CALCIUM ALGINATE-BASED ANTIBACTERIAL FILMS

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Introduction

Sodium alginate (AL) is a salt of alginic acid, a linear polysaccharide composed of 1,4-linked β-D-mannuronic acid and α-L-guluronic acid residues. Due to its gelling properties, biocompatibility, non-toxicity, biodegradability, sodium alginate is the most extensively studied material for the preparation of polymeric films. Such films have a wide variety of applications mainly in the food, cosmetic, medical and pharmaceutical industries [1]. Glycerol, sorbitol, xylitol, polyethylene glycol, or mixtures thereof are used as plasticizers of alginate films [2].

Various essential oils are used in order to prepare films with antibacterial activity. Namely, garlic, elicriso italic, lavender, eucalyptus, oregano, rosemary, chamomile blue, lemon, peppermint, cinnamon and lemongrass are incorporated in the films as active substances [3].

This study aimed to develop calcium alginate-based flexible films covered with gum of Chios mastic for the prevention of bacterial growth.

Materials and Methods

Two types of films were prepared and modified with antibacterial material. The first group of samples were prepared by dissolving AL powder in glycerol-water solution (5 wt.% glycerol) to yield a 3 wt.% AL solution. The second group of samples were prepared by dissolving hyaluronic acid (HA) in glycerol-water solution (5 wt.% glycerol) to yield a 0,5 wt.% HA solution. Further, AL powder was added into prepared solution to reach a concentration of 3 wt.%. After complete solubilisation both solutions were poured into Petri plates (30 mL/plate with a diameter of about 90 mm) and sprayed with 1% calcium lactate solution. After 10 minutes, the spraying was repeated and the plates were placed in a Binder ED53 (Binder, Germany) oven at 37°C for 72 hrs. The antibacterial properties were provided by immersing the films in a 5 wt.% solution of mastic gum in ethanol. The thickness of the films was measured with a micrometer (293 MDC-MX, Mitutoyo Co., Kawasaki, Japan). The MRS 120-3 moisture analyzer (Kern, Germany) was used to measure moisture content. The mechanical properties were determined using a universal material testing machine Zwick/Roell BDO-FB O.5 TH (Zwick, GmbH & Co, Ulm, Germany). The zone of inhibition assay on solid media was used for the evaluation of the antibacterial effect of films against Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus and Staphylococcus epidermidis*) bacteria.

Results and Discussion

Two types of flexible films were prepared: calcium alginate (Ca-AL) and its composite film with hyaluronic acid (Ca-AL-HA). The antibacterial properties of the films were ensured by immersing the films in a solution of Chios mastic gum in ethanol.

Surface coating had a direct effect on the characteristics of the films (TABLE 1). Antibacterial layer formation caused a decrease in the thickness of the films. It was found that such differences in the thickness of the films were affected by the solvent. The obtained films were swollen in water. However, the use of ethanol solution slightly shrank the films and caused a decrease in thickness.

TABLE 1. Characteristics of the films.

Film No.	Film type	Film thickness, µm ± SD	Moisture content, % ± SD
1	Ca-AL	260 ± 30	10 ± 0,9
2	Coated Ca-AL	230 ± 10	2 ± 0,2
3	Ca-AL-HA	310 ± 40	11 ± 1,2
4	Coated Ca-AL-HA	240 ± 20	3 ± 0,4

The moisture content of the films depended on the film composition. The moisture content was larger in Ca-AL-HA films due to HA ability to retain water.

The mechanical properties of formed films were investigated. The results showed that the addition of glycerol and HA into the composition of the films caused a significant reduction in the rigidity.

Films (1 cm^2 in size) coated with mastic gum were used to investigate the antibacterial properties against Gram-negative and Gram-positive bacteria. Uncoated Ca-AL, Ca-AL-HA films were studied for comparison. As expected, uncoated Ca-AL film was not effective against any test microorganisms. Uncoated Ca-AL-HA and Ca-AL coated with Chios mastic gum showed similar antimicrobial activity against *Escherichia coli*. Results showed, that the strongest antibacterial activity had Ca-AL films with HA and Chios mastic gum layer. The clear zones of inhibition directly underneath the film pieces were observed in the plates inoculated with *Escherichia coli* and *Staphylococcus aureus*. Also, a weak inhibitory effect against *Staphylococcus epidermidis*, indicated by minimal growth underneath film pieces were observed. However, *Pseudomonas aeruginosa* was resistance to all tested film compositions.

Conclusions

The observed results confirm that the preparation of calcium alginate flexible films with antibacterial properties is a complex process. The addition of plasticizer clearly improved the flexibility of the films.

The presence of HA and Chios mastic gum layer on the originally prepared films improved antibacterial properties. The films were effective against Gram-negative and Gram-positive bacteria.

References

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