

A Produção do Conhecimento **nas Ciências da Saúde 5**

Benedito Rodrigues da Silva Neto
(Organizador)



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(Organizador)

**A Produção do Conhecimento nas Ciências
da Saúde**
5

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APRESENTAÇÃO

Encerramos nesse quinto volume a coleção “A Produção do Conhecimento nas Ciências da Saúde”, com um sentimento de gratidão e dever cumprido ao apresentar uma diversidade de pesquisas sólidas e de amplo espectro fomentando o conhecimento na área das Ciências da Saúde.

Tendo em vista todo conhecimento apresentado nesta coleção, finalizamos o trabalho apresentando de forma mais multidisciplinar possível trabalhos científicos na interface de estudos ligados à saúde.

Apresentamos de forma ampla conceitos atuais em pesquisas desenvolvidas com os temas psico-oncologia, qualidade de vida biopsicosocial, perfis epidemiológicos, práticas integrativas, automedicação, novos tratamentos, promoção e educação em saúde, biotecnologias em saúde, diagnóstico, sistema de saúde pública, fatores de risco, nanotecnologia, além de revisões e estudos de caso, que poderão contribuir com o público de graduação e pós graduação das áreas da saúde.

O profissional da saúde atual precisa cada vez mais estar conectado com as evoluções e avanços tecnológicos. Além disso é necessário um comprometimento com o conhecimento, pois esse avança à passos largos dentro das pesquisas em saúde, já que descobertas e publicações de alto impacto são diárias e trazem conteúdo aprimorado e de relevância, assim a leitura de fontes que possam ir além da área específica de atuação são extremamente importantes. Como objetivo central deste volume desejamos que o leitor tenha essa possibilidade em um único volume podendo transitar de diversas formas nas áreas afins.

Assim, reforçamos a importância do aprendizado contínuo do profissional da saúde, e desejamos fortemente que esse material contribua para isso. O conteúdo de todos os volumes é significativo não apenas pela teoria bem fundamentada aliada à resultados promissores, mas também pela capacidade de professores, acadêmicos, pesquisadores, cientistas e da Atena Editora em produzir conhecimento em saúde nas condições ainda inconstantes do contexto brasileiro. Desejamos que este contexto possa ser transformado a cada dia, e o trabalho aqui presente pode ser um agente transformador por gerar conhecimento em uma área fundamental do desenvolvimento como a saúde.

Dr. Benedito Rodrigues da Silva Neto

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PRODUCTION OF NEOMYCIN AND SUNFLOWER OIL-LOADED PAA-CHITOSAN MEMBRANES - POTENTIAL APPLICATION IN VETERINARY WOUND DRESSINGS

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RESUMO: O *Staphylococcus aureus* é um dos principais agentes causadores de infecções de pele e tecidos moles. O tratamento de infecções causadas por *S. aureus* tornou-se mais difícil com o tempo devido ao surgimento de cepas resistentes a múltiplos medicamentos. A pele e as mucosas de cães e gatos têm sido frequentemente afetadas pelo *S. aureus*. A neomicina é um antibiótico restrito ao uso tópico devido à sua cocleotoxicidade e nefrotoxicidade e é empregado na prevenção de infecções bacterianas. O óleo de girassol (SO) tem sido usado no tratamento de feridas. O poli(ácido acrílico) (PAA) utilizado é empregado como agente gelificante em fármacos e na síntese de hidrogéis para liberação controlada de fármacos. A quitosana exibe atividade antibacteriana, propriedades antifúngicas, mucoadesivas e hemostáticas. Neste trabalho, membranas de PAA e quitosana carregadas com SO e neomicina foram produzidas por mistura e, na seqüência, o SO foi adicionado às membranas carregadas com neomicina. As amostras foram caracterizadas por Microscopia Eletrônica de Varredura (MEV), Difração de Raios-X (DRX) e teste microbiológico. As análises de DRX indicaram que a presença do fármaco alterou

o padrão de cristalinidade dos polímeros puros. Imagens de MEV mostraram a dispersão das partículas do fármaco nas membranas. O teste microbiológico mostrou que o número de células de *S. aureus* inoculadas nas membranas carregadas com neomicina e SO, reduziu consideravelmente em relação às membranas puras. Este resultado sugere, portanto, que os materiais desenvolvidos possuem potencial para serem aplicados como curativo, evitando a contaminação externa e impedindo o crescimento das bactérias testadas.

