"Chemical studies on ciprofloxacin derivatives as antimicrobial additives for packaging applications"

A thesis

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List of abbreviation

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CFX	Ciprofloxacin
CHS	Chitosan
CPT	Camptothecin
CP	Ciprofloxacin Piperidinone
DA	Degree of Acetylation
DDA	Degree of Deacetylation
DMF	Dimethylformamide
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FQ	Fluoro Quinolones
GlcN	Glucosamine
GlcNAc	N-Acetyl Glucosamine
IDT	Initial Decomposition Temperature
KDa	Kilo Dalton
LMWC	Lower Molecular Weight Chitosan
MDR	Multi Drug Resistant
MRSA	Methicillin Resistant Staphylococcus Aureus
μM	micro Mole

List of abbreviation

PDA	Potato Dextrose Agar
PDT_{max}	Maximum Polymer Degradation Temperature
RAR	Relative Adsorption Ratio
RTI	Respiratory Tract Infections
SAR	Structure Activity Relationship
SB	Schiff Base
SEM	Scanning Electron Microscopy
STD	Sexually Transmitted Diseases
TEA	Triethylamine
TGA	Thermo-gravimetric Analysis
TS	Tensile Strength
UTI	Urinary Tract Infections

Contents

Abstract

The development of biologically active and biodegradable films for food packaging has recently been increasing due to significant concerns about environmental pollution caused by non-biodegradable packaging materials and consumer demand for high quality food products.

Chitosan films have been successfully developed and used for food packaging such as fruits, vegetables, and meats. However, for certain food products, the limited antimicrobial activity of pure chitosan films does not reach the antiseptic level desired by packers. For example, to enhance the efficiency of chitosan films, many antimicrobial agents have been incorporated into the chitosan films to extend the shelf-life and to increase its antimicrobial activity. The incorporation of an additional antimicrobial agent could enhance its antimicrobial activity and expand the scope of its applications.

Chemical modifications are promising techniques which used for enhancement of antimicrobial activity. In this study, the author is interested in chemical modification of ciprofloxacin via Schiff base formation with chitosan polymer or glucosamine monomer. Chitosan-ciprofloxacin, ciprofloxacin-glucosamine Schiff bases and ciprofloxacin-piperidinone derivatives have been synthesized and their structures were elucidated by FT-IR and ^{1}H NMR spectroscopic analysis and their antimicrobial activities

have been evaluated. From the results, it has been found that, Schiff base (SB5) which obtained from the condensation reaction of ciprofloxacin with chitosan at the ratio of (1:1) had the best antimicrobial activity. SB5 was incorporated with pure chitosan forming biologically active chitosan films.

Key words: Biodegradable films, food packaging, chitosan films, antimicrobial activity, Schiff bases, spectroscopic analysis.

Summary

Summary

The interest in the development of biologically active and biodegradable films for food packaging has recently been steadily increasing due to significant concerns about environmental pollution. Chitosan films have been successfully developed and used for food packaging such as fruits, vegetables, and meats. The elastic and transparent chitosan films, solid mechanical properties, non-toxic, biodegradable, selective permeability for gases, biocompatible films and also have antimicrobial properties. The limited antimicrobial activity of pure chitosan films does not reach the antiseptic level desired by packers. For example, to enhance the efficiency of chitosan films against foodborne pathogens many antimicrobial agents have been incorporated into the chitosan films to extend the shelf-life and to increase its antimicrobial activity. The incorporation of additional antimicrobial agents could enhance its antimicrobial activity and expand the scope of its application. Chemical modifications one of the most promising techniques used for the enhancement of antimicrobial activity of materials. This study was divided into four parts:

1- Chitosan-ciprofloxacin Schiff bases preparation with different degrees of substitution. Structure elucidation of the Schiff's bases obtained is based on their FT-IR and ^{1}H NMR spectral data. Antimicrobial activities of chitosan, ciprofloxacin and the synthesized Schiff bases were assessed using six bacterial strains: three Gram-positive species, three Gram-negative species and one fungus species. The best biologically active Schiff base (SB5) was used for biologically active film preparation and characterized by: solubility measurement, water uptake measurement, ultraviolet spectral analysis, thermo-gravimetric analysis (TGA), mechanical properties

measurement, surface properties by scanning electron microscopy analysis (SEM) and contact angle measurement.

2- Glucosamine was condensed with ciprofloxacin to give the corresponding Schiff base, six derivatives were synthesized. The structures of the obtained seven Schiff bases were characterized using FT-IR and ^{1}H NMR spectroscopy. The antimicrobial activities of the seven ciprofloxacin-glucosamine Schiff bases were evaluated using six bacterial species and one fungus species.

