CHAPTER IV
RESULTS AND DISCUSSION

4.1 Modified Chitosan

In this part of study, chitosans which are medium molecular weight and low molecular weight have been modified by the cationic chemical (GTMAC) by various mole ratios of chitosan to GTMAC.

4.1.1 NMR results

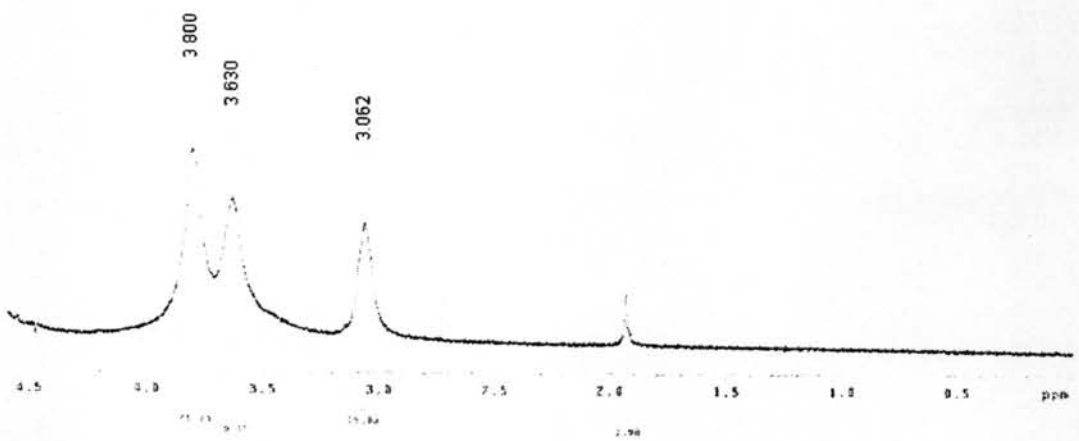


Figure 4.1 ^{1}H-NMR spectrum of low molecular weight chitosan.

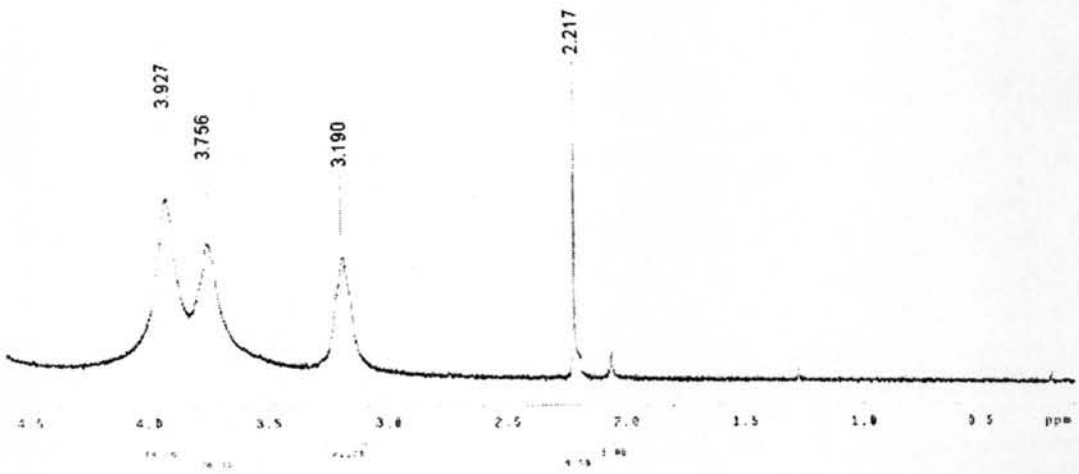


Figure 4.2 ^{1}H-NMR spectrum of deacetylated low molecular weight chitosan.

Figure 4.1 shows the ^{1}H-NMR spectrum of commercial chitosan when compare to Figure 4.2 which is the spectrum of deacetylated chitosan one can see the changing of peaks. From calculated area under ^{1}H-NMR peak, one can find the percent deacetylation of chitosan. Further deacetylation can reduced the acetyl group from about 13% to 1%. This shows that one can obtain sites of reaction of the ammonium group with GTMAC and thus enhance the water solubility of chitosan.

4.1.2 FTIR result

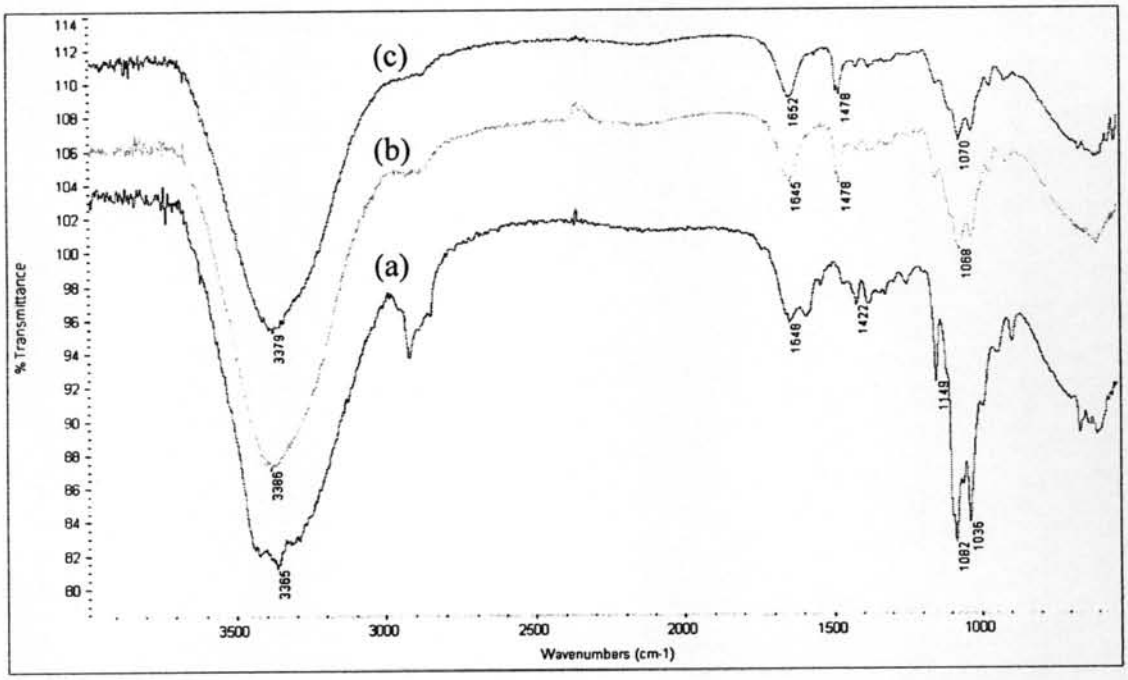


Figure 4.3 Compared FTIR spectra of Modified Medium molecular weight chitosan (a) Medium molecular weight chitosan, (b) Modified chitosan ratio 1:1, (c) Modified chitosan ratio1:1.5

From the compared FTIR spectrum (Figure 4.3), the peak at 1595 cm^{-1}, N-H bending of the primary ammine disappeared when chitosan has been modified due to the grafting of GTMAC which changes the primary ammine to the secondary amine. The peak at 1480 cm^{-1}, the C-H bending of trimethylammonium group, shows the evidence of the introduction of the quaternary ammonium group onto the chitosan backbone.