PALAVRAS-CHAVE: Membrana, Curativo, *Staphylococcus aureus*.

ABSTRACT: *Staphylococcus aureus* is a major causative agent of skin and soft tissue infections and the treatment of *S. aureus* infections has become more difficult with time due to the emergence of multi-drug-resistant strains. Skin and mucous of dogs and cats have been frequently affected by *S. aureus*. Neomycin is an antibiotic restricted to topical use as a result of its cochleotoxicity and nephrotoxicity and it is employed to prevent bacterial infections. Sunflower oil (SO) has been used in the treatment of wounds. Polyacrylic acid (PAA) has been employed as a gelling agent in drugs and in the synthesis of hydrogels for controlled release of drugs. Chitosan exhibits antibacterial activity, along with antifungal, mucoadhesive and haemostatic properties. In this work, neomycin-loaded PAA-chitosan membranes were produced by casting, and, in the sequence, SO was added (by absorption) to the neomycin-loaded membranes. The samples were characterized by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD) and microbiological test. The XRD analyses indicated that the presence of the drug altered the crystallinity pattern of the pure polymers PAA and chitosan. SEM images showed the dispersion of the drug particles on the membranes. The microbiological evaluation shows that the number of cells of *S. aureus* inoculated in SO-neomycin-loaded PAA-chitosan membranes reduced considerably compared to the unloaded membranes. This result suggests that the developed materials have a great potential to be applied as a dressing, avoiding external contamination and preventing the growth of the tested bacteria.

KEYWORDS: Membrane, Wound dressing, *Staphylococcus aureus*.

1 | INTRODUCTION

Staphylococcus aureus is a major human and animal pathogen, causing both local and systemic diseases. This microorganism is part of the natural microflora present on the surface of the skin and mucous membranes of warm-blooded animals. It may become pathogenic in some conditions, such as breakage of the skin barrier or decreased immunity, causing a variety of disorders in the bloodstream (OTTO, 2012; BRANCO, VALÉRIO, *et al.*, 2018). These gram-positive facultative anaerobic cocci are responsible for conditions ranging from skin and soft tissue infections to systemic diseases such as pneumonia, atopic dermatitis, abscess, staphylococcal scalded skin syndrome, meningitis, osteomyelitis, endocarditis, wound infections and bacteremia

(HANESSIAN, GIGUÈRE, *et al.*, 2011; HAN, KIM, *et al.*, 2018; LEI, ZHANG, *et al.*, 2018).

Infections caused by *S. aureus* are usually treated with antibiotics such as penicillin, aminopenicillins, cephalosporins, aminoglycosides, tetracyclines and chloramphenicol. In this context, neomycin is an important aminoglycoside antibiotic, effective against Gram-positive, Gram-negative bacteria and micobacteria (VASTRAD e NEELAGUND, 2011; NITANAN, AKKARAMONGKOLPORN, *et al.*, 2013). Neomycin is restricted to topical use and it cannot be administered systemically because of its cochleotoxicity and nephrotoxicity (LUZ, ANATER, *et al.*, 2014). This antibiotic inhibits the RNase P function, which consists of an essential riboprotein complex present in *S. aureus* (BLANCHARD, BROOKS, *et al.*, 2016).

Sunflower oil (SO) presents inflammatory and antimicrobial properties and it is obtained from the sunflower seed (*Helianthus annus L.*) (PORSANI, CARVALHO, *et al.*, 2016). Some researches have shown that fatty acids can help in parts of the inflammatory process, such as muscle contraction, adhesion, activation, cell death and diapedesis (HATANAKA and CURI, 2007). Cell migration and the release of arachidonic acid mediators are important steps in the beginning of the tissue healing and repairing process (CARDOSO, SOUZA, *et al.*, 2004).

Tissue engineering offers a new way to help and accelerate the regeneration and/or repair of damaged tissue. One of the strategies of tissue engineering involves the development of biomaterials that can interact with biological systems to evaluate, treat, increase, or replace any tissue, organ, or body function (O'BRIEN, 2011; RUINI, TONDA-TURO, *et al.*, 2015). Biomaterials such as scaffolds, membranes and hydrogels are some of the most used technologies in tissue engineering. In this context, biopolymers have been used for the production of biomaterials, especially in wound healing.

