Antibacterial performance evaluation for thermoplastic films containing triclosan and $CaCO_3$ fillers

Karn Silapasorn[1], Kwannate Sombatsompop[2], Apisit Kositchaiyong[1], Ekachai Wimolmala[1], Teerasak Markpin[1] and Narongrit Sombatsompop[1,*]

[1]Polymer PROcessing and Flow (P-PROF) Group,
School of Energy, Environment and Materials
King Mongkut's University of Technology Thonburi(KMUTT)
Thongkru, Bangkok, 10140, THAILAND

[2]College of Industrial Technology
Department of Civil and Environmental Engineering Technology
King Mongkut's University of Technology North Bangkok(KMUTNB)
Bangkok, 10800, THAILAND

***Corresponding author: N. Sombatsompop*
Tel:(662)470-8645 Fax : (662)470-8647
Email: narongrit.som@kmutt.ac.th

Abstract

Triclosan and calcium carbonate ($CaCO_3$) were incorporated in various thermoplastics and their anti-bacterial performances were studied through Halo and Plate-Count-Agar(PCA) tests. The thermoplastics used were polyethylene (LDPE, MDPE, HDPE), polypropylene (PP), polystyrene (PS) and poly(vinyl chloride) (PVC). *Escherichia coli (E.coli,* ATCC 25922) and *Stapphylococcus aureus* (*S.aureus,* ATCC 25923) were used as the testing bacteria. The color index results suggested that introducing triclosan did not change the color of all thermoplastics used. The anti-bacterial results showed that the inhibition radius increased with increasing triclosan for non-polar thermoplastics like LDPE, MDPE, HDPE, PP and PS films whereas the opposite effect was observed for polar PVC film. The anti-bacterial efficacies of the triclosan decreased in the order of LDPE > MDPE > HDPE > PP > PS > PVC and this was confirmed by the triclosan releasing and FT-IR results. The addition of $CaCO_3$ appeared to reduce the inhibition radius and % *E.coli* reduction for the HDPE and PS matrices whereas the addition of $CaCO_3$ improved the inhibition zone and the % *E.coli* reduction for the PVC.

***Keywords*:** Antimicrobials; thermoplastics; halo test; plate count agar; polymer composites.

Introduction

Because of the increasingly high demands for hygienic thermoplastic products, attention has been extensively placed on active thermoplasticic materials in food packaging applications, the medical professions, and household products. The thermoplastics used for such applications include polyolefin, poly(vinyl chloride), polyamide, and polyurethane and are usually incorporated with antimicrobial agents [1-3]. There are a number of antimicrobial agents available and used, including Carbendazim, 2-Hydroxypropyl-3-Piperazinyl-Quinoline carboxylic acid Methacrylate, Silver and silver substituted zeolite, Triclosan, Benzoic acid Benzoic anhydride, Sorbic acid, Potassium sorbate, Nisin, Lysozyme, Glucose oxidase, Cinnamic Caffeic and *p*-Coumaic acid [3-10]. The efficacies of these antimicrobial agents are dependent on mixing method, contact time, carrier and thermoplastic types, standard testing methods [5].

A number of scientific research evidences have been made on antimicrobial efficacies of polymeric packaging products, mostly considering effects of polymer matrix selection, type and loading of anti-bacterial agents and processing conditions Review on 8-10. Ai-Hua *et. al.* [8] synthesized triclosan-loaded PS/TiO_2 nanocapsules and using ethanol as a media for releasing studied. It indicates that the amount of triclosan in ethanol increased with the elongation of time.It can be concluded that the as-prepared triclosan-loaded TiO_2 nanocapsules are useful sustained-release material for potential applications in cosmetic, drug delivery system, and so on. Zhang *et. al*. [9] used plasma immersion ion implantation to modify PVC surface for coating triclosan and bronopol more effectively and enhance the antibacterial properties. The result show that the plasma-modified PVC with antibacterial agents exhibits good antibacterial properties. Joerger *et. al*. [10] found that bacteriocin nisin was the

