BLEND EFFECT OF PCL ON ANTIMICROBIAL ACTIVITY OF DIFFERENT MOLECULAR WEIGHT CHITOSAN

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Abstract

Background: Studies in the past have shown the inhibitory capability of chitosan on the growth of several microbial organisms such as *E. coli, L. monocytogenes, S. typhimurium, P. aeruginosa, S. aureus.* Polycaprolactone(PCL), which is synthetic polyester, makes for an interesting study in the preparation of polymer blends. Few other studies of PCL-chitosan blend towards oral pathogens such as *S. mutans* and A. actinomycetemcomitans have shown that adding PCL to chitosan can lower the antimicrobial activity of pure chitosan and results in the presence of microbes on the surface of the polymer film.

Purpose of Study: The current study was undertaken to develop a new polymer material that comprises desirable properties from different polymers which are not possessed in individual polymer.

Study Design: Experimental

Method: Low molecular weight chitosan-PCL blends and medium molecular weight chitosan with PCL of different proportions such as 100:0, 80:20, 75:25, 60:40, 50:50, 20:80 were synthesized. The protocol for synthesizing polymer blends was carried out in the fume hood. *S.auerus* was used for screening antibacterial activity of chitosan-PCL blend. The zone of inhibition was measured in diameter (mm). The number of colonies was counted by comparing the test plates with control and data was recorded.

Result: Analysis of crystallinity has shown that when the concentration of chitosan was high, there were not many pits and holes present on the film. However, when the concentration of chitosan decreased, we could see more holes and pits there.

In low molecular weight chitosan-PCL blend, based on *S.aureus* colony count, antimicrobial activity was low for 100% chitosan but the antimicrobial activity rose rapidly in 80:20 chitosan-PCL blends, showing that the addition of PCL increased antimicrobial activity. Low molecular weight Chitosan-PCL blends of 75:25, 60:40 and 50:50 proportions showed 100% inhibition.For medium molecular weight chitosan-PCL blends, antimicrobial activity on *S. aureus* based on colony count, number of colonies increased in 80:20 chitosan-PCL ratio and at the ratio of 75:25 and 60:40, no *S.aureus* colonies were found, depicting 100% inhibition. However, the addition of PCL beyond this point did not show antimicrobial activity.

Conclusion: The addition of PCL to medium molecular weight chitosan enhanced the antimicrobial effectiveness on *S.aureus* until particular ratio of PCL, after that the effect was decreased when the concentration of PCL in the blends increased. This result was in contrast to the antimicrobial testing of low molecular weight chitosan-PCL blends. Low molecular weight chitosan with PCL blends showed better inhibition when the concentration of PCL was higher.

Keywords: Antimicrobial, Chitosan, molecular weight, blend

Introduction

Natural polymer, synthetic polymer, or polymer blends produced from a combination of two different polymers possess antimicrobial activity and are of interest in current research. Keratin, starch, chitosan, cellulose and chitin are examples of antimicrobial polymers that have been studied (Correlo et al 2005; Sionkowska, 2011) and some of these polymers, especially chitosan, have been used in biomedical applications (Toan, 2011).The modification of polyacrylonitrile (Alamri et al 2012), blending of chitosan with PVP (Li J et al 2010), extraction of chitosan from shrimp waste (Martinez-Camacho et al., 2010) and blending of chitosan with PEO (Zivanovic et al 2007) are some of the past research carried out on antimicrobial polymers. Antimicrobial polymers have applications in various aspects of human life such as drug delivery in medical field, tissue engineering, scaffold-synthesis and packaging materials in the food industry (Sionkowska, 2011; Sahoo et al 2010). Antimicrobial activity is an important property of polymers that can find application in all these areas due to their ability to inhibit the growth of bacteria, fungus and other harmful microorganisms such as S. aureus, E. coli and A. niger that has the potential of contaminating the food, causing food spoilage and other infections in the human body (Coma et al 2003; Martinez-Camacho et al 2010).

