CAFEi2012-160

KINETIC AND RELEASE RATE OF LAURIC ACID FROM STARCH-BASED FILM IN FOOD STIMULANT

Siti Nur Hana Mamat[1], Eraricar Salleh[2] & Mohd Harfiz Salehuddin[3]

[1,2,3]Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor Malaysia.
Email:[1] snhana87@gmail.com; [2]eraricar@cheme.utm.my; [3]mharfizutm@gmail.com

ABSTRACT

This study presented information of kinetic and release rate of lauric acid in starch-based film blending S: C: LA ratio 1: 9: 0.08 to 5:5:0.4. The diffusion method was applied in order to investigate the kinetic and the release rate of lauric acid towards microbial from starch film blending using Gas Chromatography (GC). For kinetics test, the result from release mechanism is used and plotted on graph to determine the type of lauric acid kinetics. The best formulation of LA for water stimulant is 2:8:0.16 which is 3.38x10-4 s-1 as the inhibition process can occur at early stage and 3:7:0.24 which represents fatty food is 1.65x10-4 s-1. The film ratio of 5:5:0.40 has higher release rate of lauric acid in both acid and alcohol food stimulants and obey Higuchi release model that shows the release of lauric acid increase rapidly until it reach a stable state. As conclusion, the entire food stimulant affected the optimum release rate of lauric acid after 6 hours lead longest lag phase time by good diffusion coefficient.

Keywords*: Kinetic Release, Chitosan, Lauric Acid, Antimicrobial, Food Packaging*

INTRODUCTION

The main purpose of packaging is to protect the food from spoilage and pathogenic microorganism, oxygen, water and vapour light which might contaminate the foods. The antimicrobial packaging have been use to control microbial growth in a food ingredient using packaging materials and edible films and coating that contain antimicrobial agents and sometimes by using the techniques that modify the atmosphere within the package [1].

Antimicrobial packaging is a type of packaging designed to release active agents to inhibit growth of microorganism inside the package. When antimicrobial agents (organic acids, chemical antimicrobial, natural antimicrobial, fatty acid) are incorporated into a film, the film may then have the ability to prevent or inhibit microbial growth [2].

Most of plastics used in packaging industry nowadays were made from petroleum and this was lead to the environmental pollution where this kind of plastics is non-degradable. Due to this factor, great interest triggers in films made from edible, renewable and natural polymers such as starch has been produced. Starch-based films exhibit physical characteristics similar to synthetic polymers which are transparent, odorless, tasteless, semipermeable to CO2 and resistant to O2 passage [3]. The physical and functional properties of starch films can be improved by blending with other biopolymers, hydrophobic substances and/or antimicrobial compounds [4].

One of the most common biopolymers that have been used in packaging industry is chitosan. Chitosan is a natural biopolymer obtained from the deacetylation of chitin [poly-β-(1-4)-N-acetyl-D-glucosamine], a major component of shells of crustaceans such as crab, shrimp and crawfish. Chitosan has been found to be non-toxic, biodegradable, biofunctional, biocompatible and was reported by several researchers to have strong antimicrobial and antifungal activities. The potential of chitosan to act as food preservative of natural origin has been widely reported on the basis of in vitro trails as well as through direct application on real complex matrix foods [5].

Beside chitosan, lauric acids also work well as antimicrobial agent in packaging film. This is due to the facts that lauric acids have antimicrobial effects against gram positive bacteria and yeast. It also suggested that fatty acids were bacteriostatics and may be potential microbial inhibitors in foods using a systematic approach with other antimicrobials [6]. Another characteristic on lauric acids are it is a medium length-long chain fatty acid which found in the form of glycerides in a number of natural fats, coconut oil and palm-kernel oil. It is advantageous in food processing as it acts as a kind of preservatives, staving off oxidation and spoilage [3].

