# STUDY ON ANTIMICROBIAL ACTIVITIES OF QUARTERNARY AMMONIUM MODIFIED CHITIN-PAA GELS

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**Abstract**

Chitin-PAA gel was prepared by a 2-step process composed of esterification between carboxyl groups of acrylic acid monomer (AA) and hydroxyl groups of chitin and polymerization of the attached AA. The highly water-absorbable resulting product was further modified with either glycidyltrimethylammonium chloride (GTMAC) or chitosan quarternary ammonium derivative (CS-GTMAC) to impart the antimicrobial activity. The chemical structure and degree of substitution (DS) of the resulting products were characterized by FTIR and NMR. CS-GTMAC samples with varied molecular weight and DS were used in this study to investigate their effects on the antimicrobial efficacy of the hydrogels. To study the antimicrobial activity of each gel, the minimum inhibition concentration (MIC) against Escherichia coli (gram negative) and Staphylococcus epidermis (gram positive) was evaluated using a broth dilution method with respect to that of the starting material, chitin-PAA gel.

## Introduction

Quaternary ammonium salts are widely used as disinfectants, and quaternary ammonium (glycidyl trimethylammonium chloride (GTMAC)) modified chitosan has been well known to inhibit the growth of bacteria [1-2]. Previously, the water absorbable chitin-PAA dressing was synthesized in our laboratory [3]. The dressing possessed a satisfactorily effective wound healing ability. In this study, the chitin-PAA gel was supplemented its antimicrobial activity by incorporating with GTMAC via either direct chemical bonding or blending with GTMAC-modified chitosan. The effects of factors such as molecular weight of chitosan and degree of GTMAC substitution (DS) on antimicrobial efficacy were investigated. The antimicrobial activities, against *Escherichia coli* (gram negative) and *Staphylococcus epidermis* (gram positive), of the modified gels were evaluated and compared with that of the chitin-PAA gel.

## Material and Methods

*Synthesis*

Chitin-PAA gel and chitosan with different molecular weights (93,000 and 452,000 Dalton, from Qingdao Heppe Biotechnology Co., Ltd, China) were reacted with GTMAC (from Fluka) in aqueous solution at various mol ratios of amino to epoxide. The reactions were performed at 70°C for 18 h. The resulting products, GTMAC modified chitin-PAA (chitin-PAA-GTMAC), GTMAC-modified chitosan (CS-GTMAC) and also the blend of chitin-PAA gel and CS-GTMAC (chitin-PAA/CS-GTMAC blend), were then investigated for their antimicrobial activities.

*Antimicrobial activity*

The minimum inhibitory concentration (MIC) of CS-GTMAC against *Escherichia coli* and *Staphylococcus epidermis* was determined by turbidimetric method. The assessment of antimicrobial activity of the modified gels was carried out by colony counting on incubated agar plates.

## Results and Discussion

*Structural Analysis*

1. Structural analysis of CS-GTMAC

As seen in Fig.1, a decrease in the peak intensity at 1520 $cm^{-1}$, corresponding to amino groups of chitosan, and an appearance of a new peak at 1478 $cm^{-1}$, corresponding to methyl groups of GTMAC, verified the reaction between amino groups of chitosan and epoxides of GTMAC. New peaks arisen in $^{1}$H-NMR spectrum of CS-GTMAC (Fig.2b) also confirmed that the chemical modification of chitosan with GTMAC was successful. The degree of substitution of quaternary ammonium group (DS) of each CS-GTMAC determined by $^{1}$H-NMR and a titration method was presented in Table 1.

Table 1 Degree of substitution of quaternary ammonium group.

| Sample | Degree of substitution | |
|---|---|---|
| | $^{1}$H-NMR | Titration |
| CS93k-GTMAC (1:1) | 0.1 | 0.3 |
| CS93k-GTMAC (1:4) | 0.4 | 0.7 |
| CS452k-GTMAC (1:1) | 0.3 | 0.7 |
| CS452k-GTMAC (1:4) | 0.5 | 0.7 |