Materials made of chitosan are widely used in tissue engineering. Chitosan is a biopolymer derived from the deacetylation of chitin and the deacetylation process is conducted by chemical hydrolysis under alkaline conditions or by enzymatic hydrolysis in the presence of specific enzymes, such as chitin deacetylase. After cellulose, chitin is the second most abundant biopolymer available and it can be found in the exoskeleton of crustaceans, insects and fungal cell walls (CROISIER and JÉRÔME, 2013).

This biopolymer is biodegradable, hydrophilic and it can be physically altered, which is a great advantage as it can be shaped into hydrogels, scaffolds, fibers, membranes and nanoparticles. Chitosan has an intrinsic antibacterial activity, low toxicity, properties of mucoadhesion, along with hemostatic and antimicrobial properties and analgesic effects. Due to these properties, some studies have described the use of antibiotic-loaded chitosan-based dressings for the treatment of wounds and tissue infections (AHSAN, THOMAS, *et al.*, 2018; BARANWAL, KUMAR, *et al.*, 2018).

Synthetic polymers have been successfully used in tissue engineering since they can be produced with properly architecture and their degradation characteristics are controlled by altering the polymer itself and its composition. One of the main

drawbacks of using these materials is the risk of rejection by the body due to its reduced biocompatibility. The majority of natural polymers, however, are biologically active and provide good cell adhesion and growth but they have poor mechanical properties. Owing to these disadvantages of synthetic polymers, they have been associated with natural polymers in order to increase their biological capacity (O'BRIEN, 2011).

Poly (acrylic acid) (PAA) is a synthetic polymer obtained by the free radical polymerization of acrylic acid, by the use of photoinitiators or radiation by γ rays. The polymerization is usually done in aqueous medium, and, as it is an exothermic reaction, the concentration of AA should not exceed 25% to allow the system control (ABDELAL; MAKKI and SOBAHI, 2012).

This synthetic polymer, according to pH, is anionic and hydrophilic, due to the carboxyl groups in its chain, which can make hydrogen bonds with water molecules. It exists as a liquid at pH 5 and as a gel at pH 7. This polymer is known as a carbomer and is the main gelling agent used in medicines and cosmetics (VILLANOVA, ORÉFICE and CUNHA, 2010; KADAJJI, BETAGERI, 2011).

PAA has mucoadhesive properties due to the fact of the hydrogen bonding with mucin (SOGIAS; WILLIAMS; KUTUTORYANSKIY, 2008). It is used to synthesize hydrogel matrices, which are capable of absorbing from 10% (arbitrated low value) up to thousands of times the value of their dry weight when immersed in water (HOFFMAN, 2012). The production of PAA-based hydrogels was tested in controlled drug delivery systems in the mouth considering its mucoadhesive properties. These hydrogels swell reversibly according to the pH and temperature of the medium in which they are placed (KUTYŁA, BOEHM, *et al.*, 2013).

In the present work the polymers chitosan and PAA were associated for the development of membranes that can be loaded with drugs for the inhibition of *S. aureus*. The drugs used were neomycin and SO, due to their unique properties, in order to produce a biomaterial that assists the healing of wounds and in the treatment of skin infections of dogs and cats.

2 | MATERIALS AND METHODS

The experimental sequence employed for the production of neomycin-SO-loaded PAA-chitosan membranes is schematically represented in Figure 1.