Various studies have shown the inhibitory capability of chitosan on the growth of several bacteria, yeast and fungus such as *E. coli, L. monocytogenes, S. typhimurium, P. aeruginosa, S. aureus* (Coma et al., 2003; Kim, Min, Kim et al 2011) and S. sclerotium, B. cinerea, M. fructicola, R. stolonifer, A. niger (Li et al 2001; Sebti et al 2006) in culture media. This was supported by Martinez-Camacho et al. (2010), who did the fungistatic inhibition study of chitosan on A. niger which can cause allergy in humans (Klich 2009). Due to these properties, chitosan is commonly utilized in medical products that help with tissue regeneration, wound recovery and to reduce infection by microorganisms in addition to its use in the food industry (Liu et al 2004; Aider 2010). Notwithstanding that, the antimicrobial properties of chitosan are affected by many intrinsic and extrinsic factors, including charge, molecular weight of chitosan, pH, temperature, and ionic strengths of the medium (Kong et al 2010; Martinez-Camacho et al., 2010).

In addition, PCL which is synthetic polyester makes for an interesting study in the preparation of polymer blends. The reason is because PCL is a hydrophobic, neutral charge polyester which possesses good mechanical properties and a low melting point that allows for easy processing (Liu et al 2004; Sarasam et al 2012). Hence, PCL was chosen to blend with chitosan in this research in order to produce an antimicrobial polymer that consists of the properties of both chitosan and PCL. However, past studies of a PCL-chitosan blend towards oral pathogens such as S. mutans and A. actinomycetemcomitans have shown that adding PCL to chitosan can lower the antimicrobial activity of pure chitosan and result in the presence of microbes on the surface of the polymer film (Sarasam et al 2012). This might have been caused by the combination ratio of chitosan and PCL, or the incubation temperature and period as proposed by No et al (2006). Thus, in our study, antimicrobial activity on different concentrations and different molecular weights of chitosan-PCL blends was carried out to determine the polymer having the highest antimicrobial capability.

The current study was undertaken to develop a new polymer material that comprises desirable properties from different polymers which are not displayed in individual polymers. Thus the study focused on the effects of different molecular weight Chitosan-PCL blends on antimicrobial activity.

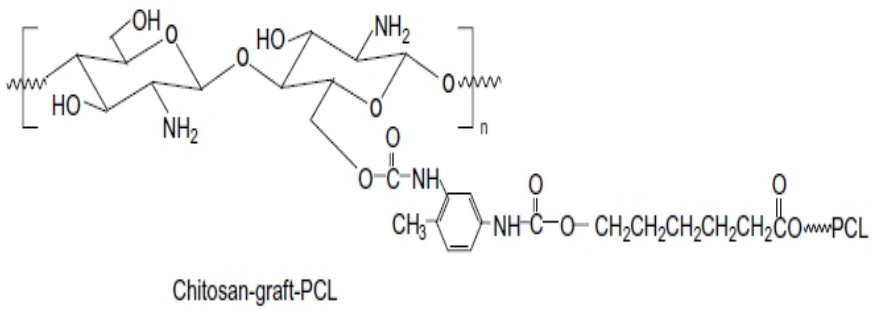

Figure 1: Chemical structure of PCL and chitosan blend

Experimental Study

Materials and Methods

Chitosan, Polycaprolactone (PCL), 1 M acetic acid, glacial acetic acid, distilled water, magnetic stirrer, spatula, polarized microscope, oven, Teflon coated pan, Schott bottle, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, incubator, Mueller Hinton Agar, nutrient broth, L loops, micropipette, micropipette tips, petri dishes, aluminium foil, cotton, boiling tubes, beakers, droppers, test tubes rack, spectrophotometer, disk, parafilm, alcohol lamp, 70% ethanol, forceps.

Preparation of film

The low & medium molecular weight chitosan with PCL blends in proportion of 100:0, 80:20, 75:25, 60:40, 50:50, 20:80 were synthesized. The protocol for the synthesizing of polymer blends synthesize was carried out in the fume hood.

Chitosan was dissolved in 1 M acetic acid while PCL was dissolved in glacial acetic. After that, 4 ml of chitosan solution was removed and poured into a Teflon coated pan to form 100:0 chitosan films. PCL solution was then slowly added (drop by drop) to the remaining chitosan solution to prepare a chitosan-PCL (80:20) blend. The mixture was continuously stirred during the addition of PCL to prevent the formation of clumps. During the dissolving of chitosan and PCL and the mixing of chitosan with PCL, low heat was applied and the fume hood was switched off to prevent evaporation of the polymer solution. Then, after both polymers were completely mixed, it was poured into a teflon coated pan to form film and left to cool at room temperature in the fume hood.