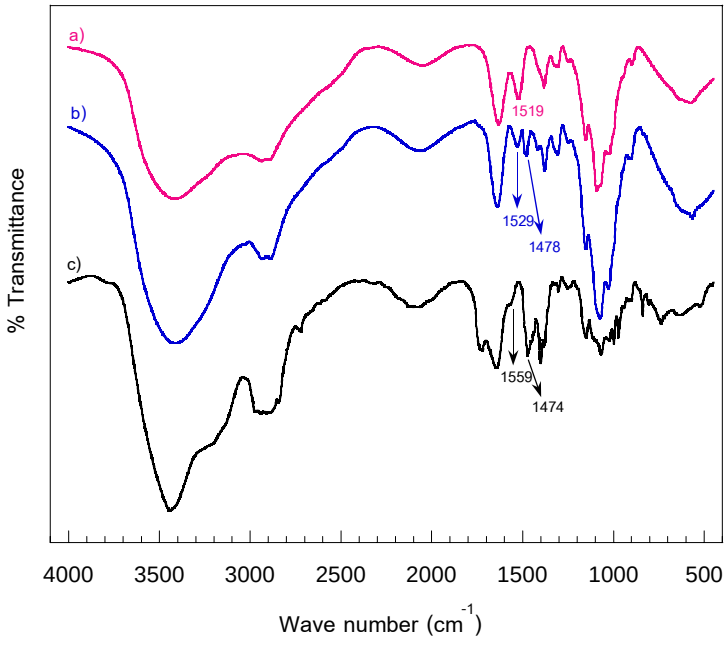


**Figure 1 :** FTIR spectra of a) chitosan (CS452k, Mw = 452,000 Dalton), b) CS-GTMAC with 1:1 mol ratio of amino to epoxide, and c) CS-GTMAC with 1:4 mol ratio of amino to epoxide.

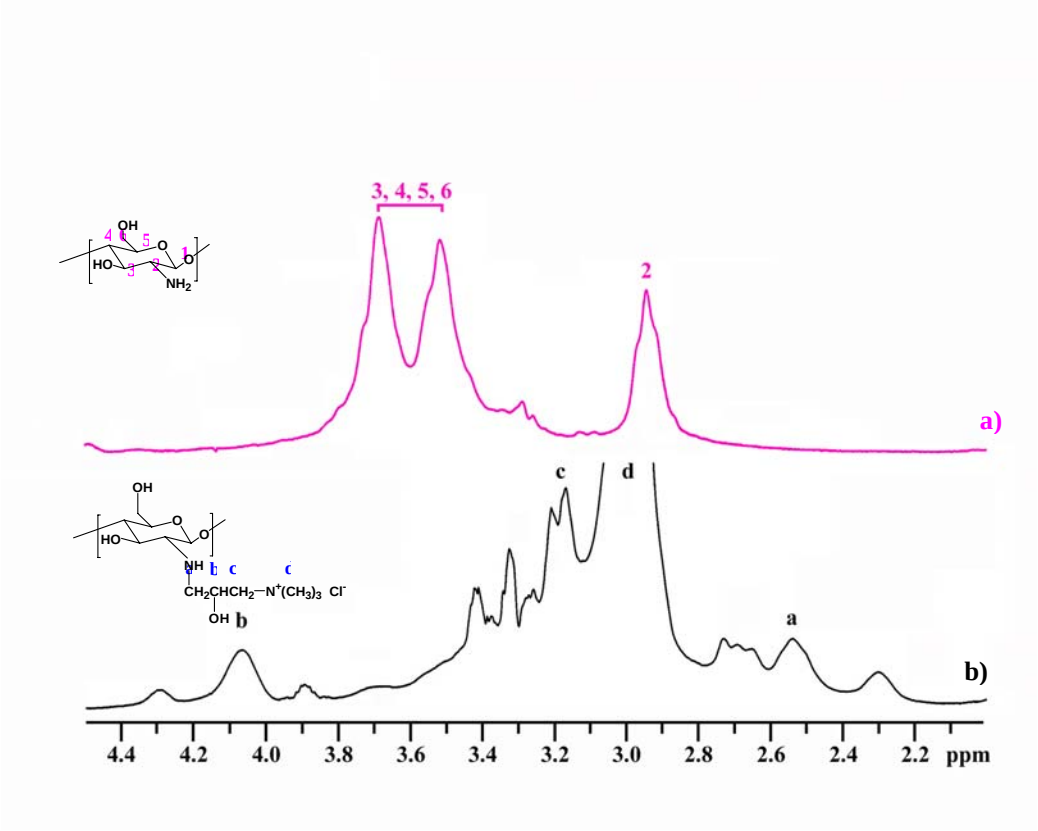


**Figure 2 :** $^{1}$H-NMR spectra of a) chitosan (CS), and b) CS-GTMAC.

2. Structural analysis of Chitin-PAA-GTMAC

The new peak at 1479 cm$^{-1}$ appearing in the FTIR spectra of chitin-PAA-GTMAC (Fig.3) indicated the evidence of the reaction between chitin-PAA and GTMAC. This was in agreement with the $^{15}$N-NMR results (Fig.4) which showed a strong signal of N-methyl group of GTMAC at 48 ppm dominating N-acetyl signal of chitin-PAA at 122 ppm.