The most important part in maintaining the food quality and safety is the release rate of the antimicrobial agent. A rapid release of an antimicrobial agent from film to food surface may reduce the success of packaging application considerably since this cause subsequent diffusion of the agent from food surface to internal parts which is less critical than the food surfaces for microbial growth and contamination [7]. In order to design an effective and efficient active packaging, it is essential to know the diffusion rates of an active substance from packaging to the food matrix. If the release of antimicrobial substance is too slow, the antimicrobial packaging systems will not be effective. The food product may be spoiled as the growth of microorganisms may be faster than the liberation of antimicrobial substance [8-9].

Mechanism of Lauric Acid from Starch-Based Film

Lauric acid micro domains were dispersed within the starch matrix in the blend films or by water diffusion where; water molecules from food penetrate into the matrix of film leading to its swelling; as the water diffuses from the outer solution into the matrix, the meshes of the polymeric network become increasingly wider, allowing the active compound (lauric acid) to diffuse through the matrix into the outer water solution, until a thermodynamic equilibrium between the two phases is reached [10].

If the void volume of solid food products is assumed as a kind of headspace, most food packaging systems represent either a package/food system or a package/headspace/ food system. In Figure 1, diffusion between the packaging material and the food and partitioning at the interface are the main migration phenomena involved in this system. Antimicrobial agents may be incorporated into the packaging materials initially and migrate into the food through diffusion and partitioning [11].

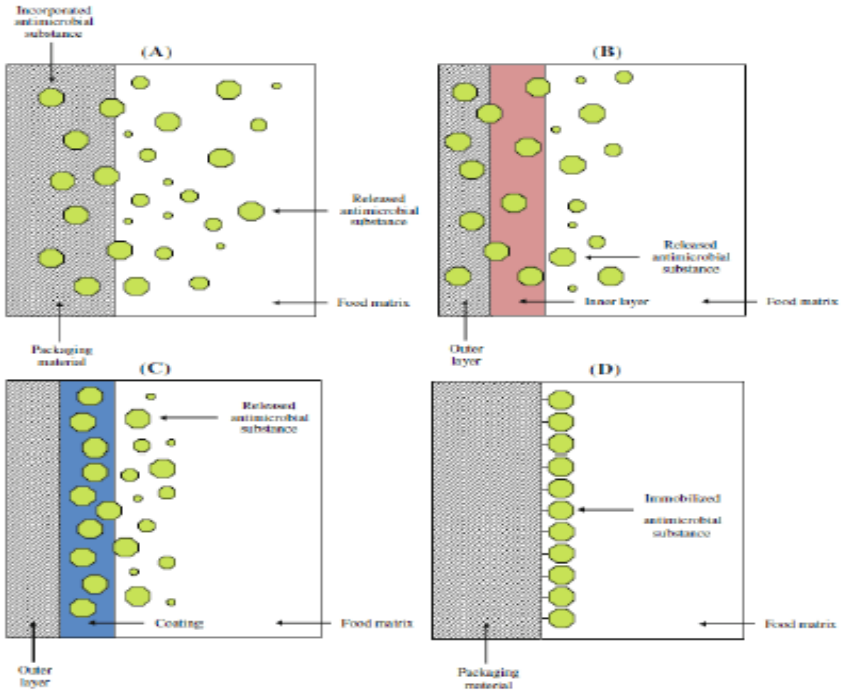

Fig. 1: Food Packaging Systems and Migration Phenomena [12].

Kinetic Modeling

Kinetic models that are based on elementary reactions offer the best accuracy and reliability. As migration experiments are time-consuming, expensive, and often difficult, the use of mathematical models to predict migration is gaining interest. Migration in polymers is known to follow physical diffusion laws. This opens the possibility of a mathematical description of migration and the use of such models for prediction.

Previous researches have used different mathematical model approach to study the release of certain AM or additives from the film. Besides to predict certain released agent, it also able to predict the release characteristic of the additives. There are some model usually stated in many journals such as first order, zero order, Hixson-crowell release model and Higuchi release model. Mathematical modeling of the diffusion process could permit prediction of the AM agent release profile and the time during which the agent remains above the critical inhibiting concentration. With a higher diffusivity and much larger volume of the food component compared to the packaging material, a semi-infinite model in which the packaging component has a finite thickness and the food component has infinite volume could be practical [2].