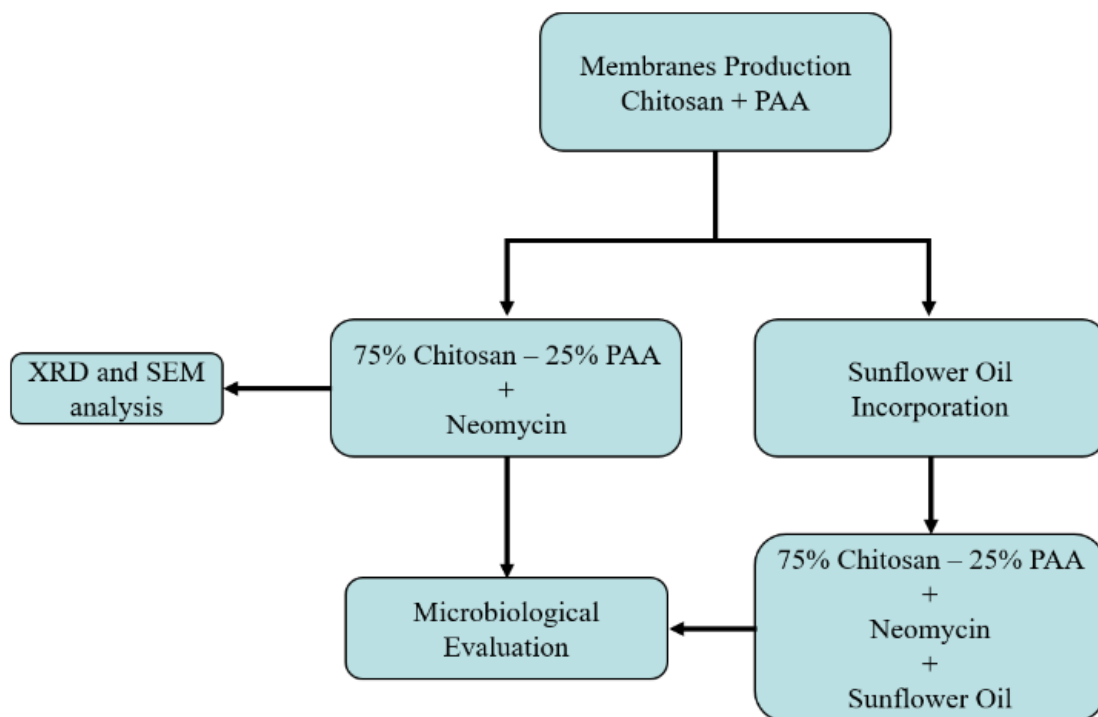


Figure 1 - Schematic representation of the experimental sequence used for the membranes production.

2.1 PAA-chitosan membrane and neomycin-loaded PAA-chitosan membrane preparation

The films were produced in the Materials Development Laboratory (LADEMAT - DEQ - UFRRJ) using chitosan (Sigma-Aldrich low molecular weight) and PAA (Sigma-Aldrich Mv ~ 4,000,000).

To prepare PAA-chitosan membranes, PAA solution (0.02 g/mL) and chitosan solution (0.02 g/mL) were mixed following the volumetric ratio of PAA: chitosan equal to 75:25. The neomycin loaded membranes were produced as follow: neomycin powder (3×10^{-7} g/mL) was added on the top of the chitosan and PAA solution and the mixture was stirred continuously. The resulting mixture was verted into silicone molds (113 cm²) and dried in a microwave oven (1620 W for 30 seconds).

2.2 SO incorporation

After drying, the films were cut into squares (1 x 1 cm), and the SO was added (0.0375 mL SO/cm² membrane) by absorption, as shown in Figure 2.



Figure 2 – SO absorption by the neomycin-loaded membrane.

2.3 Morphological analysis by scanning electron microscopy (SEM)

For the morphology evaluation, neomycin-loaded membranes were analyzed by SEM (TM3000, Hitachi), in the Synthesis of Nanomaterials Laboratory (DEQM – PUC-RIO). The ImageJ software was used to measure the diameters of the particles presented in SEM obtained images.

2.4 Microstructural analysis by X- ray diffraction (XRD)

In order to study the effect of the drug on the polymers microstructure and to evaluate the effect of the polymer on maintaining the microstructure of the drug, the membranes were also analyzed by XRD (D8 Discover, Bruker), with Cu K α source ($\lambda= 0.154$ nm) at 40 kV and 40 mA, in the scattering range of $2\theta = 5^\circ - 60^\circ$ in steps of 0.02° using 2 s for each step, in the X-Ray Diffraction Laboratory (DEQM- PUC-RIO).

The chitosan and PAA powders were also analyzed by XRD (Mini Flex II, Rigaku), with Cu K α source ($\lambda= 0.154$ nm) at 30 kV and 15 mA, in the scattering range of $2\theta = 2^\circ - 60^\circ$ in steps of 0.02° using 1 s for each step, in the Catalysis Laboratory of the Chemical Engineering Department, UFRRJ.