antimicrobial most commonly incorporated into films, followed by food-grade acids and salts, chitosan, plant extracts, and the enzymes lysozyme and lactoperoxidase. They suggest that antimicrobial films still face limitations and are perhaps still best viewed as part of a hurdle strategy to provide safe foods. Zhang *et. al.* **[11]** found polyethylene surface modification by plasma immersion ion could improve coating efficiency of an antibacterial agent on surface and this resulted in higher antibacterial performance because of its higher polarity on polyethylene surface. Park *et. al.* **[12]** indicated that thermoplastic synthesized from vinyl monomer derivatives in different forms of phenol and benzoic acid would give different bacteria inhibition zones. The polymers with greater glass-transition temperature yielded lower antimicrobial activities. Chung *et. al.* [13] suggested that the effect of triclosan in styrene-acrylate co-thermoplastic on diffusion performance using water, ethanol and *n*-hexane as diffusion media. It was found that the efficiency for killing bacteria was dependent on the diffusion ability in the media and diffusion in ethanol exhibited the highest anti-microbial efficiency. Camilloto *et al* [14] developed polyethylene antimicrobial extruded films containing triclosan of 2000 and 4000 mg kg^{-1}. The films efficacies were studied against *Escherichia coli, Staphylococcus aureus, Listeria innocua, Salmonella choleraesuis* and *Pseudomonas aeruginosa* growth using agar diffusion test and by monitoring the inhibition of *E. coli* and *S. aureus* inoculated on sliced cooked ham. They found that the addition of triclosan did not affect the mechanical properties of the films. Films containing triclosan showed an antimicrobial effect for *E. coli* and *S. aureus* detected by formation of an inhibition halo, but this was not the case for *L. innocua, S. choleraesuis* and *P. aeruginosa*.

The chemical structures of polymers used as packaging products are considered as one of the important factors to affect the anti-microbial performance of the products. This may involve polarities, amorphous and crystalline structures of the polymers, polymer density, as well as type and loading of filler incorporated within the polymer matrices. Iconomopoulou *et al* [7] suggested a significant parameter for controlled release antibacterial agent. Triclosan, antibacterial agent, has been incorporated into high density polyethylene (HDPE) films that have been subsequently uniaxially drawn at different draw ratios.They found that the relevant release rate from the drawn specimens was lower than the non-stretched samples depending on the molecular orientation developed during the drawing process. Kalyon *et al* [15] used Triclosan as antibacterial agent and incorporated into Polystyrene through melt-mixing. They assessed the antibacterial efficacy of triclosan-incorperated polymer disks against *Escherichia coli* and *Bacillus thuringiensis* in liquids in contact with the polymer. They found that triclosan at the surface of the disks dissolves into the liquids, and the rest of the triclosan, immobilized in the disks, does not contribute to the antibacterial effectiveness of triclosan-incoperated polymer. Zhang *et* al [9] used the Plasma immersion ion implantation (PIII) method to modify medical-grade PVC coated by triclosan and bronopol to enchance the antibacterial properties. The antibacterial properties were evaluated utilizing the method of plate-counting of *Escherichia coli* and *Staphylococcus aureus*. The result shows that the plasma-modified PVC with triclosan has better antibacterial performance against *E.coli* than bronopol.

The anti-bacterial performances of triclosan-incorporated thermoplastics have been studied extensively and separately for individual thermoplastics [8-10, 14], and mostly in unfilled thermoplastic systems; i.e., studies on the effects of the molecular structures of

thermoplastics on the anti-bacterial performance of triclosan/thermoplastic blends are still rare and open for discussion, especially in thermoplastic composites. In this work, the effects of triclosan content on the anti-bacterial performance of various thermoplastics with and without $CaCO_3$ filler was investigated. *Escherichia coli (E.coli*, ATCC 25922) and *Stapphylococcus aureus* (*S.aureus*, ATCC 25923) were used as the testing bacteria.