MATERIALS AND METHODS

Materials

For wheat starch and acetic acid (glacial 100%) used to dissolve chitosan was purchase from Merks (Malaysia). Medium molecular weight chitosan was from Sigma-Aldrich (Malaysia). Meanwhile, lauric acid was 99% pure bought from HmbG chemicals (Malaysia).
B. Methods

Preparation of Antimicrobial Starch-Based Film

Starch-based film was formed using casting process [13]. A control film, without lauric acid or chitosan was formed using mixtures of starch (5.0g), glycerol (2.5g) and water (92.5g). Chitosan was dispersed in 400mL of distilled water into which 20mL of glacial acetic acid was added to dissolve the chitosan. The solution of starch (S), and chitosan (C) with the different ration 5:5:0.40, 4:6:0.32, 3:7:0.24, 2:8:0.16, and 1:9:0.08 starch/chitosan/lauric acid (w/w/w) were prepared by adding glycerol (half amount of the starch) and 8% lauric acid (LA was added based on the percentage of starch. The solution was mixed by gentle stirring with a magnetic stir bar until the starch dissolved. Stirring and heating were ended when the solution reaches temperature of 80-86□C. 15mL of the film forming solution was pipette and spread evenly into petri disk bottom (100 x 15mm) and allowed to air-dry at room temperature overnight.

Release Test:

Antimicrobial Agent Release using Incubators

For the analysis of water and oil food stimulant, compression molded film samples of approximately 5 ×5 cm were immersed in a sealed vessel of 200 mL of N-heptane and 100ml of distilled water and were placed in an incubator shaker (InnovaTM 4230, New Brunswick Scientific, U.S.A.) maintained at 25□C. The amount of AM agent released was monitored until equilibrium was attained. An aliquot of the solution was analyzed by GC at different time intervals in 5min, 15min, 30min, 1hr, 3hr, 6hr, 24hr. The release of AM agents from the extruded films into the food stimulants was investigated by immersing *ca.* 0.5 g (5 pieces, 5× 5 cm) of weighed film sample into 100 mL of N-heptane, distilled water in a sealed vessel.

The analysis of acid and alcohol food stimulant will be test using the starch-based films containing each antimicrobial agent were placed and covered the food spoilage. The film samples were cut in 3cm x 3cm square shape and put inside conical flask with food stimulant. Food stimulants are liquids used for practical reason to simulating the real food. Two of food stimulant that used are ethanol (role as alcoholic foods), and acetic acid (role as acidic foods). Each 3cm x 3cm film is dipped inside 200ml of food stimulant and incubated

at 37°C for 48 hours with 200 rpm. Within different time interval, the sample of film was collected to analyze the concentration of lauric acid that left in the sample.

2.2) Quantification of AM Agents by Gas Chromatography

The concentration of AM agent in the prepared samples was determined by gas chromatography (GC). For the water and oil food stimulant, the sample of film was extracted using N-haptane and distilled water of the extract of a precisely known volume was sampled for GC analysis using a Varian Star 3400-CX GC equipped with fused fatty acid capillary column DB-5 (30x0.25 mm inner diameter, film thickness 0.25 μm, J. & W. Scientific, USA). The GC was operated using the following conditions: injection volume: 1.0 μL; initial column temperature: 80□C; heating rate: 5□C min-1; injector temperature: 250□C; split ratio 1:100; FID detector temperature: 300□C; and carrier gas: helium. While for the acid and alcohol food stimulant, the concentration of lauric acid in the prepared samples was determined by gas chromatography (GC) that is FID-GC equipped with fused silica capillary column DB-5 (30 × 0.53 mm inner diameter, film thickness 1.0 μm, Sigma-Aldrich Co. LLC). The GC was operated using the following conditions: injection volume: 1.0 μl; initial column temperature: 110°C; heating rate: 8°C min-1; injector temperature: 220°C; split ratio 1:100; FID detector temperature: 250°C; and carrier gas: hydrogen. Standard curve of lauric acid was prepared making different concentration of lauric acid sample. The pure sample was diluted to make concentration gradient. Sample then run with GC and the result analyzed. The graph of area of the peak versus concentration was calibrated. The curve was calibrated using Microsoft Excel. The concentration of AM agent was calculated from standard curves.