2.5 Antibacterial activity

The antibacterial activity of the membranes was evaluated according to a modified ASTM Method E2180- 07 (2012). This analysis was made at Food Microbiology Laboratory (DTA – UFRRJ). A suspension of *S. aureus* cells (ATCC 6538) was initially prepared, with the turbidity adjusted on the MacFarland 5 scale corresponding to about 10^8 CFU/mL. 1 mL of this solution was transferred to 100 mL of the agar paste to obtain 10^6 CFU/mL. The membranes were plated onto 24-well plates and added with 200 microliters of the inoculated agar paste. The plates were incubated at 30 °C for 24 h. After incubation, the samples were transferred to Falcon tubes and 1.8 mL of

buffer (this was considered the 10^{-1} dilution) was added to the tubes. It was prepared subsequent decimal dilutions up to 10^{-4} . The test was made for pure PAA-chitosan membranes, SO-loaded PAA-chitosan membranes, neomycin-loaded PAA-chitosan membranes and neomycin-SO-loaded PAA-chitosan membranes.

3 | RESULTS AND DISCUSSION

In the present work, PAA-chitosan, SO-loaded PAA-chitosan, neomycin-loaded PAA-chitosan and neomycin-SO-loaded PAA-chitosan membranes were successfully obtained, as shown by the photos in Figure 3. The membranes presented different colors by naked eye examination, due to the incorporation of the sunflower oil.

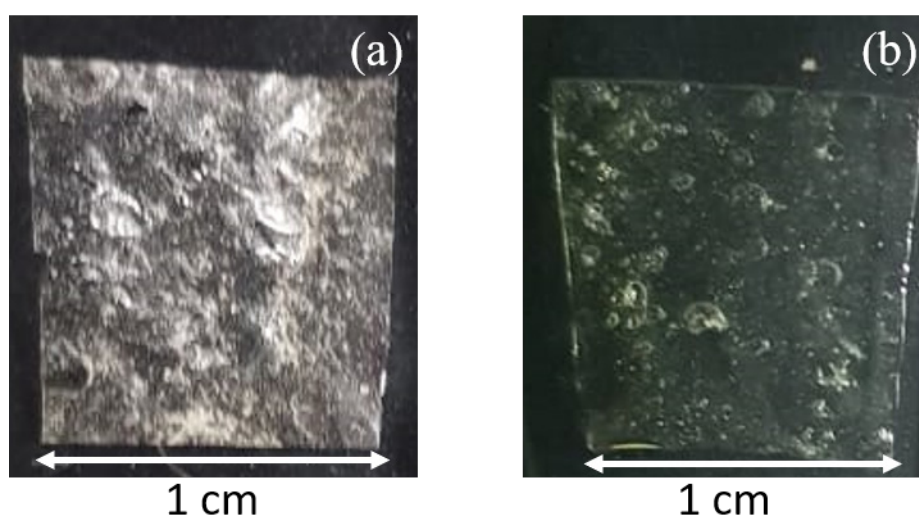


Figure 3 – (a) neomycin-loaded PAA-chitosan membranes and (b) neomycin-SO-loaded PAA-chitosan membranes.

The raw materials and the resulting membranes were analyzed by XRD and the diffractograms are depicted on Figure 4 and Figure 5, respectively.

The diffractogram of pure PAA (Figure 4) presented characteristics broad peaks at $2(\theta)$ equal to: 18.87° and 35.76° . The wide peaks suggested that PAA has low degree of crystallinity. This result is in agreement with the literature (BEKIN, SARMAD, *et al.*, 2014; TODICA, STEFAN, *et al.*, 2014; YAMAGUCHI, NAKANISHI, *et al.*, 2015).

The diffractogram of pure chitosan (Figure 4) presented characteristics peaks at $2(\theta)$ equal to: 20° and 10° . This result was also observed by Mendonça *et al.* (2013), Abdeen, Mohammad and Mahmoud (2015) and Dey *et al.* (2016).

The diffractogram of the neomycin-loaded membranes presented characteristics peaks at $2(\theta)$ equal to: 9.46° , 19.06° and 28.84° . It is possible to notice that the characteristics peaks of the pure polymers are not observed. The peaks shown in Figure 5 are related to the mineral talc, present in the neomycin powder used to load the membranes, as shown by Pérez-Maqueda *et al.* (2004) and Kogure *et al.* (2006).

The characteristic peak from neomycin was not observed, accordingly to the literature (NUGRAHANI, 2015).