Experimental

Materials & Chemicals

The six thermoplastics used were a low-density polyethylene (LDPE, 1902F, SCG Public Co.Ltd.,Thailand), a medium-density polyethylene (MDPE, M380RU/RUP, Thai Polyethylene Co.Ltd.,Thailand), a high-density polyethylene (HDPE, HD6000F, PTT Thermoplastic Marketing Co.Ltd.,Thailand), a polypropylene (PP-401S, SCG Public Co.Ltd.,Thailand), polystyrene (PS, Styron-656D267, Siam Polystyrene Co.Ltd., Thailand) and poly(vinyl chloride) (PVC, SIAMVIC 258RB, V.P.Wood Co.Ltd., Thailand). Calcium carbonate ($CaCO_3$, Hicoat-410, Sand and Soil Industry Co.Ltd., Thailand) was used as filler. Triclosan (2,4,4'-trichloro-2'-hydroxlydiphenylether, 24 USP, Koventure Co.Ltd., Thailand) was used as the anti-bacterial agent. The specifications, including suppliers, grades, physical properties and chemical structures, for all materials used are given in Table 1. *Escherichia coli (E.coli,* ATCC 25922) and *Stapphylococcus aureus* (*S.aureus*, ATCC 25923), as gram negative and gram positive, respectively, *were* used as the testing bacteria.

Specimen preparation

All thermoplastic specimens were prepared in film test-pieces which were prepared by blending triclosan and thermoplastic using an internal mixer (Haake Rheomix5000, Germany) to obtain a good dispersive blend, before made into the film form by compression molding. The processing temperatures for LDPE, MDPE, HDPE, PP, PS and PVC were 160, 170, 180, 210, 150 and 170^{o}C, respectively. The blend was placed on a square mould at the desired mold temperature and then preheated for 10min under a pressure of 100kg/cm^2 before cooled down to an ambient temperature to obtain the resultant film of 0.2mm thick. The films were made in a circular disc of 6 mm in

diameter for the halo test, and in a square piece of 5x5cm^2 for the plate count agar(PCA) method as detailed later. The triclosan concentration used was between 0-1.5x10^{4}ppm.

Antibacterial efficacy evaluations

Halo test: Inhibition zone was examined to qualitatively assess the anti-bacterial efficiency through the growth of bacteria by diffusion of antibacterial agent within the agar media. In this work, the soft agar technique was introduced to prepare the testing media. The testing inoculum used was *Escherichia coli* (ATCC 25922) and *Stapphylococcus aureus* (ATCC 25923). The soft agar was performed by mixing, in the bottle, nutrient agar and nutrient broth at the ratio 50:50. After that the agar was calibrated at an initial concentration of 10^6 cfu/ml using a UV spectrometer. The prepared soft agar of 5ml was poured on the solidified nutrient agar as substrate. The triclosan/thermoplastic test-pieces were carefully placed over the soft agar at a determined position. Finally, the plates were incubated at 37^{o}C±0.5^{o}C for 24 h. The results were reported in terms of "inhibition zone radius" as given by **Equation 1 [5]**. D_C and D_S were diameters of inhibition zone and testing specimen, respectively.

$$Inhibition\ \text{zone radius} = \frac{D_C - D_S}{2} \qquad \text{(Eqn. 1)}$$

Plate Count Agar (PCA) method: Plate Count Agar (PCA) is appropriate for quantitative evaluation of bacteria-colony reduction by triclosan incorporated specimens [Ref]. The PCA test followed the ASTM E-2149 (2001) test method. The growing medium for *E. coli* was nutrient broth (NB) and peptone solution (prepared by 1 g / L peptone, pH 6.8 – 7.2). The bacteria were inoculated overnight in 5 ml of NB at 37^{o}C . Tenfold serial dilution was used for diluting bacteria cell suspensions to 10^6 cfu/ml so that the colony forming was in the range of 30 – 300. The required

bacteria suspensions were then mixed with 45 ml of peptone before adding the film specimens into the solution. The reciprocal shaker at a speed of 100 – 120 rpm at 37^{o}C ± 0.5^{o}C was used to shake the solution. Contact time, defined as the time that the antibacterial incorporated thermoplastic specimens were shaken in the peptone solution which contained the tested bacteria (*E.coli* and *S.aureus*), used was 60 minutes throughout this work. After that, 100 µL of the bacterial solution was put over the agar in sterilized Petri dishes. Finally, the inoculated plates were kept at 37^{o}C ± 0.5^{o}C overnight before evaluating the anti-bacterial efficacy. The results were reported in terms of percentage reduction of bacteria-colony using **Eqn.2**[6]. The releasing rate of triclosan from the thermoplastics was performed by measuring the UV absorbance of triclosan solution, the experimental procedure being detailed elsewhere[7-8]

$$R = \frac{A - B}{A} \times 100 \qquad \text{(Eqn.2)}$$

where, R is the percent reduction of bacteria(%), A is the average number of bacterial colonies from triclosan unfilled thermoplastic(CFU/ml), and B is the average number of bacterial colonies from triclosan filled thermoplastic(CFU/ml).