Antimicrobial Activity Assay

Bacterial strains and Inoculum preparation

In this study the bacterial strains, *S.auerus* was used for screening antibacterial activity of chitosan-PCL blend. All the cultures were

maintained in the nutrient agar slant at 2^0 - 8^0C. The bacterial strain was inoculated on nutrient broth and incubated overnight at 37^0C. The *S.auerus* culture was maintained at (Optical Density) 0.004 OD.

Determination of antibacterial activity by disc diffusion and colony count method

According to Fernandes et al (2008), Tareq et al (2013), chitosan possesses good antimicrobial activity against gram-positive and gram-negative bacteria. The antimicrobial activity of chitosan is based on the cell surface of the bacteria. Thus, in this research, we tested out the antimicrobial activity of chitosan and chitosan-PCL blend solution with gram-positive bacteria (*S.auerus*). The antibacterial property of different molecular weight chitosan blended with PCL at different concentration ratio such as 100:0, 80:20, 75:25, 60:40, 50:50 and 20:80 were determined by using disc diffusion method. Disc-assay was found to be a simple, cheap and reproducible practical method (Maidment et al 2006). A suspension of each sample tested micro-organism diluted was spread on a solid agar medium in Petri dishes (Mueller-Hinton agar). The sterile discs dipped in Chitosan and Chitosan-PCL blend solution at different concentrations were placed in the inoculated plates. They were then incubated at 37^0C for 24hrs. The zone of inhibition was measured in diameter (mm). The number of colonies was counted by comparing the test plates with control and data was recorded

S. aureus ATCC 25922 was used to test for the antimicrobial activity of low and medium molecular weight chitosan with PCL blend. The bacteria was first cultured in Muller Hinton Broth (MHB) and incubated at 37^oC for 24 hours. Then, active culture was inoculated into 10 ml of MNB and incubated at 37^oC for 15 hours. The culture was diluted with MHB/PDB to obtain bacterial count of 5-10 x 10^5 CFU/ ml. Then, the films were submerged into culture tubes containing 9 ml sterile phosphate buffer (0.5 M, pH 7.08) inoculated with 1 ml of ca. 106 CFU/ml *S. aureus*, vortexed and incubated for 6 hours at 25^oC. The survival of *S. aureus* was determined by using pour plate method.