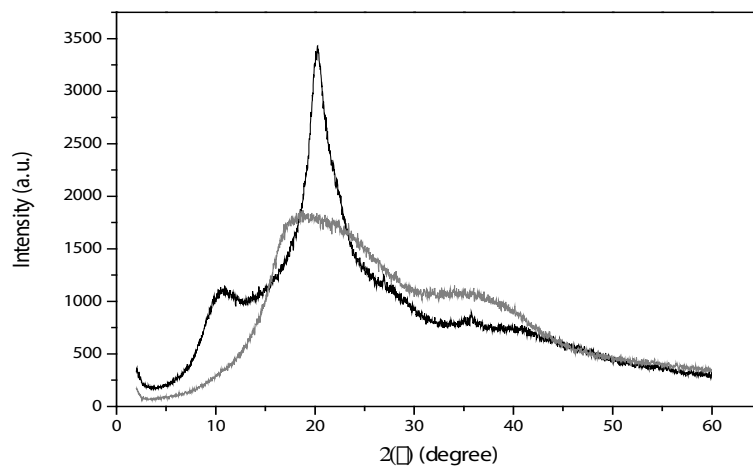


Figure 4 – X-ray diffractogram of chitosan (black solid line) and PAA (gray solid line).

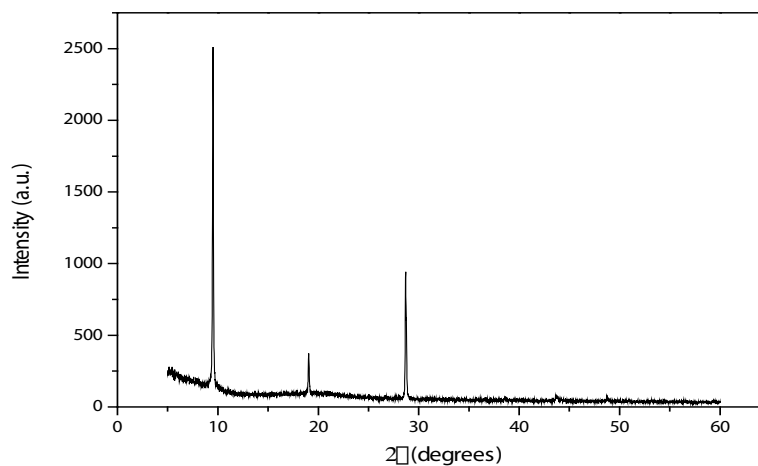


Figure 5 – X-ray diffractogram of neomycin-loaded PAA-chitosan membrane.

The membranes were analyzed by SEM and the resulting images are shown in Figure 6. It is possible to observe that the neomycin powder was dispersed in the polymers and exhibited an irregular size distribution.

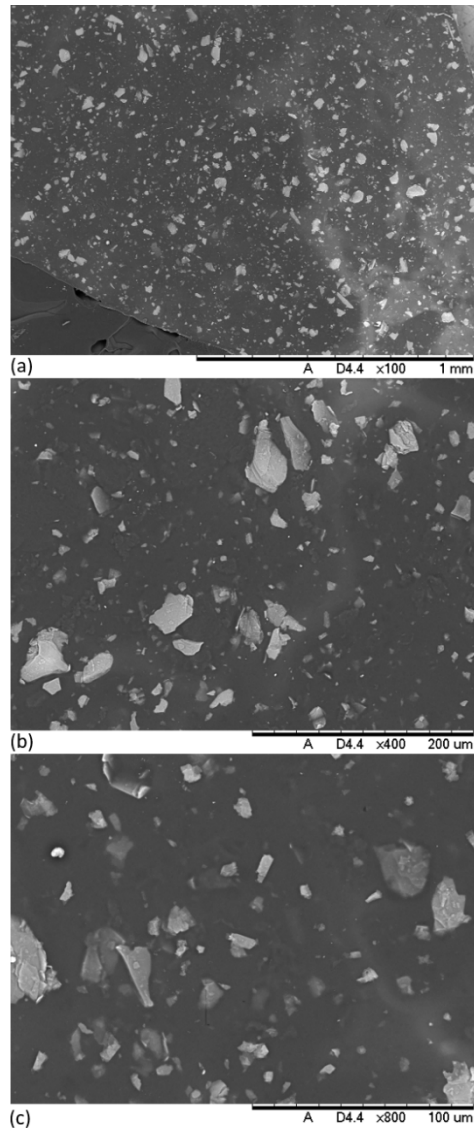


Figure 6 - SEM images of neomycin-loaded PAA-chitosan membrane.

The Figure 6 (b) was analyzed by ImageJ, to evaluate how the particles were dispersed in the membrane, and it was found that 95% of the particles have an area between 0.44 and 100 μm^2 and the cumulative and relative distribution are shown in Figure 7.