4.2 Charge density of modified chitosan

Charge density of cationic chitosan was verified from the titration with Particle Charge Detector (MÜtek). Using opposite charge of solution, PVSK to titrate with cationic chitosan samples. The charge density of modified low molecular weight chitosan is 6.5 meq/g. while modified medium molecular weight chitosan is 4.5 meq/g. It means that, the modified low molecular weight has higher site of cationic than modified medium molecular weight so the ability of attaching to the bacterial cell wall is higher in modified low molecular weight than modified medium molecular weight. This is the result from the molecular weight of chitosan, the low molecular weight chitosan can grafted more cationic chemical than medium molecular weight chitosan due to the distance between each site of reaction can effect each other by repulsion force, so the modified low molecular weight chitosan has less site repulsion to each other than modified medium molecular weight chitosan.

4.3 Water soluble of modified chitosans

A variety chitosan to GTMAC mole ratios has been investigated for water solubility. The results obtained are given in Table 4.1

Table 4.1 The water soluble ability of modified chitosan

Chitosan to GTMAC ratio	Water solubility
1:1	insoluble
1:1.5	insoluble
1:4	soluble

At the low ratios of chitosan to GTMAC (1.1 and 1.5) cannot be water soluble because of not sufficient in the ammonium group on the chitosan base polymer.

4.4 Adsorption of modified chitosan on pulp

In this part the adsorption of modified chitosans on pulp were studied. The modified chitosan that used in this part must have the mole ratio of chitosan to GTMAC equal to 1:4. Because of the previous result in part 4.3 shows that the modified chitosans which have the mole ratio others than 1:4 were not soluble in water. These modified chitosans were adsorbed on pulp at temperature of 30°C for 1 hour.

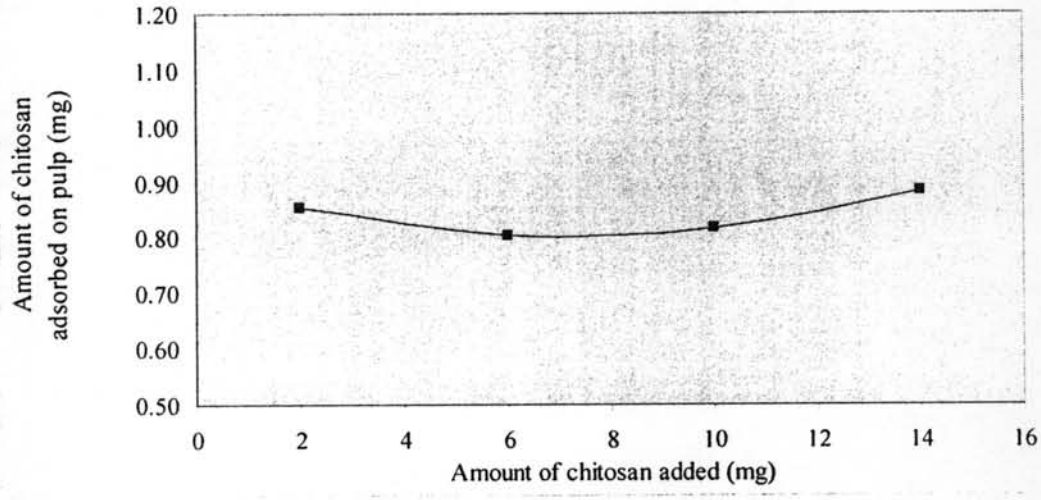


Figure 4.4 The amount of modified low molecular weight chitosan adsorbed on pulp.

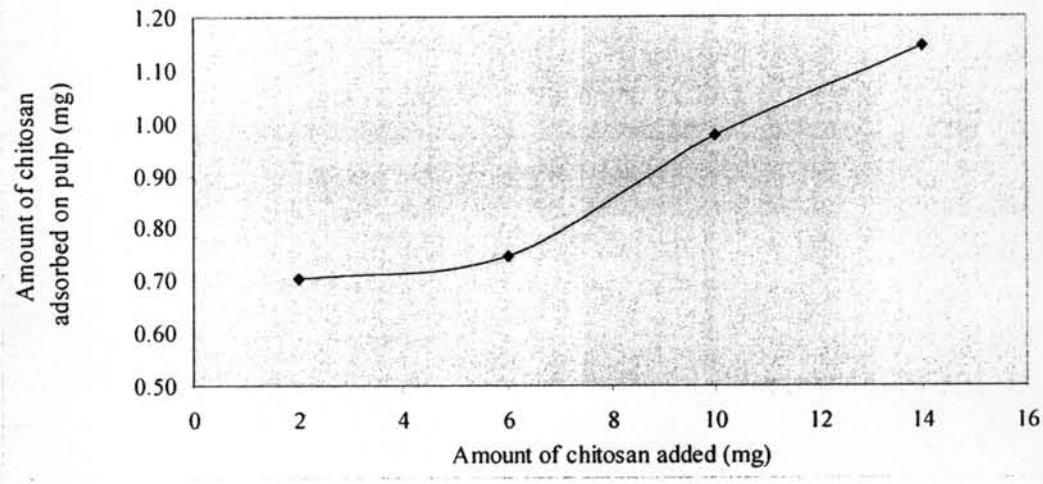


Figure 4.5 The amount of modified medium molecular weight chitosan adsorbed on pulp.

Figure 4.4 shows that the adsorption amount of modified low molecular weight chitosan on pulp is around 0.8-0.9 mg/ g fiber even though the added amount of modified chitosan was varied from 2-14 mg. The adsorption amount of modified chitosan on pulp is relatively low and it is saturated around 0.8 mg/ g fiber for the

modified low molecular weight chitosan. For the modified medium molecular weight chitosan (Figure 4.5) the adsorption amount increases with the higher amount of chitosan added to the system. According to the charge density of modified chitosans, the higher charge density can obstruct the modified chitosan from being adsorbed on pulp. The charge density related to molecular weight of modified chitosans (modified low molecular weight chitosan has higher charge density than modified medium molecular weight chitosan).

As a result of the increasing charge density of modified chitosan, the adsorption was so strong that the cellulosic fiber surfaces, reversal of the sign of the surface charge occurs. This overcompensated charge of cellulose surfaces led cellulose particles to restabilization.