Kinetic Test

For kinetics test, the result from release mechanism is use and plot on graph to determine the type of lauric acid kinetics. To determine the order of release of lauric acid from the film by graphical method that is zero order release model, first order model, Miltz release model and Higuchi release model from the dissolution data, a graph was plotted with remaining lauric acid concentration vs. time to confirm the order release, and a graph was plotted with log remaining lauric acid concentration vs. time to confirm first order release. If a straight line or linearity is observed, it can be confirmed that the lauric acid release follows the corresponding order kinetics. Regression co-efficient of the graph was found out to confirm the correlation between X and Y axis [14].

RESULT AND DISCUSSION

Antimicrobial Agent Migration in Food

Antimicrobial agent migrations was tested by using different food simulant which are water (represent water contained food), oil (represent fatty food), acetic acid (represent acidic food) and ethanol (represent alcohol food). The relevant of study the release of antimicrobial agent are to control the amount and the rate at which the active compound is released from the film to foodstuff. In the migration of antimicrobial mechanism in incorporated film, the AM agent is allowed to be migrating from the film surface to inhibit bacteria at the food surface during extended exposure.

Direct addition of AM into food will result immediate reduction of bacteria population, but it cannot retain long enough to inhibit any afterward bacterial attacks. Inversely, constant and long lasting AM release will be able to takes account of recovery of injured cell or the growth of the cell that were not destroyed by direct addition. It is important to achieve the most excellent migration rate of AM agent to food surface. The minimum and gradual migration is favored, but concentration of AM agent released also to be considered. Thus, mathematical model were takes part. The mathematical model was developed that could represent the release kinetics of the antimicrobial agent in different food stimulant [15].

It basically describes the releasing of antimicrobial agent from film to inhibit the growth of the bacteria by either reducing the growth rate and maximum growth population or extending the lag phase of the target microorganism or by inactivating microorganism by contact. The migration rate of AM such as chitosan and lauric acid might be slower because of the co-extruded characteristic formed when incorporating the film with additives.

Release Characteristics of Lauric Acid in Food Stimulant

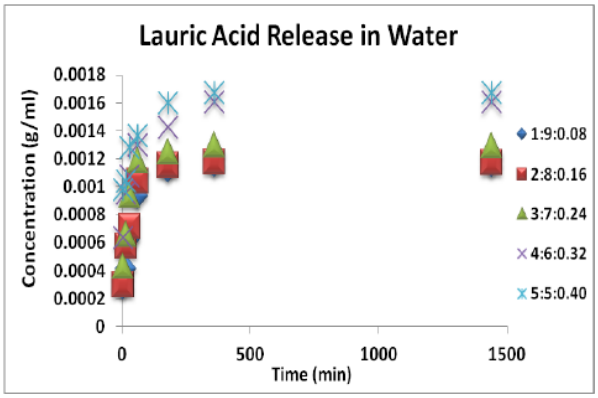

Figure 2: Lauric Acid Releases versus Time in Water Stimulant.

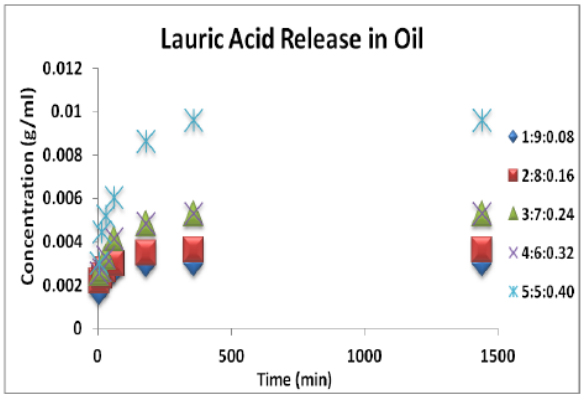

Figure 3: Lauric Acid Releases versus Time in Oil Stimulant.