Results & Discussion
Crystallinity

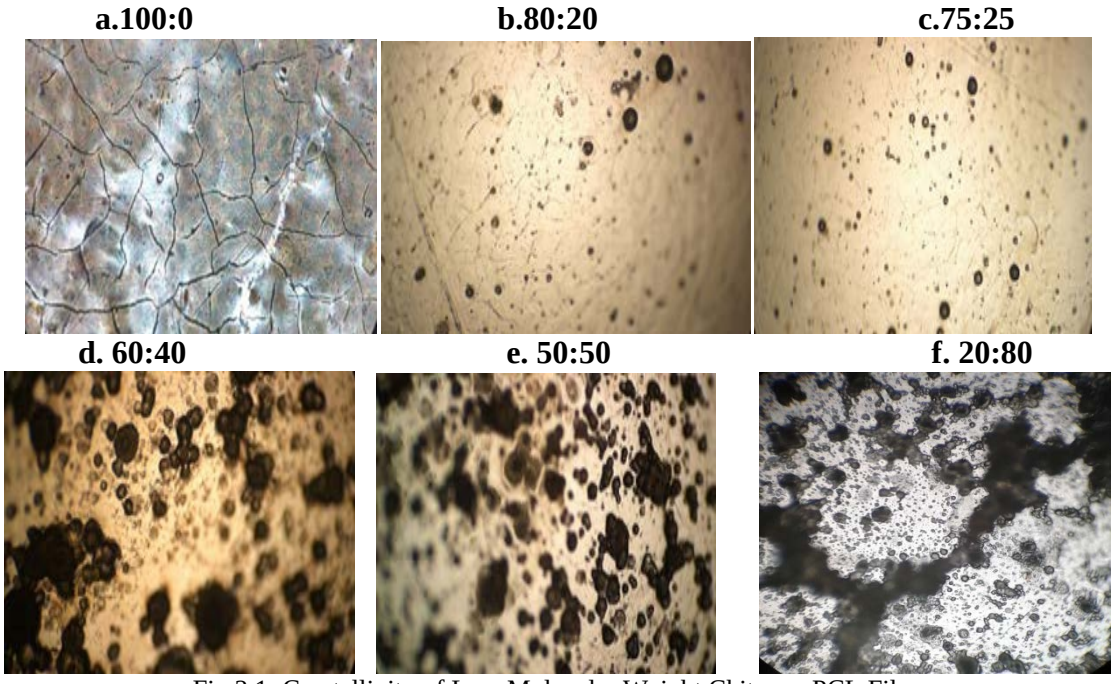

Fig 2.1: Crystallinity of Low Molecular Weight Chitosan-PCL Film

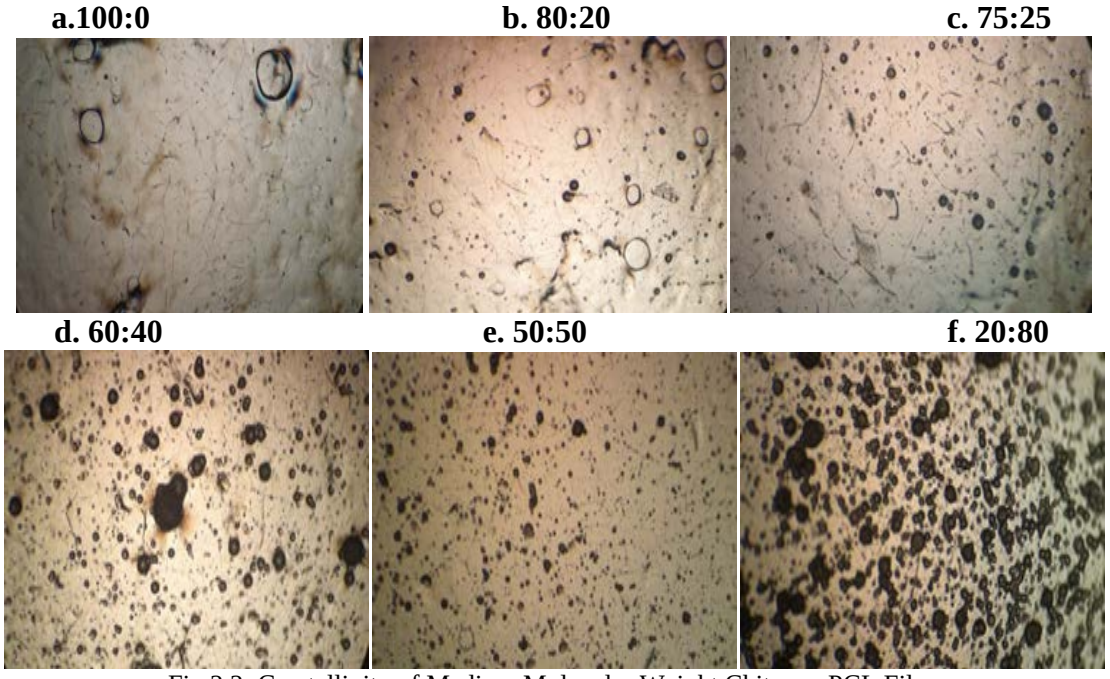

Fig 2.2: Crystallinity of Medium Molecular Weight Chitosan-PCL Film

Antimicrobial Activity Assay