4.5 Mechanical and Optical improvement of paper

Both modified low molecular weight and medium molecular weight chitosans were used to form complexes with an anionic polymer (Carboxymethyl cellulose, CMC). The resulting complexes were designated as MLC and MMC for the modified low molecular weight chitosan and modified medium molecular weight chitosan, respectively

In this section the effects of five variables were studied namely effect of washing stage, drying method, molecular weight of chitosan, molecular weight of CMC and modified chitosan to CMC ratio.

The mechanical property of paper was determined using the tensile index of the paper whereas the optical property was examined via the paper brightness in this study.

4.5.1 Effect of washing stage

In the first stage, chitosan was adsorbed on pulp for 1 hour followed by adsorption of CMC as second stage. To eliminate the effect of excess chitosan in the solution, a washing stage was proposed. A washing stage represents the stage that

chitosan adsorbed pulp was rinsed by deionized water before adsorbs CMC in second stage.

4.5.1.1 Mechanical property of paper

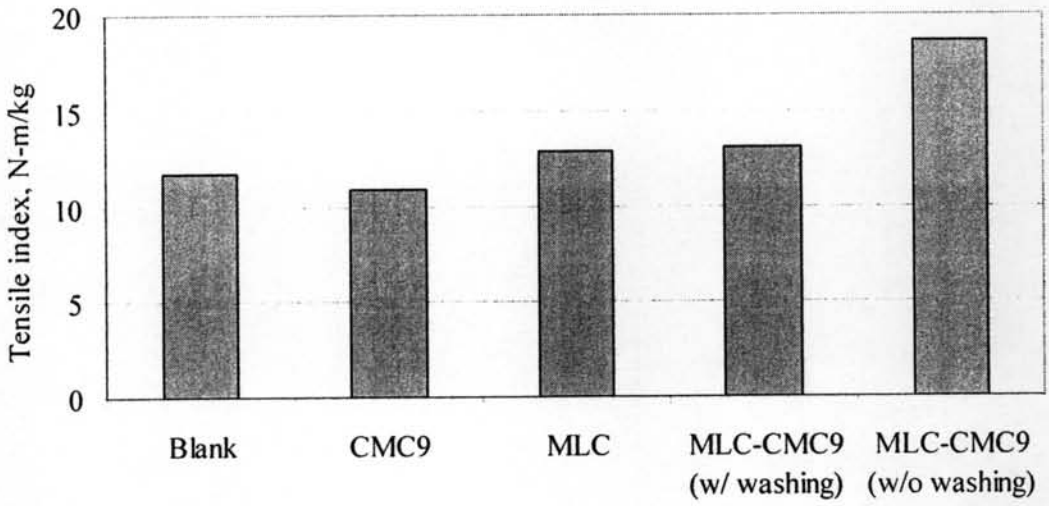


Figure 4.6 Comparison of tensile index of the paper in the presence and absence of washing stage.

As the chart shown the tensile index of various conditions, for the CMC9 and chitosan have no washing stage because only one polymer has been adsorb on pulp. CMC9 reduced the tensile index of the paper, due to the same negative charge of CMC9 and fiber surface create the repulsion force between fiber surfaces. In other hand the MLC, MLC-CMC9 complex increase the tensile index of the paper.

The adsorption of MLC which is cationic polymer bind the fiber surface together and create more strong in bonding between fiber. When washing stage between chitosan and CMC has been applied the increasing in tensile index of the fiber is almost the same as MLC, the result due to the low adsorption ability of MLC on pulp.

Method of no washing stage has applied to compare the washing stage of the adsorption. Significant improvement of tensile index has found without washing stage. As expected, when the rest MLC has not been remove from the system the complex of MLC- CMC can be form to reduce the charge of MLC which can synergize MLC to attach more on the fiber surface and increase the bonding between fiber surfaces.

4.5.1.2 Optical property of paper

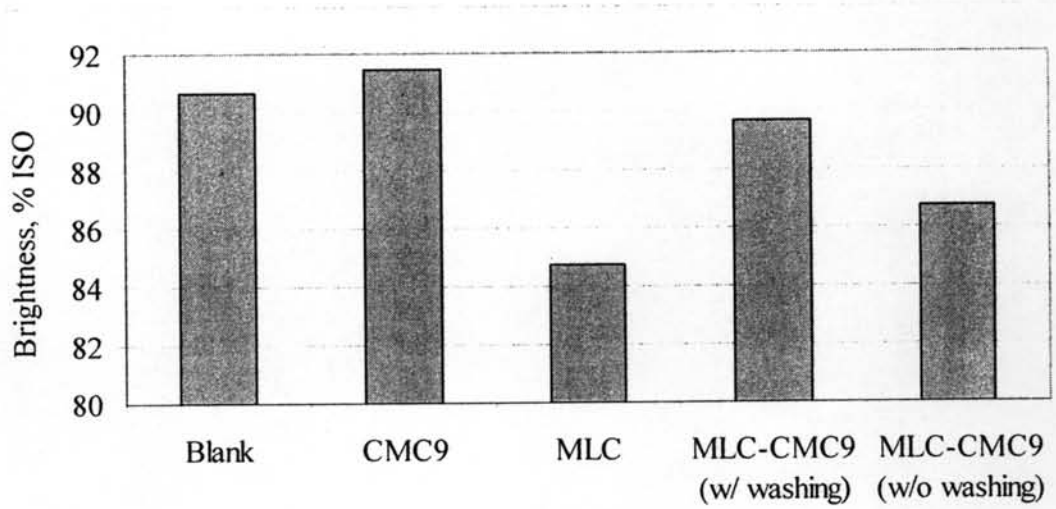


Figure 4.7 Brightness of paper compare with washing stage and without washing stage of adsorption.

The results suggest that the MLC has a negative effect on the brightness of paper, the more adsorption of chitosan, the less brightness of paper will appear. Chitosan polymer possesses its color, when adsorbed on the fiber, it will cause the reduction in brightness. In addition, the complex of chitosan-CMC eliminated some effect of chitosan color. CMC can increase the brightness of paper because of white color itself. From the results the MLC-CMC9 with washing stage has higher brightness than MLC-CMC 9 without washing stage indicating that the amount of chitosan adsorbed on fiber for the former is lower.

4.5.2 Effect of drying method

Drying process is considered as the last process of paper making industry. For the handsheet, it is however normally dried at standard room conditions (50%RH, 23°C) for 24 hours. This study compared the effects of drying method between normal air dry condition and instant dry at 110°C for 30 minutes.