Materials characterizations

Triclosan releasing study: The releasing rate of triclosan form testing specimens was performed by measuring the UV absorbance of triclosan solution **[7]**. The specimens were put in the flask containing 50 ml of ethanol solution (95% v/v) and then the flask was shaked under the same conditions with shake flask method (37^{o}C, 100 rpm for shaking speed and 0.5 and 1.0 h for contact time). After determined contact time, the ethanol solution was pipetted for 20 ml into cuvet to measure UV absorbance value using UV-Vis spectrophotometer (HACH DR/4000U) at the wavelength of 287-290

nm. The absorbance value was translated in triclosan concentration using a calibration curve.

Color change test: The color changes of triclosan filled thermoplastic were observed using UV-Vis spectrophotometer. The CIE-LAB color system, *L*a*b** coordinates, were collected and calculated based on a D65 light source *L** represents the lightness whereas *a** and *b** are the chromaticity coordinates. The higher the *L** value the lighter the sample. The *a** coordinate represents red-green coordinate while the *b** coordinate represents yellow-blue coordinate. The total color changes or discolorations of the UV-weathered specimens was calculated from differences of lightness and chromatic coordinates (*ΔE*) of filled and unfilled thermoplastic piece were calculated based on a D65 light source. The equation 3 was used to express the lightness and chromatic alteration of specimen.

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \qquad \text{(Eqn. 3)}$$

Fourier Transform Infrared (FT-IR) analysis: The FT-IR spectrum of triclosan filled polymer were used to investigate the functional change and /or interaction between triclosan and polymer matrix. FTIR Spectrometer Spectrum One, Perkin Elmer, which having resolution of 4 cm^{-1} and scanning region between 400 and 4,000 cm^{-1} was performed in this work.

Results and discussion

Physical appearance of thermoplastic films added with triclosan

This section studied the physical appearance of the thermoplastic films with no triclosan addition and with 1.5 x 10^4 ppm triclosan. This was achieved by assessing the changes in color and light transmission whose results are given in **Table 2**. It can be seen that all the color indices (*L**, *a**, *b**, *ΔE*) and %light transmission for al lthermoplastic films with and without triclosan were very similar, the differences being within the experimental errors. This suggested that the addition of triclosan had no significant effect on the physical appearances of all thermoplastic films.

Effect of triclosan content on inhibition zone

Figure 1 shows the effects of triclosan content on inhibition zones for all thermoplastic films for *E.coli* and *S.aureus* bacteria. The results suggested that different thermoplastics had different effects on the inhibition radius. That was, LDPE, MDPE and HDPE and PP films showed relatively high inhibition radius compared to PS and PVC films. The greater inhibition radius only indicated the ability of the triclosan to diffuse from the thermoplastic to react with and kill the bacteria. The differences in inhibition radius for all thermoplastics used could be explained by a number of factors including (i) triclosan-thermoplastic compatibility [Ref], (ii) thermoplastic free volume [Ref] and (iii) rigidities of the thermoplastics [Refs].First, it seems that the triclosan had greater ability to diffuse in the PE and PP compared to the PS and PVC. It is thought that the triclosan could interact better with PS due to the benzene groups in PS and with PVC due to the polarities of triclosan and PVC molecules. These interactions could probably cause difficulty for triclosan to diffuse away from the PS and PVC films, thus less inhibition radius. Second, the

differences in inhibition radius between LDPE, MDPE, HDPE and PP were caused by differences in free volume; i.e., the greater the free volume the higher the triclosan to diffuse[7]. That was why the LDPE had the greatest inhibition radius**.** Finally, the rigidities of the thermoplastics decreased in the order of PVC > PS > PP > PE as a result of the size and polarity of the side-groups on the backbones of the thermoplastics used [Ref]. That was, the higher the rigidity of the thermoplastics the lower the diffusion of the tricloson, and thus smaller inhibition zone.