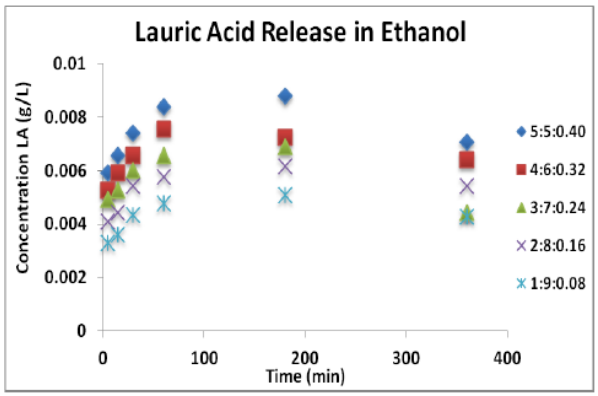

Figure 4: Lauric Acid Release versus Time Ethanol Food Stimulant.

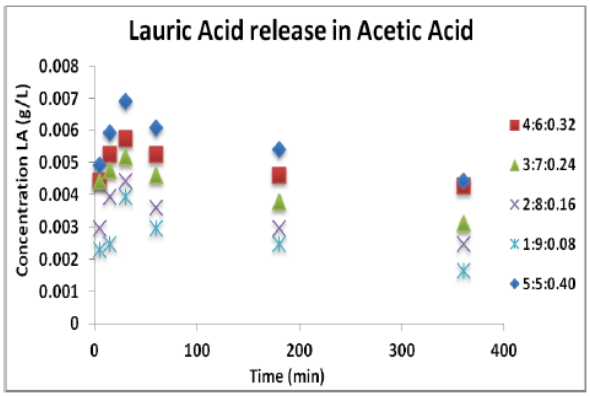

Figure 5: Lauric Acid Release versus Time in Acetic Acid Stimulant.

Figure 2, 3, 4 and 5 shows the patterns of all film ratio release from the concentration of 1:9:0.08 until 5:5:0.40 increasing as time increase and at certain point the graph remain constant for all food stimulants. The film ratio 5:5:0.40 have the highest lauric acid release concentration which is 1.67x10-3 mg/L while film ratio 1:9:0.08 gave the lowest release concentration of lauric acid 1.15x10-3 mg/L at the same time compared to others films in water stimulant. For oil stimulant at the same time, the film ratio 5:5:0.40 also gave the highest lauric acid release concentration which is 9.60x10-3 mg/L while film ratio 1:9:0.08 have the lowest release concentration of lauric acid 2.98x10-3 mg/L compared to others. The optimum time of lauric acid release rate in ethanol (alcohol) is 180 minutes (3 hour) meanwhile the optimum time of lauric acid in acetic acid (acidic) is 60 minutes (1 hour). After the optimum time, the concentration lauric acid continues to drop in ethanol due to Fischer esterification reaction (Figure 6) which convert lauric acid (carboxylic acid) and ethanol (alcohol) to ethyl laurate (ester). Although ratio of 5:5:0.40 release the higher lauric acid concentration in ethanol (alcohol) food stimulant and acetic acid (acidic), the lower ratio especially ratio 1:9:0.08 in other hand has the most consistent release throughout the period of time. This is due to ionic interactions between preservative and chitosan are likely to exist, which are expected to reduce the intramolecular electrostatic repulsion in the chitosan molecules and facilitated formation of intramolecular hydrogen bonding where it allow the lauric acid to release consistently [16]. The optimum point for release concentration of lauric acid in Figure 3and 4 were at 360 minutes (6 hours) as the release concentration of lauric acid at that time was the highest before the graph remains constant toward the end. It indicates that the films were in the best protection period against microorganism at that time as the antimicrobial is gradually release and kills the microorganism by diffusing into and distrupting the cell [17]. The packaging incorporated antimicrobial agent slowly released at the food

surface where they remain at high concentration for extended period of time [18]. Consequently, the optimum release of lauric acid for all films ratio is during 6 hours period and the release remains constant afterward.