4.5.2.1 Mechanical property of paper

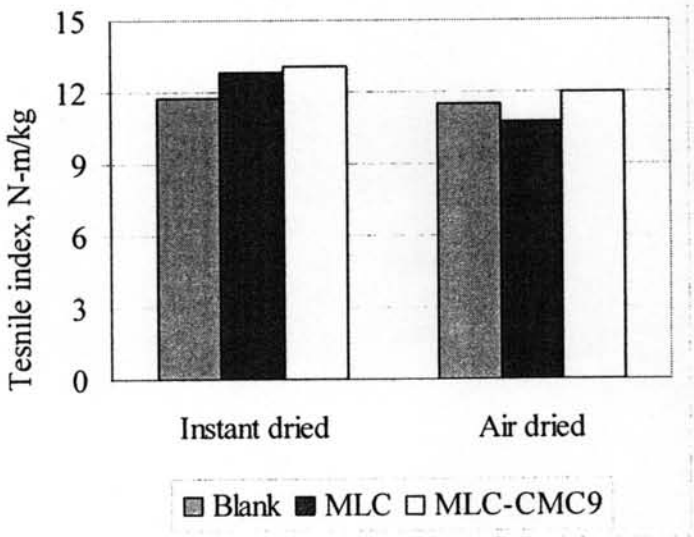


Figure 4.8 Comparison of tensile index obtained by instant dried and air dried paper.

Figure 4.8 shows comparison of tensile index obtained by instant dried and air dried papers. The instant dried papers had more profound improvement of tensile index than normal air dried papers. When water has been removed from the matrix of fibers, the direct fiber-fiber bonds have been created. The fibers obtain higher hydrogen bonding between surface than the normal air dried which have major bond from the hydrogen bonding between fiber surface and water molecule

4.5.2.2 Optical property of paper

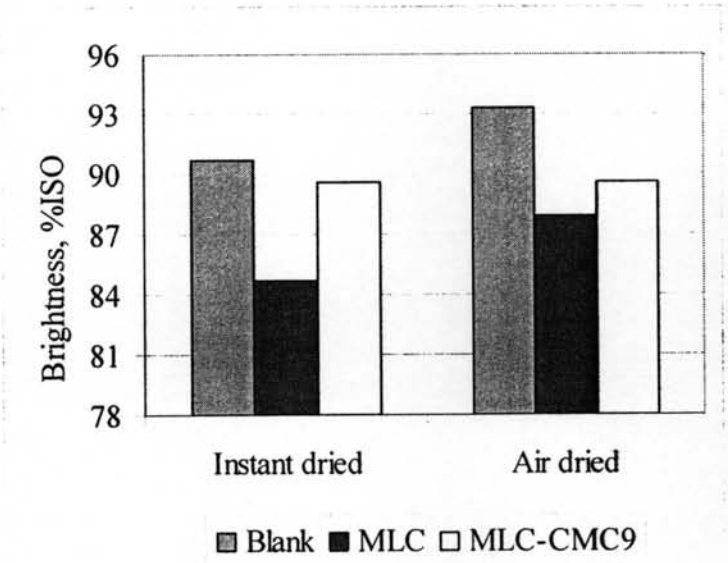


Figure 4.9 Brightness of paper for instant dried compare to air dried papers.

The trends of the brightness when using instant dried is less than normal air dried. Using heat for instant dried can removed water between fibers the color of paper was decreased by heating.

In conclusion, the instant dried method gives the higher improvement in mechanical property than air dried method however; there has drawback of using heat to dry the paper which is the reduction in brightness of paper.

4.5.3 Effect of modified chitosan molecular weight

Modified chitosan which has the different molecular weight had been study. Forming complex with CMC to synergist the adsorption of modified chitosan on pulp fiber has been investigated.

4.5.3.1 Mechanical properties of paper

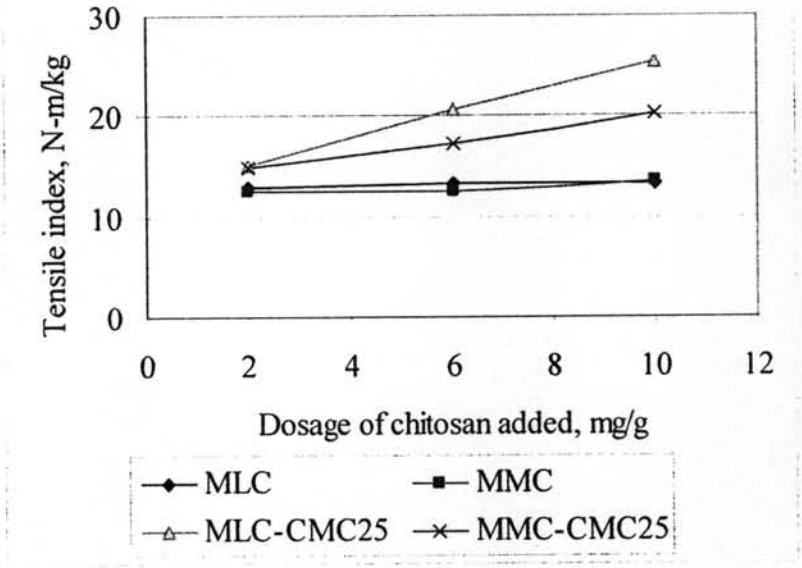


Figure 4.10 Tensile index of modified chitosan by varying molecular weight of chitosan.

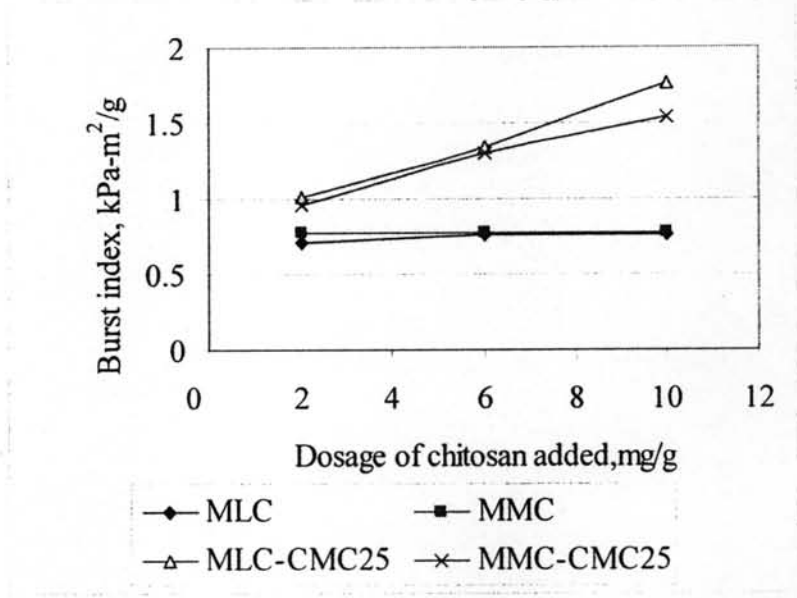


Figure 4.11 Burst index of modified chitosan by varying molecular weight of chitosan

The mechanical properties both tensile index and tear index give the same trends. The different between these two indexes is the character of rupture in the test but both can indicate the same result. Both graph show the index versus the different amount of modified chitosan adding in the adsorption process.

