canela

SIGMA-ALDRICH®

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3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Product Specification

Product Name:
Cinnamon leaf oil - Ceylon origin, FCC, FG

Product Number: **W229210**
CAS Number: 8015-91-6

TEST	Specification
Appearance (Color)	Yellow to Brown
Appearance (Form)	Liquid
Refractive index at 20 °C	1.529 - 1.537
Infrared spectrum	Conforms to Structure
Specific Rotation	-2 - 1 °
Assay	80.00 - 88.00 %
Specific Gravity	1.030 - 1.050
Solubility (Turbidity)	Clear
Solubility (Color) 1ml/1.5ml 70% Ethanol	Yellow to Brown
Arsenic (As)	≤ 3 ppm
Cadmium (Cd)	≤ 1 ppm
Mercury (Hg)	≤ 1 ppm
Lead (Pb)	≤ 10 ppm
Expiration Date Period 5 Years	----------

Specification: PRD.0.ZQ5.10000029935

C- Especificações do cinamaldeído

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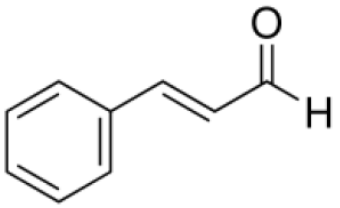
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Product Specification

Product Name:
Cinnamaldehyde - natural, ≥95%, FG

Product Number:	**W228613**
CAS Number:	104-55-2
MDL:	MFCD00007000
Formula:	C9H8O
Formula Weight:	132.16 g/mol



TEST	Specification
Appearance (Color)	Colorless to Yellow
Appearance (Form)	Liquid
Refractive index at 20 ° C	1.614 - 1.623
Infrared spectrum	Conforms to Structure
Purity (GC)	≥ 95 %
Specific Gravity	1.046 - 1.052
Acid Value	≤ 5.0 ml
Arsenic (As)	≤ 3 ppm
Cadmium (Cd)	≤ 1 ppm
Mercury (Hg)	≤ 1 ppm
Lead (Pb)	≤ 10 ppm
Expiration Date Period 5 Years	----------

Specification: PRD.2.ZQ5.10000009149