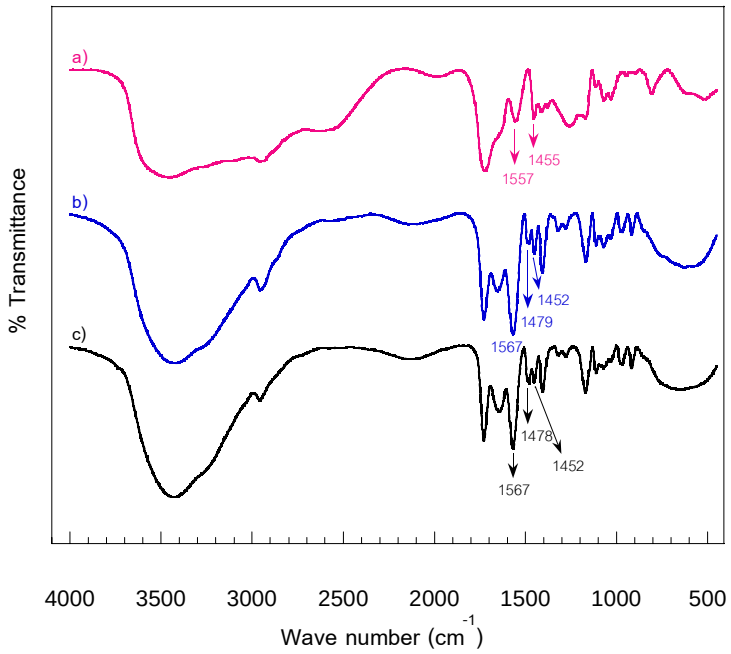


**Figure 3 :** FTIR spectra of a) chitin-PAA, b) chitin-PAA-GTMAC with 1:4 mol ratio of amino to epoxide, and c) chitin-PAA-GTMAC with 1:20 mol ratio of amino to epoxide.

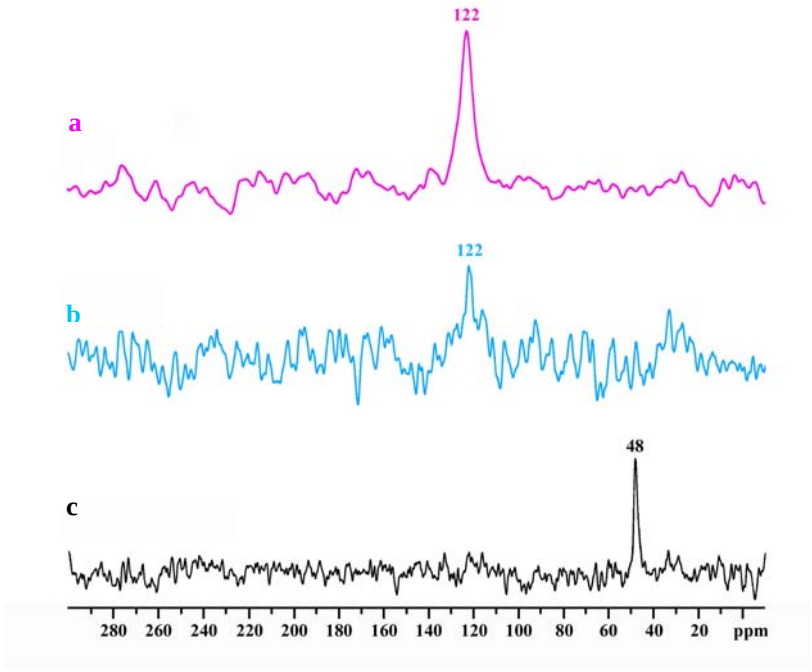


**Figure 4 :** $^{15}$N-NMR spectra of a) chitin, b) chitin-PAA, and c) chitin-PAA-GTMAC.

*Antimicrobial activity*

The lowest concentration of each sample that can inhibit the bacterial growth (MIC) is presented in Table 2. All CS-GTMAC showed effective antimicrobial activities against both *E. coli* and *S. epidermis*. The observed MIC was affected by both molecular weight of chitosan and DS but not significantly. The low molecular weight chitosan with a high DS seemed to enhance the antimicrobial activity best.

Table 2. Minimum inhibitory concentration (MIC) of various samples.

| Sample | Minimum inhibitory concentration (% w/v) | |
|---|---|---|
| | *Staphylococcus epidermidis* | *Escherichia coli* |
| CS93k-GTMAC (1:1) | 0.0029 | 0.0059 |
| CS93k-GTMAC (1:4) | 0.0015 | 0.0029 |
| CS452k-GTMAC (1:1) | 0.0029 | 0.0029 |
| CS452k-GTMAC (1:4) | 0.0029 | 0.0059 |

The amount of viable bacteria (CFU/ml) after in contact with the modified gels is shown in Table 3. It seemed that chitin-PAA-GTMAC had a greater antimicrobial activity than chitin-PAA/CS-GTMAC blend. CS-GTMAC tended to form ionic complex with chitin-PAA resulting in the deswelling of the blend. The complex possibly suppressed the function of the quarternary ammonium salt in inhibiting the bacterial growth, thereby resulting in a poorer antimicrobial activity compared to chitin-PAA-GTMAC.

Table 3. Antimicrobial activities of samples against *Staphylococcus epidermis*.

| Sample | Viable bacteria (CFU/ml) |
|---|---|
| Control | $6.13 \times 10^8$ |
| Chitin-PAA | $1.05 \times 10^8$ |
| Chitin-PAA/CS93k-GTMAC (1:4) blend | $3.83 \times 10^7$ |
| Chitin-PAA-GTMAC (1:20) | $1.00 \times 10^7$ |

## Conclusion

The chitin-PAA-GTMAC gel was successfully prepared. The gel showed a greater antimicrobial activity than both the starting chitin-PAA gel and its blend


## Acknowledgements

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## References


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