Comparing the MLC to MMC, although adding high amount of MLC and MMC in the system the result comes out constantly in tensile index and burst index. These are the consequent of the low adsorption of chitosan on pulp. Also, both of MLC and MMC, give the nearly the same result as each other.

Complex of chitosan-CMC can improved in both tensile and burst index, and the trends are increase when having more addition amount of MLC and MMC. MLC complex has higher effective than MMC complex from the binding ability of MLC which has higher in charge density create higher in mechanical properties of paper.

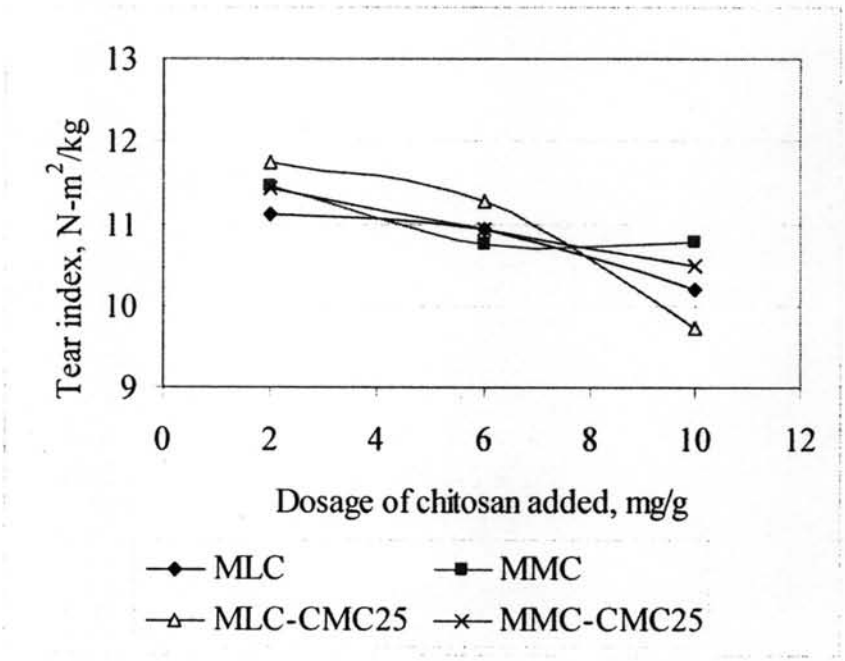


Figure 4.12 Tear index of modified chitosan by varying molecular weight of chitosan.

From the Figure 4.12, tear index of all the combining conditions give the reverse trend from tensile and burst index. Normally, when the tensile index has been improved the tear index has been reduced. From the impact of various factors such as in different combination of chemical system will give the different in tear index trend. The studying of this reverse trend is over scope of this research work. The results only show the decreasing trend of adding chitosan in the system.

4.5.3.2 Optical property of paper

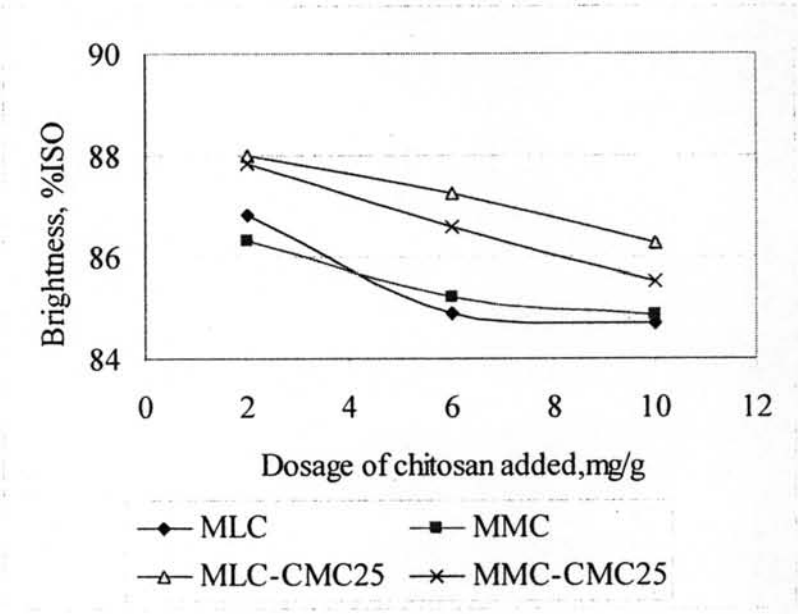


Figure 4.13 Brightness of paper by varying in molecular weight of chitosan.

Again, adsorption of chitosan on pulp results in brightness decreasing. And CMC can eliminate some effect by enhance more brightness of paper. But still less than blank sample which the brightness is 90.66 %ISO.

4.5.4 Effect of molecular weight of CMC

There are 2 types of CMC used in this study which are CMC which has 90,000 in molecular weight and 250,000 in molecular weight. Further label will define CMC which has MW. 90,000 and MW. 250,000 as CMC9 and CMC25 respectively.

4.5.4.1 Mechanical properties of paper

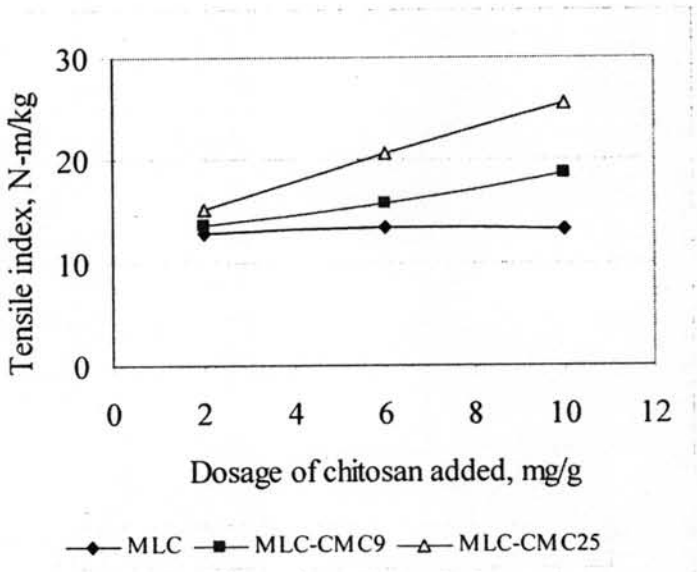


Figure 4.14 Tensile index of modified chitosan by varying molecular weight of CMC.