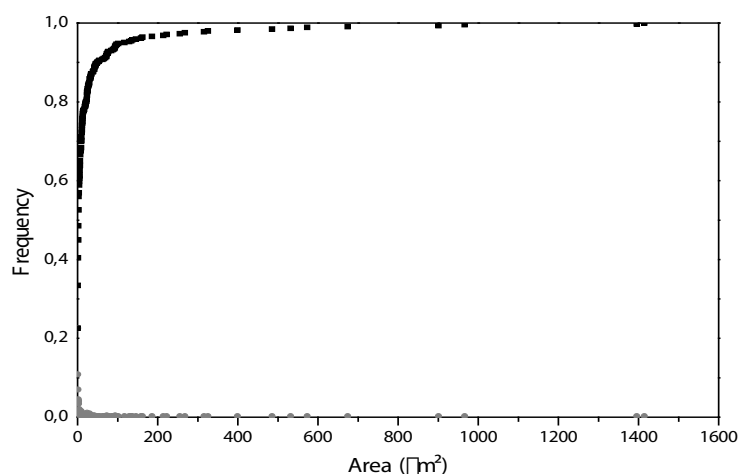


Figure 7 – Cumulative (black scatter) and relative (gray scatter) distribution of neomycin particles in the membrane.

A biological test was performed to evaluate the effect of sunflower oil and neomycin addition on the membranes antibacterial activity. It was initially inoculated approximately 6.70×10^6 CFU/mL cells of *S. aureus* for PAA-chitosan and SO-loaded PAA-chitosan membranes and 1.11×10^7 CFU/mL cells of *S. aureus* for neomycin-loaded PAA-chitosan and neomycin-SO-loaded PAA-chitosan membranes. The number of cells obtained after 24 h of incubation may be visualized in Table 1.

Membranes	CFU/mL
PAA-chitosan	1.33×10^3
SO-loaded PAA-chitosan	$< 5.00 \times 10^2$
neomycin-loaded PAA-chitosan	$< 1.00 \times 10^3$
neomycin-SO-loaded PAA-chitosan membranes	$< 1.00 \times 10^3$

Table 1 – Antibacterial activity of the membranes.

It is possible to observe that all membranes showed antibacterial activity. However, the best result was obtained by the SO-loaded PAA-chitosan. It is important to cite that more detailed studies will be conducted to better comprehend the interactions between membranes, loaded-drugs and the microorganisms.

PAA, when associated with chitosan, may improve the bacterial activity of this polymer. This result is in concordance with the study of Noppakundilokrat *et al.* (2013). They developed chitosan grafted polymers hydrogels with poly(acrylic acid)/hydroxyethyl methacrylate and poly(acrylic acid)/hydroxyethyl methacrylate/mica. It was observed that the chitosan grafted hydrogel with PAA presented the best results, with the highest value of relative inhibition (%). The biological activity of chitosan depends on its molecular weight. The charge, the size and conformation of the polymer chain may interfere in its antimicrobial efficiency (FERNANDES, FRANCESKO, *et al.*,

2013). The oil addition, may, potentially, alter the polymers chain conformation due to the material properties, such as chitosan charge.

The neomycin-loaded PAA-chitosan and neomycin-SO-loaded PAA-chitosan membranes presented the same number of cells after 24 h of incubation. Merlusca *et al.* (2018) studied the antibacterial activity against *S. aureus* of chitosan– poly(vinyl alcohol)–neomycin sulfate films and they observed that the inhibitory effect of the drug-loaded films was significantly higher than the one of the unloaded films. Preethika *et al.* (2016) evaluated the antimicrobial activity of neomycin functionalized chitosan stabilized silver nanoparticles and it was inferred that the chitosan stabilized silver nanoparticles loaded with neomycin showed an enhanced antimicrobial activity when compared to the chitosan nanoparticles and the pure silver nanoparticles.

4 | CONCLUSIONS

The neomycin-SO-loaded PAA-chitosan membranes were successfully prepared and it is possible to observe that the membranes align the properties of the constituent materials. According to the results obtained by the microbiological test, it is suggested that the developed membranes have potential to be used for the intended application of assisting the healing of wounds and the treatment of skin infections in dogs and cats, since all the developed membranes inhibited the growth of *S. aureus*.

5 | ACKNOWLEDGMENTS

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