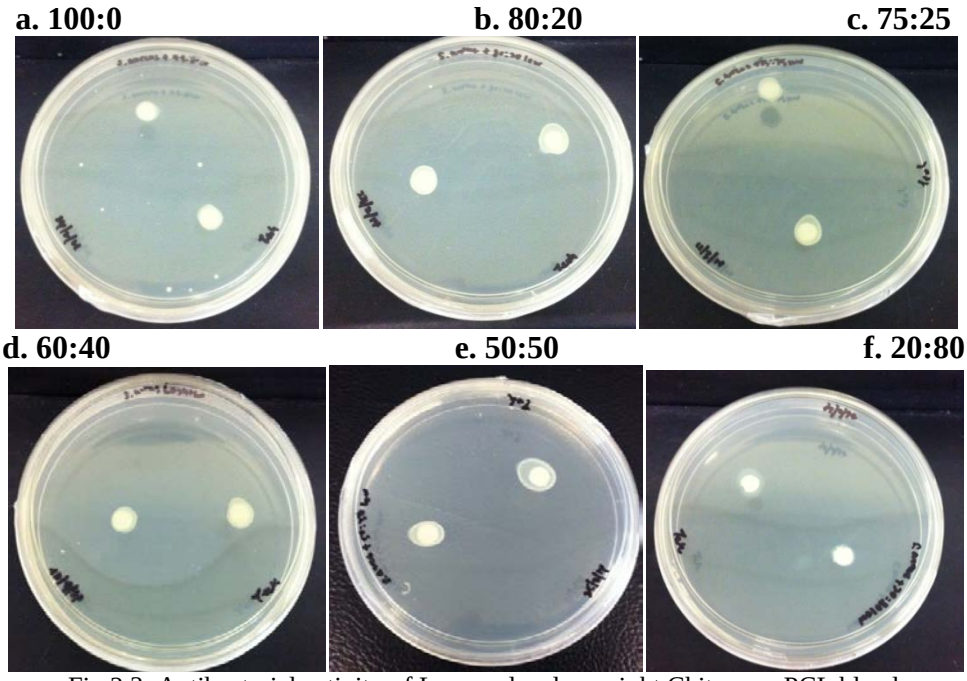

Fig 2.3: Antibacterial activity of Low molecular weight Chitosan - PCL blend against S.aureus

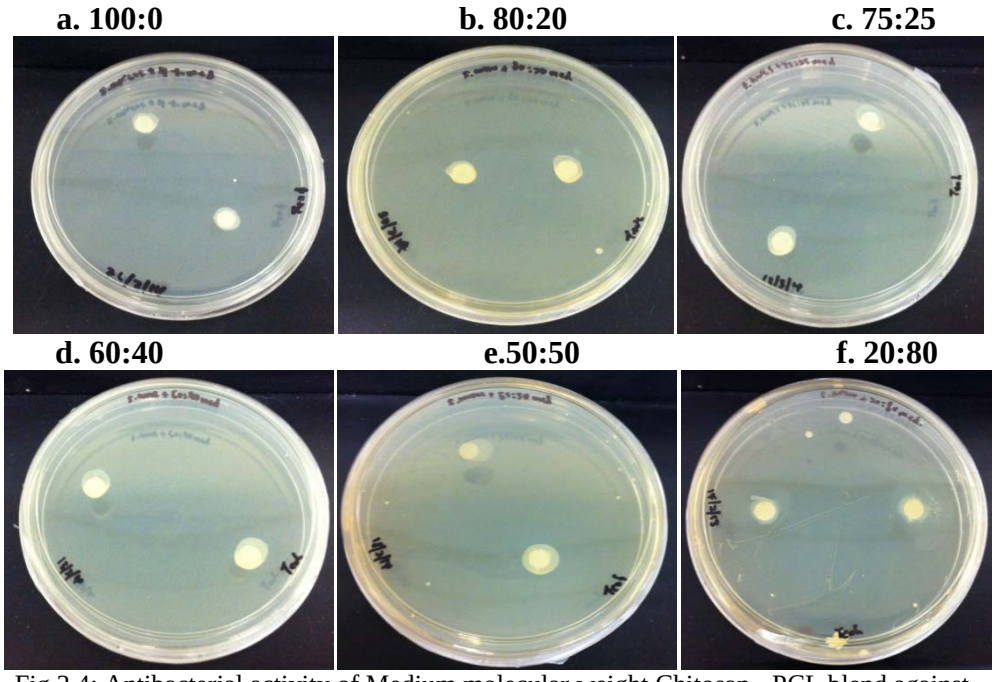

Fig 2.4: Antibacterial activity of Medium molecular weight Chitosan - PCL blend against S.aureus