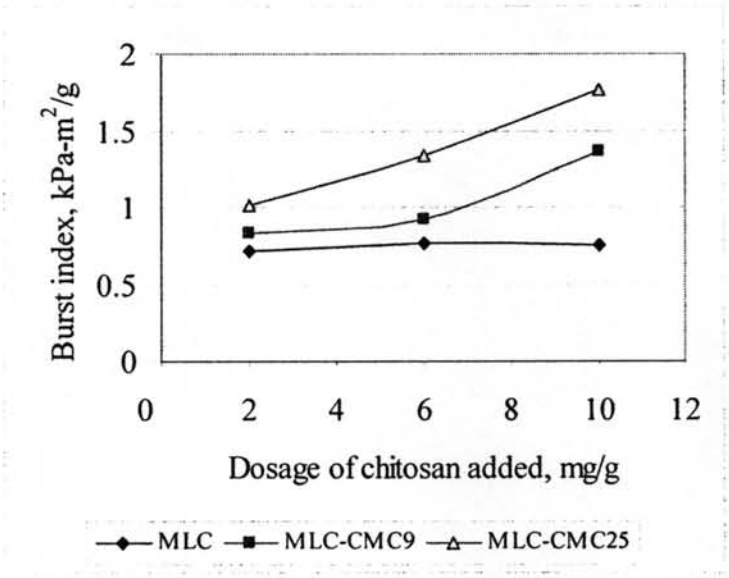


Figure 4.15 Burst index of modified chitosan by varying molecular weight of CMC.

Tensile index and burst index give the same trend which increases in tensile and burst index with higher dosage of chitosan added in the system. The complex of MLC-CMC25 give higher improves efficiency of mechanical properties. This is result from the longer chain in CMC25 can have the possibility of linkage between fiber interface more than shorter chain of CMC9. Even though giving the lower improvement, the CMC9 still can increase in tensile index and burst index.

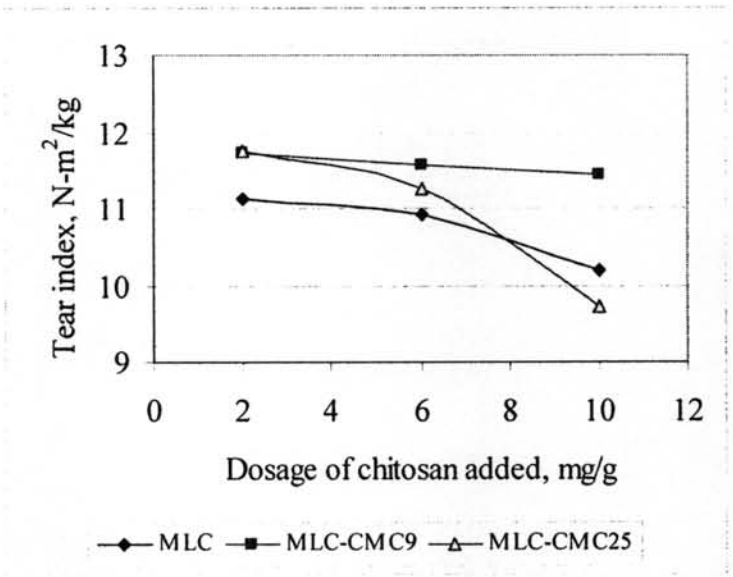


Figure 4.16 Tear index of modified chitosan by varying molecular weight of CMC.

Again, the same explanation in the section of 4.5.3.1, the trend of tear index when using different in CMC molecular weight gave the reverse trend as in tensile and burst index.

4.5.4.2 Optical property of paper

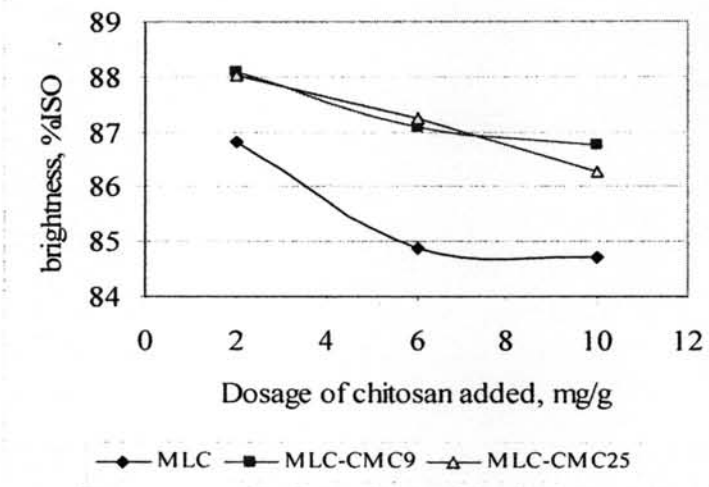


Figure 4.17 Brightness of paper by varying the molecular weight of CMC.

From Figure 4.17 when CMC was added in the system, the brightness of paper is higher compare to the system without CMC. The CMC9 and CMC25 give the same result in brightness.

4.5.5 Effect of CMC to modified chitosan charge ratios

In this section the different charge ratio of CMC to chitosan has been studied varying from 0.5, 1 to 1.5 ratios.

4.5.5.1 Mechanical properties of paper

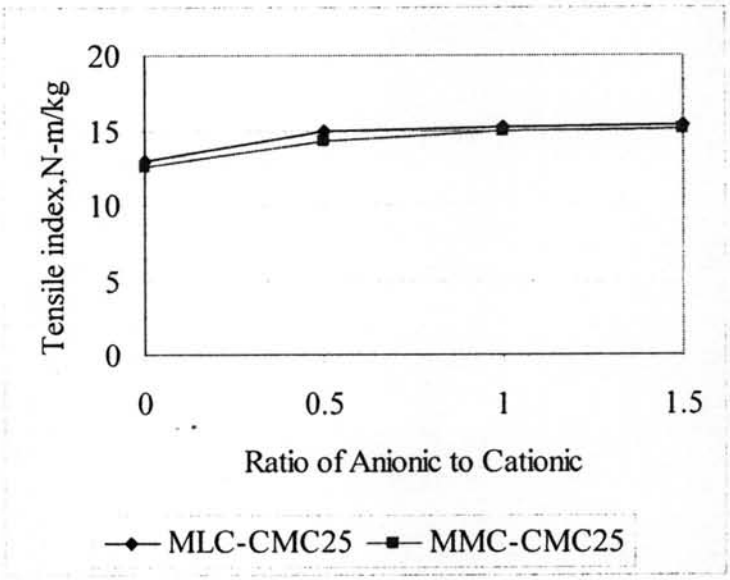


Figure 4.18 Tensile index of complex chitosan-CMC25.