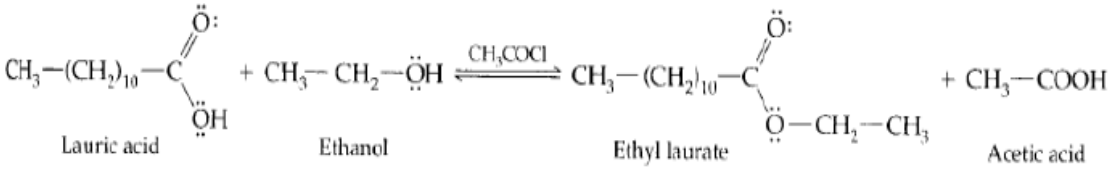

Figure 6: The Reaction of Fisher Esterification.

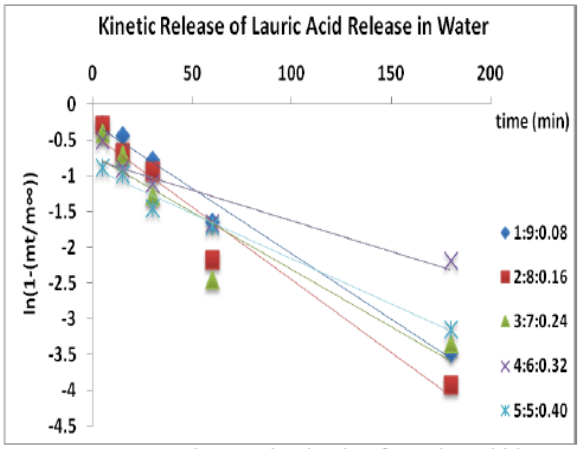

Figure 7: The Graph Kinetic of Lauric Acid in Water Stimulant.

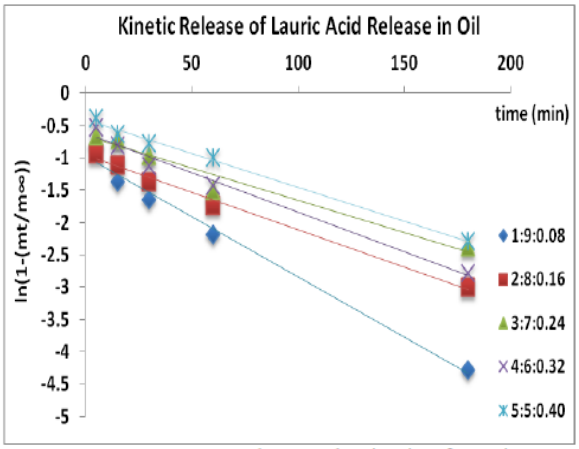

Figure 8: The Graph Kinetic of Lauric Acid in Oil Stimulant.

The graphs of release test showed all ratios of film were decreased concentrations of lauric acid against time. The optimal release time for each ratio is at the time of 6 hours. After 6 hours, the release of lauric acid is constant. This shows that the first order model can be used in finding the release rate for each ratio of the film. Plots of ln(1 - mt/m) versus time for SC 1:9, SC2:8 and SC 3:7 film are shown in figure 7 and 8 respectively. The linearity of these plots confirms the data are adequately described only for long-term migration in first order model. Plots of ln(1 - $mt/m\infty$) versus time for SC 1:9, SC 2:8, SC 3:7, SC 4:6, and SC 5:5 SC8:2 film in both water and oil food stimulant using first-order kinetic approach show the linearity. These systems are also consistent with first order kinetics because the modeling approach has the regression (R_2) of approximate to 1.