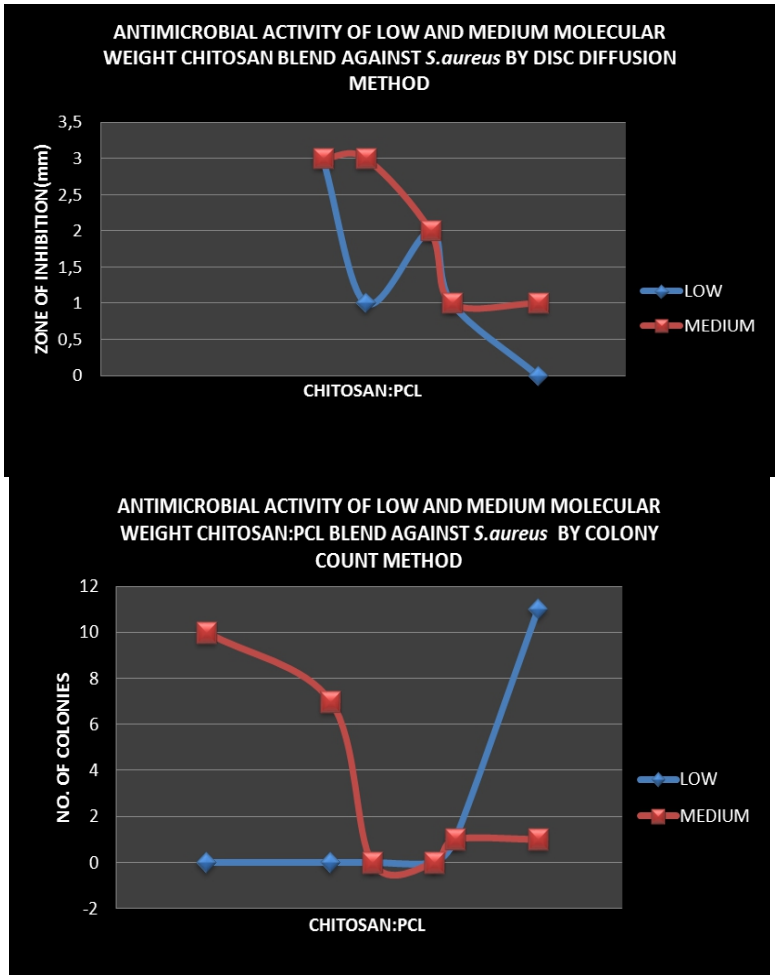

Figure 2.5: Graph of Low And Medium Molecular Weight Chitosan-PCL Blends against S. aureus.

The results showed that some films were yellowish in colour. This was due to the colour of chitosan present in the film. Thus, when the concentration of chitosan was reduced, the yellowish colour of the chitosan-PCL film (both low and medium molecular weight) was lighter and the films became transparent.

Crystallinity

Polymer crystallization has a significant effect on the structure and physical properties of the films. The crystalline structure was viewed under a polarized microscope and the results were shown. Fig. 2.1 and 2.2 illustrates the shape and distribution of the crystals within chitosan/PCl films. From the findings, we could observe that when the concentration of chitosan was high, there were not many pits and holes present on the film. However, when the

concentration of chitosan decreased, we could see more holes and pits there. The reason is that as the concentration of PCL increases, the homogeneity of chitosan is reduced, crystals form less in the film and the number of pits and holes increase.

Antimicrobial Activity Assay

Blending two polymers is an approach to develop new biomaterials exhibiting combinations of properties that could not be obtained by individual polymers1. Blends made from synthetic and natural polymers can imbibe the wide range of physicochemical properties and processing techniques of synthetic polymers as well as the biocompatibility and biological interactions of natural polymers.

According to Fernandes et al (2008), Tareq et al (2013), chitosan possesses good antimicrobial activity against bacteria, both gram-positive and gram-negative bacteria. The antimicrobial activity of chitosan is based on the cell surface of the bacteria. In this research, chitosan and Chitosan-PCL blends were prepared and tested for their antimicrobial effectiveness on Gram positive (*S. aureus* ATCC 25923) bacteria. From the results gathered, it was found that antimicrobial effectiveness varied in both low and medium molecular weight PCL-chitosan blends.