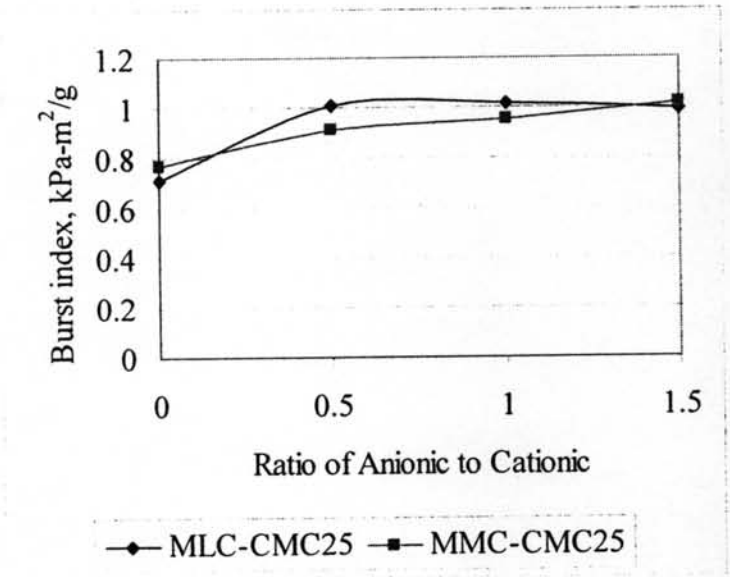


Figure 4.19 Burst index of complex chitosan-CMC25.

From both graph of tensile index (Figure 4.18) and burst index (Figure 4.19), when higher ratio of Anionic CMC applied the mechanical properties enhanced until level off at equal ratio. This means the extra amount of CMC cannot bind with the modified chitosan to increase the mechanical properties.

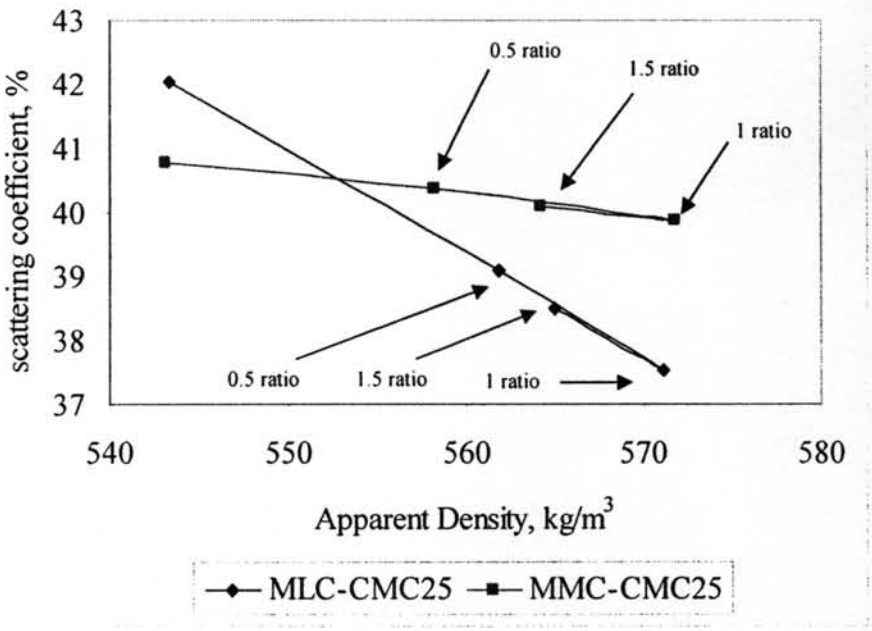


Figure 4.20 The graph of apparent density versus scattering coefficient.

As the result shown in Figure 4.20 the apparent density which is calculate from the basis weight of each paper divide by thickness of each paper. Adding the extra charge of CMC to the chitosan-CMC complex will cause increased in apparent density to some degree before starting perform oppositely. First of all, CMC which has been added to the system can full-fill the pore between fiber cause the higher in apparent density but when the ratio of CMC reach 1.5 the extra charge induce the repulsion force between fiber. Finally, the apparent density of the paper perform invest trend.

4.5.5.2 Optical property of paper

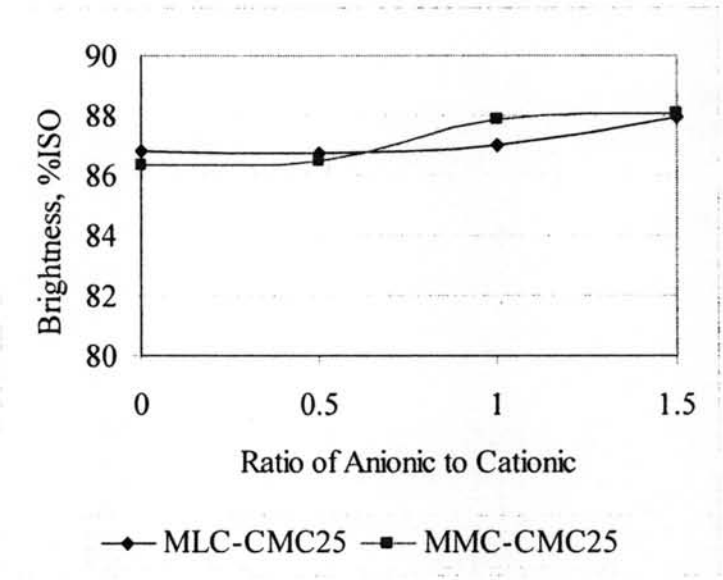


Figure 4.21 Brightness of paper with different CMC ratios.

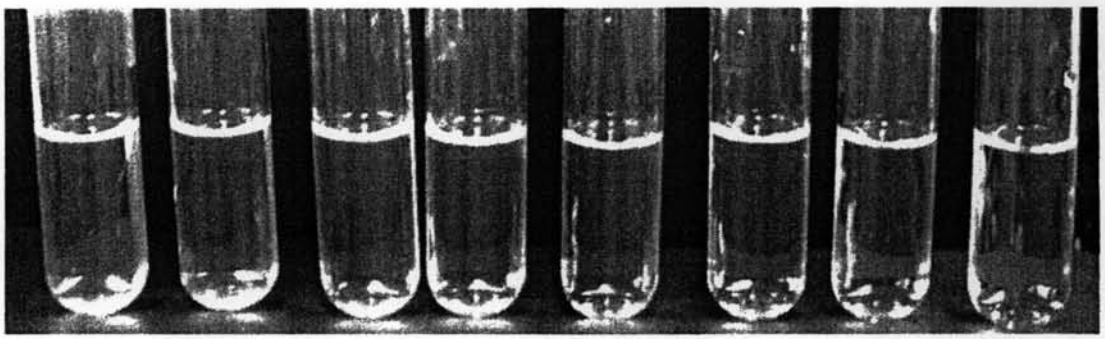
The increasing in brightness when higher ratio of CMC added into the system resulted from the higher CMC that can adsorb on the fiber.