Considering the effect of triclosan content on the inhibition zone, one may expect that increasing triclosan loading would result in an increase in inhibition zone. This was not always true according to the results obtained in this work. The inhibition zone increased with increasing triclosan content for the non-polar thermoplastics (PE, PP and PS) whereas the opposite effect was observed for the polar PVC. This observation has confirmed the effect and explanation on the molecular compatibility as mentioned earlier. The decreases in inhibition zone as a result of increasing triclosan content in PVC could be explained through chemical and physical interactions between triclosan and PVC molecules. The chemical interaction involved a polar-polar interaction between the PVC and triclosan molecules. Evidence to support such interaction used the FT-IR spectra for pure triclosan, neat PVC, and triclosan/PVC blend samples as given in **Figure 2**. The polar-polar interaction between triclosan and PVC was evidenced by two shifted peaks, which were the peak of O-H stretching at 3525 cm^{-1} in R(Cl)-OH in triclosan/PVC blend, and that of C-Cl stretching at 865 cm^{-1} of PVC in the triclosan/PVC blend. This observation was similar to the work by Zhang et al.[9] who worked on the antibacterial properties of bronopol- and triclosan-coated PVC. The physical interaction between triclosan and PVC molecules was associated

with a change in diffusion rates of triclosan through the PVC matrix at different triclosan levels. That was, the diffusion rate of triclosan was likely to differ depending on the amount of triclosan contained in the PVC matrix. This explanation is schematically given by **Figure 3**, showing triclosn diffusion rates in the triclosan/PVC blend with low and high triclosan contents. It was believed that the blend with high triclosan content was expected to have higher polar-polar interaction between PVC and triclosan molecules and this would probably retard further diffusions of triclosan to kill the bactetia at the sample surface. This was why the inhibition zone of PVC with higher triclosan content was lower than that with lower triclosan content.

Effect of triclosan content on percentage reduction of bacteria

The Plate Count Agar (PCA) method was used to *quantitatively* substantiate the Halo test results as discussed in **Figure 1**. **Figure 4** shows the percentage reductions of *E.coli* for different thermoplastics for different triclosan loadings for *E.coli* and *S.aureus* bacteria, calculated using Equation 2. An example of living bacteria colony for HDPE and PVC matrices filled with different triclosan contents using *E.coli* as a testing bacterium by the PCA method is given in **Figure 5**. It can be seen that the changing trends of the PCA results in **Figures 4 and 5** for all thermoplastics corresponded well with those of the inhibition zone results in **Figure 1.** The triclosan exhibited a most effective anti-bacterial agent for non-polar polymers with high free volume structure, like LDPE in this work. The main reason was the triclosan diffusion, which were influenced by triclosan-thermoplastic polar interaction, thermoplastic free volume and rigidities of the thermoplastics as detailed earlier. The results and explanations for the triclosan diffusion in different thermoplastics were substantiated *qualitatively* by the triclosan releasing rate results given in **Figure 6** for *E.coli*. It can be seen that the higher the triclosan content the

greater the triclosan releasing rates for non-polar thermoplastics, most pronounced effect being observed for LDPE matrix. However, a slightly decreasing rate of triclosan release with triclosan content was observed for PVC matrix. It should be noted that the magnitude changes in the inhibition zone and releasing rate were different for all thermoplastics with various triclosan contents. This was because these two results (Figures 1 and 4) were performed in two different environments, inhibition zone result in the agar and the triclosan releasing result in the ethanol solution.

Effect of $CaCO_3$ incorporation

In this section, three thermoplastics, namely HDPE, PS and PVC, were selected based on their differences in crystalizabilities and polarities. **Figures 7 and 8** shows the effect of $CaCO_3$ content on inhibition radius and percentage reduction, respectively, for *E.coli* and *S.aureus* for three selected thermoplastics (HDPE, PS and PVC). It was found that the addition of $CaCO_3$ into the selected thermoplastic films had two different effects on the inhibition radius and % bacteria reduction depending on the type of thermoplastics used. In addition, an increase in the $CaCO_3$ filler had no definite trend. In the first case, the addition of $CaCO_3$ reduced the inhibition radius and % bacteria reduction, this being the case for HDPE and PS matrices. This could be explained by asserting that the presence of $CaCO_3$ prohibited the diffusion of the triclosan through the HDPE and PS films. In the case of the PVC film, the addition of $CaCO_3$ improved the inhibition radius and % bacteria reduction. This could be associated with the polar-polar interaction between the PVC and triclosan which was interfered by $CaCO_3$ filler. If this was the case, the releasing of triclosan to kill the bacteria would be facilitated.