Higuchi Modelling for Release of Lauric Acid in Acidic and Alcohol Food Stimulant

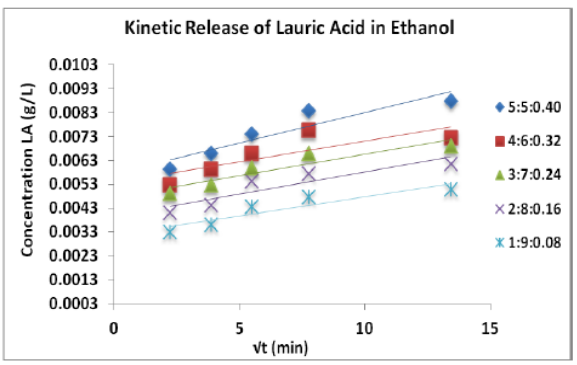

Figure 9: Graph of The Higuchi Kinetics Release of Film in Ethanol.

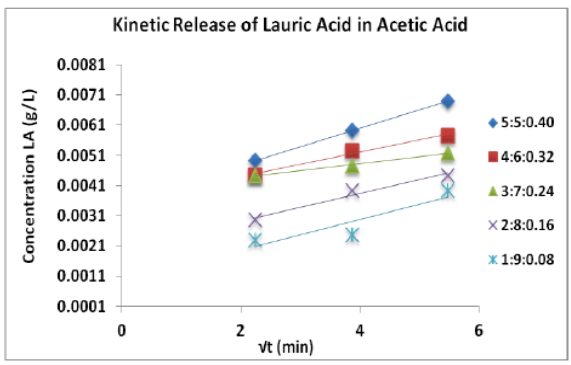

Figure 10: Graph of The Higuchi Kinetics Release of Film in Acetic Acid.

The kinetic release of lauric acid is analyzed by observing the graph in Figure 9 and 10 produced based on Higuchi model and steeper slope on graph will shows that the ratio produces higher kinetics release. Ratio of 5:5:0.40 observed to have higher kinetic release of lauric acid in both food stimulants since it has a steeper slope and high value of release rate than other ratio. The higher concentration of lauric acid may contribute to this result [198]. Also from the value of R2, the release of lauric acid is best fitted in Higuchi release model that stated the molecules of lauric acid that leach out of the system are rapidly until it reach a stable state.

From the results obtained the film formulation of 5:5:0.40 is preferred as it can release lauric acid at high concentration at short period because high initial rate of release of antimicrobial agent could inhibit the microbial growth. However, it is only optimum for 6 hours in ethanol (alcohol) and 1 hour in acetic acid (acidic food). The lower ratio of lauric acid has the consistent release rate of lauric acid to food which make it long term migrate process.

CONCLUSION

The kinetic rate and diffusion coefficient for water food stimulant is highest on 2:8:0.16 film ratio and for the oil stimulant give kinetic rate highest at 3:7:0.24 film ratio. The linearity of the plots from Miltz modeling for water and oil stimulants confirms the data are adequately described only for long-term migration in first order model. The film ratio of 5:5:0.40 has higher release and kinetic rate of lauric acid in both food stimulant which ethanol and acetic acid based on slope of graph that represent release rate. Also from the value of R_2 in the table, the release of lauric acid is best fitted in Higuchi release model that stated the molecules of lauric acid that leach out of the system are increase rapidly until it reach a stable state where high initial rate of release of antimicrobial agent could inhibit the microbial growth at the early stage of storage. However, film with low concentration of lauric acid especially 1:9:0.08 have more consistent release of lauric acid that more suitable for long migrate term. Based on the release analysis, the film ratio of 1:9:0.08 until 5:5:0.40 is more suitable in alcohol than in acidic food because the optimum time in alcohol is 6 hours higher than optimum time in acetic acid that is only 1 hour.

ACKNOWLEDGEMENT

The authors would like to thank the Research Management Centre (RMC), UTM for financial support of this project (Vot Number Q.J130000.2644.05J36).

REFERENCE

[1] Dutta, P. K., S., Mehrotra, G. K. and Dutta, J. (2009). Perspectives for Chitosan Based Antimicrobial Films in Food Application. *Food Chemistry*. 114, 1173-1182.

[2] Han Jung H., (2000). Antimicrobial Food Packaging, VOL. 54, NO. 3, Dept.of Food Science, University of Manitoba, Winnipeg, Canada.