4.6 Antimicrobial results

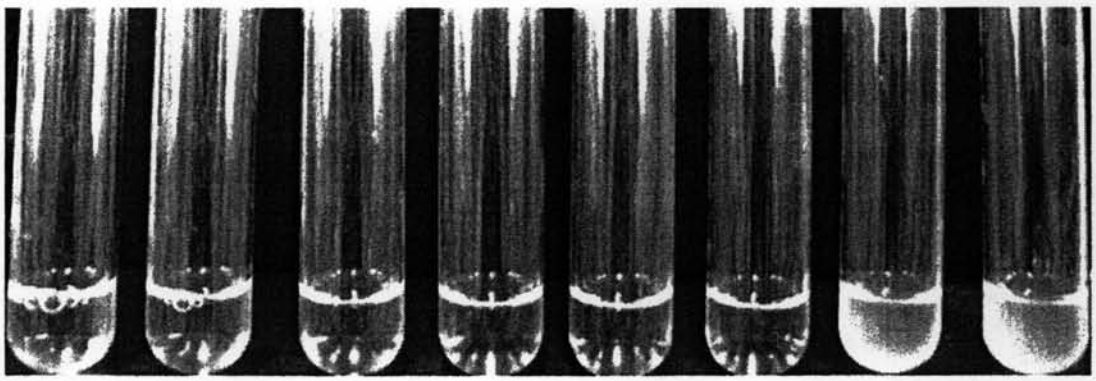
Property of antimicrobial has been study from 2 types of tests, which are Minimum Inhibitory Concentration (MIC) and the Biocide efficiency tests with shaking method.

4.6.1 Minimum Inhibitory Concentration (MIC)

The MIC of MLC and MMC have been found in this test, to determine biocide efficiency of modified chitosan. Because of using CMC in the previous part to form complex, the complex chitosan-CMC also has been study in this test.



(a) Controlled sample



(b) Modified sample

Figure 4.22 MIC test of MLC compare controlled sample(a) with modified sample (b)

Figure 4.22 shows the antimicrobial activity of modified chitosan after 18 hours incubated the turbid solution indicate the alive of bacterial (*E.Coli*) in the solution. Comparing with controlled sample, which have no bacterial in the solution, can see the different in last 2 tube samples.

Also the activities of unmodified chitosan have been test with dissolve in acetic condition because the chitosan cannot water soluble.

Table 4.2 MIC of chitosan samples

Conditions	MIC (ppm)
Low MW. Chitosan (acid condition)	62.5
Medium MW. Chitosan (acid condition)	62.5
Acid solution (for being the control of chitosan in acid condition)	62.5
MLC	31.25
MMC	62.5
MLC-CMC9	125
MMC-CMC9	125

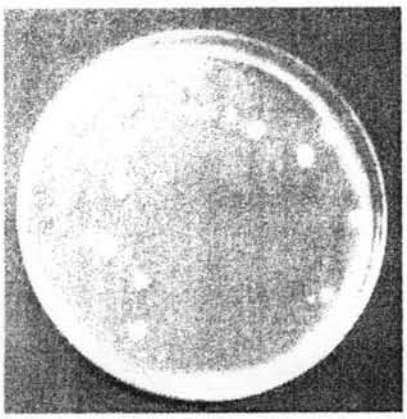
Both unmodified low molecular weight and medium molecular weight chitosan give MIC at 62.5 ppm. Unmodified chitosans have been dissolve in acid solution, so the acid solution has been tested MIC to eliminate the biocide activity of acid solution. The results found that the unmodified chitosan and the acid solution has the same MIC. This shows activity of unmodified chitosans are come from the acid solution.

Modified low molecular weight chitosan has the lower MIC than modified medium molecular weight chitosan. According to the higher in charge density of modified low molecular weight chitosan creates more site of attaching to the bacterial.

Forming complex of chitosan-CMC will increase the MIC values. As the theoretical, if the complex forms the charge of modified chitosan will reduce which cause decreasing in antimicrobial activity. The positive charge is important to the antimicrobial activity.

4.6.2 Biocide efficiency test with shaking method

(a) Controlled sample



(b) Modified low molecular weight chitosan

Figure 4.23 Agar plates of solution from blank sample(a) and modified low molecular weight chitosan solutions (b).

Biocide activity of modified chitosan has been proven by shaking method. The agar plates of blank sample shows the huge amount of bacterial colony in the plate compare to the modified chitosan solution sample. This indicates the biocide activity of modified chitosan on pulp.

Table 4.3 Colony forming unit (CFU) of bacteria

Sample	colony of bacterial, CFU	%bacteria reduction
Controlled sample	410000	-
MLC	31500	92.3%
MLC-CMC9	43500	89.4%
MLC-CMC25	31500	92.3%
MLC-CMC9 (3steps)	19500	95.2%
MMC	18000	95.6%
MMC-CMC9	30000	92.7%
MMC-CMC25	32500	92.1%
MMC-CMC9 (3steps)	0	100.0%

The amount of bacterial colony has been reduced in the range of 89.4% - 100% of reduction. These results show the good effective of the biocide of modified low molecular weight chitosan and modified medium molecular weight chitosan.

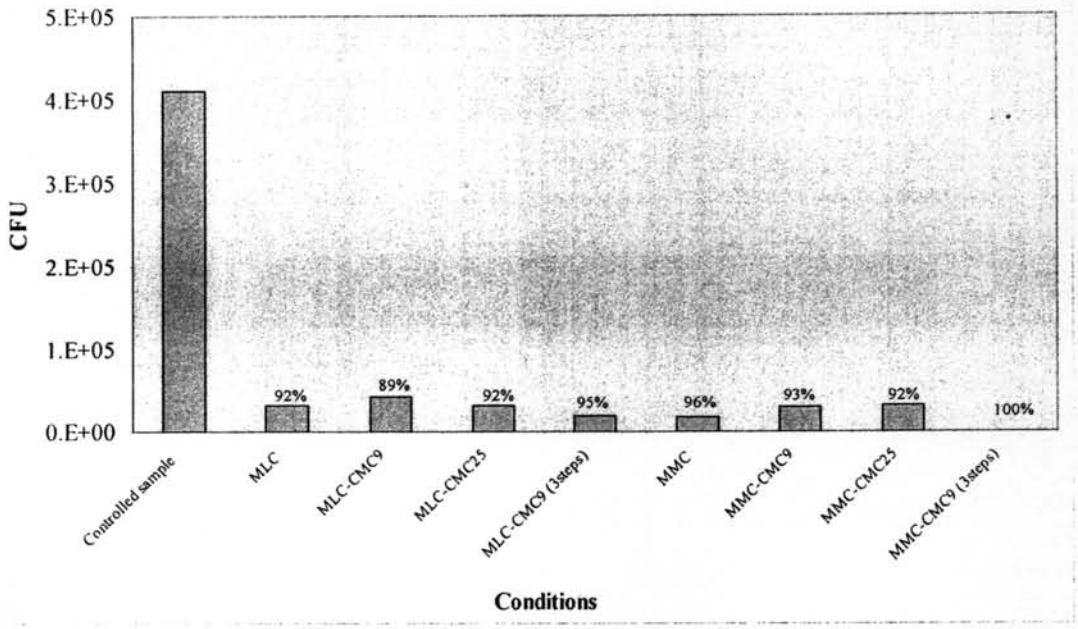


Figure 4.24 The chart indicate % bacteria reduction of modified chitosan on pulp.