In low molecular weight chitosan-PCL blend, based on *S.aureus* colony count, antimicrobial activity was low for 100% chitosan but the antimicrobial activity rose rapidly in 80:20 chitosan-PCL blends, showing that the addition of PCL increased antimicrobial activity. Low molecular weight Chitosan-PCL blends of 75:25, 60:40 and 50:50 showed 100% inhibition. This showed that the effectiveness of antimicrobial activity on *S.aureus* increased with increase in concentration of PCL. When comparing this data with the zone of inhibition, the results were similar as the zone of inhibition of *S.aureus* increased with the chitosan-PCL blends from 100% to 80:20, 75:25, 60:40 and 50:50, with the largest zone of inhibition in 50:50 chitosan-PCL ratios. This result further confirmed that at low molecular weight, the antimicrobial effectiveness of chitosan-PCL blends increases with the addition of PCL up to 50%.

For medium molecular weight chitosan-PCL blends, antimicrobial activity on *S. aureus* based on colony count, number of colonies increased in 80:20 chitosan-PCL ratio and at the ratio of 75:25 and 60:40, no *S.aureus* colonies were found, depicting 100% inhibition. However, the addition of PCL beyond this point did not show antimicrobial activity as *S. aureus* colonies were present at chitosan-PCL ratio of 50:50 and 20:80. The results of this study was further confirmed with the measurement of the zone of inhibition where the zone of inhibition increased from 100% chitosan to 80:20, 75:25 and 60:40 ratio.

This showed that the addition of PCL to medium molecular weight chitosan did increase antimicrobial effectiveness on Gram positive bacteria until particular ratio of PCL, after that the effect was decreased when the concentration of PCL in the blends increased (Figure 2.4). This result was in contrast to the antimicrobial testing of low molecular weight chitosan-PCL blends. Low molecular weight chitosan with PCL blends showed better inhibition when the concentration of PCL was higher (Figure 2.3).

The results in this study is of great significance as Gram positive bacteria such as *S.aureus* are usually pathogenic and exhibit great antibiotic resistance in clinical applications. Therefore, using the specific chitosan-PCL ratio in blends for drug delivery as well as in tissue engineering scaffolds, unwanted growth of pathogens could be curbed. These findings would also be useful in determining the application of chitosan-PCL blends in food packaging applications as antimicrobial activity is necessary to keep unwanted food pathogens at bay.

Different studies have shown different results in antimicrobial testing on chitosan with different types of bacteria. There is a contradiction between our result of antimicrobial testing on 100:0 chitosan with the result reported by Tareq et al (2013) in which their chemically deacetylated chitosan from shrimp shell demonstrated better inhibition against gram-negative bacteria (*E. coli*) than gram-positive bacteria (*S. aureus*). However, in their study, they did not mention the molecular weight of chitosan used. Thus, we can suggest that the different set of results might be due to the different molecular weight of chitosan used. Furthermore, it could also be due to other reasons such as differences in the pH and temperature of media, age of the bacteria, and concentration of the chitosan used in the research (Kong et al 2010).

Conclusion

The addition of PCL to medium molecular weight chitosan enhanced the antimicrobial effectiveness on *S.aureus* until particular ratio of PCL, after that the effect was decreased when the concentration of PCL in the blends increased. This result was in contrast to the antimicrobial testing of low olecular weight chitosan-PCL blends. Low molecular weight chitosan with PCL blends showed better inhibition when the concentration of PCL was higher. Therefore, we can conclude that 60:40, 75:25, 80:20 and 100:0 medium molecular weight chitosan-PCL blends have better antimicrobial property and are desirable polymer materials for industrial application.

Acknowledgement

We would like to acknowledge the Research Management Centre of Nilai University in giving permission & facilitating this research. We are

also grateful to Mr Teoh Chen Yik; final year BSc (Hons) Biotechnology student in assisting our experiment & Mr Manirao Ramachandra Rao of Aircraft Maintenance Engineering, Nilai University for helping in statistical analysis.

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