[3] Salleh, E., Muhammad, I. I. and Khairuddin, N. (2009). Structural Characterization and Physical Properties of Antimicrobial (AM) Starch-Based Films. World *Academy of Sciences, Engineering and Technology*. 55, 432-440.

[4] Ayranci, F. and Tunc, S. (2003). A Method for the Measurement of the Oxygen Permeability and the Development of Edible Films to Reduce the Rate of Oxidative Reactions in Freshs Foods. Food *Chemistry*. 80, 423-431.

[5] V. Coma, I. Sebti, P. Pardon, A. Deschamps and H. Pichavant (2001). "Antimicrobial Edible Packaging Based on Cellulosic Ethers, Fatty Acids and Nisin Incorporation to Inhibit *Listeria Innocua* and *Staphylococcus Aureus*." *J. Food Prot*, 64 (4), pp 470-475.

[6] Beuchat, L.R and Golden, D.A. (1989). Antimicrobial Occurring Natually Naturally in Foods different molecular weight. Carbohydrate Polymers 54: 527-530. Food Technol. 43, 134-142.

[7] Appendini, P., Hotchkiss, J.H. 2002. "Review of Antimicrobial Food Packaging", *Innovative Food Science & Emerging Technologies*. Vol. 3, pp. 113.

[8] Han, J. H. (2003). Design of Antimicrobial Packaging Systems. *International Review of Food Science and Technology*. 11, 106-109.

[9] Luis Bastarrachea, Sumeet Dhawan, Shyam S. Sablani, (2010). Engineering Properties of Polymeric-Based Antimicrobial Films for Food Packaging, Springer Science+Business Media.

[10] Rejane C. Goy, Douglas de Britto, Odilio B. G. Assis (2009). A Review of the Antimicrobial Activity of Chitosan, *São Carlos, SP, Brasil*

[11] Mastromatteo, M., Barbuzzi, G., Conte, A., Del Nobile, M., (2008). Controlled release of thymol from zein based film. Innovative Food Science and Emerging Technologies 10 (2), 222–227.
[12] Luis Bastarrachea, Sumeet Dhawan, Shyam S. Sablani, (2010). Engineering Properties of Polymeric-Based Antimicrobial Films for Food Packaging, Springer Science+Business Media.
[13] Salleh, E., I. I. Muhamad, and N. Khairuddin (2007). Preparation, Characterization and Antimicrobial Analysis of Antimicrobial Starch-Based Film Incorporated with Chitosan and Lauric Acid. Asian Chitin Journal. 3: 55-68.
[14] Dhanya K , K Santhi, S A Dhanaraj and C I Sajeeth, (2011). Formulation And Evaluation Of Chitosan Nanospheres As A Carrier For The Targeted Delivery Of Lamivudine To The Brain, Department of Pharmaceutics, Grace College of Pharmacy, Kodunthirappully, Palakkad, Kerala, India. Faculty of Pharmacy, AIMST University, Malaysia.
[15] Gemili S., 2007.Ppreparation and Characterization of,Antimicrobial Polymeric Films for Food Packaging Applications. Ms. Sc. Thesis. Deparment of Chemical Engineering, Izmir Institute of Technology, pp. 11-91.
[16] Chen, M. H., Yeh, G. H., & Chiang, B. H. (1996). Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. Journal of Food Processing and Preservation, 20, 379–390.
[17] Torres, J.A., Bouzas, J.O., and Karel, M. (1985). *Microbial stabilization of intermediate*
[18] *moisture food surfaces. III. Effectiveness of surface preservative concentration and surface pH control on microbial stability of an intermediate moisture cheese analog*. J. Food Proc. Preserv. 9: 107-119
[19] Denyer, S. P. (1995). *Int. Biodeterior. Biodegrad.*, 36, 227.
[20] Ouattara, B., Simard, R. E., Piette, G., Begin, A. and Holley, R.A. (2000). Diffusion of Acetic and Propionic Acids from Chitosan-Based Antimicrobial Packaging Films. *Journal Food Science*. 65(5), 768-773.