Conclusion

Triclosan was found to be suitable for non-polar polymers with high free volume structure, with the effectiveness of the triclosan added in the thermoplastics decreasing in the order of LDPE > MDPE > HDPE > PP > PS > PVC. Triclosan-polymer compatibility, free volume structure and rigidities of thermoplastics were observed to have significant effects on the anti-bacterial performance of triclosan in the thermoplastics used. The effect of increasing $CaCO_3$ content on inhibition zone and percent reduction had no definite trend; i.e., the addition of $CaCO_3$ reduced the inhibition radius and %reduction of *E.coli* in the PE and PS matrices, but the effect was opposite for the PVC matrix. *E.coli* and *S.aureus* bacteria showed similar antibacterial efficacy for all thermoplastics used through the effects of triclosan and $CaCO_3$ contents.

Acknowledgments

The authors would like to thank National Research Council of Thailand (NRCT) the Thailand Research Fund (RTA5280008) for financial supports of this work. The use of instruments and laboratory, College of Industrial Technology, King Mongkut's University of Technology North Bangkok (KMUTNB) is appreciated.

References

1. Niall A. Antimicrobials in plastics: a global review. Plast Addit Compound 2001;3:12-3.
2. Holme M. Biocide use in plastics continues to rise. Addit Polym 2006;6:11-2.
3. Appendini P. Hotchkiss J.H. Review of antimicrobial food packaging. Innovat Food Sci Emerg Technol 2002;3:113-26.
4. Quintavalla S. Vacini L. Antimicrobial food packaging in meat industry. Meat Sci 2002;62:373-80.
5. Chammanee P. Sombatsompop K. Kositchaiyong A. Sombatsompop N. Effects of anti-bacterial agents, sample preparation and contact time on anti-bacterial efficacy in MDPE film. J Macromol Sci Phys 2009;48:755-65.
6. Lee H.J. Jeong S.H. Bacteriostasis and skin innoxiousness of nanosize silver colloids on textile fabric. Text Res J 2005;75:551-6.
7. Iconomopoulou S.M. Voyiatzis G.A. The effect of the molecular orientation on the release of antimicrobial substances from uniaxially drawn polymer matrixes. J Contr Release 2005;103:451-64.
8. Ai-Hua P. Zheng-Wu S. Gui-Sheng Y. Preparation of TiO_2 nanocapsules for loading and release of antimicrobial triclosan molecules. Mater Lett 2007;61:2757-60.
9. Zhang W. Chu P.K. Ji J. Zhang Y. Liu X. Fu R.K.Y. Ha P.C.T. Yan Q. Plasma surface modification of polyvinyl chloride for improvement of anti-bacterial properties. Biomaterials 2006;27:44-51.
10. Joerger, R.D. Antimicrobial films for food applications: a quantitative analysis of their effectiveness. Pack. Technol. Sci 2007;20:231-273.

11. Zhamg, W., Chu, P.K., Ji, J., Zhang Y., Fu, R.K.Y. and Yan Q., 2006, "Antibacterial Properties of Plasma – modified and Triclosan or Bronopol Coated Polyethylene", **Thermoplastic**, Vol. 47, pp. 931-936.
12. Park, E.S., Moon, W.S., Song, M.J., Kim, M.N., Chung, K.H. and Yoon, J.S., 2001, "Antimicrobial Activity of Phenol and Benzoic Acid Derivative", Intl Biodeter Biodegrad, Vol.47, pp. 209-214.
13. Chung, D., Papadakis, S.E. and Yam, K.L., 2003, "Evaluation of A Thermoplastic Coating Containing Triclosan as The Antimicrobial Layer for Packaging Material", Intl J Food Sci Technol, Vol. 38 pp. 165-169.
14. Camilloto G.P., Soares N.D.F.F, Pires A.C.D.S, Paula F.S.D. Preservation of sliced ham through triclosan active film. Pack Tech Sci 2009;22:471-477.
15. Kalyon, Y.D. and Olgun, U., 2001, "Antibacterial Efficacy of Triclosan-incorporated Polymers", **American Journal of Infection Control**, Vol. 29, No. 2, pp.

List of Table and Figures