3- 3-hydroxy piperidin-2-one ring formation from ciprofloxacin-glucosamine Schiff base derivatives. The structures of the obtained seven ciprofloxacin- piperidinone derivatives were characterized using FT-IR and ^{1}H NMR spectroscopy. The antimicrobial activities of the seven ciprofloxacin -piperidinone derivatives were evaluated using six bacterial species and one fungus species.

4- Preparation of active antimicrobial packaging films based on incorporation of the best biologically active chitosan-ciprofloxacin Schiff base (SB5) as an antimicrobial additive on pure chitosan. Physical properties measurement by testing mechanical, solubility and water uptake properties. Structural properties measurement by FT-IR, ^{1}H NMR and surface properties by SEM. The films were tested for antimicrobial properties using six bacterial species and one fungus species.

The results obtained in this study can be summarized as follows:

1- Characterization of the prepared chitosan-ciprofloxacin Schiff bases:

a. The infrared spectra of the five Schiff bases obtained showed the appearance of strong absorption bands at 1618, 1600, 1598, 1608 and 1577 cm^{-1} which are attributed to the $\upsilon_{C=N}$ of the imines formed.

b.The ^{1}H NMR spectra of Schiff bases presented significant alterations in relation to the original chitosan. The proton assignments of the synthesized Schiff base were: 0.9 ppm (CH_3 of the acetyl group), 1.2-1.5 ppm (H-2', 3'), 2.4-2.9 ppm (H-2'', 3''), 5.3 ppm (H-1·), 5.4-6.3 ppm (H-2·-6· of glucosamine ring) and aromatic protons 7-8 ppm (H-2, 5, 8).

c.The antimicrobial activities of the synthesized five chitosan - ciprofloxacin Schiff bases obtained were measured, it has been found that Schiff base (SB5) formed by the condensation reaction of ciprofloxacin with chitosan at (1:1) ratio among all synthesized Schiff bases exhibited the highest antibacterial activity specially against *Staphylococcus aureus* species.

2- Characterization of the prepared ciprofloxacin-glucosamine Schiff bases:

a. The infrared spectra of the seven Schiff 's bases obtained showed the appearance of strong absorption bands at 1630, 1640, 1642, 1690, 1635, 1640 and 1630 cm^{-1} which are attributed to the $\nu_{C=N}$ of the imines formed.

b. The proton assignments of the synthesized Schiff base were: 0.9 ppm (CH_2 of the methylene group) of the glucosamine moiety, 1.2-1.5 ppm (H-2',3') of the piperazin ring , 2.4-2.9 ppm (H-2'',3'') of the cyclopropyl ring, 5.3 ppm (H-1•) and 5.4-6.3 ppm (H-2•-6•) of the glucosamine moiety and 7-8 ppm of the aromatic protons (H-2,5,8).

c.The antimicrobial activities of the synthesized seven Schiff bases obtained were measured, Schiff base (SB12) exhibited the higher antibacterial activity toward all bacterial strains at concentration 25 µg/ml.

3-Characterization of the prepared ciprofloxacin-piperidinone derivatives:

a. The infrared spectra of the seven ciprofloxacin-piperidinone derivatives obtained showed the appearance of strong absorption bands at 1685, 1690, 1795, 1725, 1695, 1690 and 1630 cm^{-1} which are attributed to the $\nu_{C=O}$ of the piperidinone carbonyl formed.

b. The proton assignments of the synthesized piperidinone derivatives were: 0.9 ppm (CH_2 of the methylene group) of the glucosamine moiety, 1.2-1.5 ppm (H-2',3') of the piperazin ring , 2.4-2.9 ppm (H-2'',3'') of the cyclopropyl ring, 5.3 ppm (H-1•) and 5.4-6.3 ppm (H-2•-6•) of the glucosamine moiety and 7-8 ppm of the aromatic protons (H-2,5,8).

c. The antimicrobial activities of the synthesized seven piperidinone derivatives obtained were evaluated. It is evident that the antimicrobial activity of the piperidinone derivative (CP7) exhibited the highest antibacterial activity toward all bacterial strains at concentration 25 µg/ml compared with ciprofloxacin itself except at concentration 25 µg/ml ciprofloxacin itself exhibited the higher antibacterial activity toward the Gram-negative species Escherichia coli.

4- Characterization of the prepared active films for packaging applications.

a. The antimicrobial activities of non-modified chitosan and three developed chitosan films obtained were evaluated. It is evident that the antimicrobial activity of the developed chitosan film (F3) which obtained from the incorporation of SB5 into non-modified chitosan film (F0) at concentration 3.0 g/100 g exhibited the highest antibacterial activity.

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