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Computational Fluid Dynamics Simulations of Gas-liquid Two-phase Flow Characteristics through a Vertical to Horizontal Right Angled Elbow

N. Z. AUNG[1]* AND T. YUWONO[2]

Having a clear understanding on the phase distribution of gas-liquid two-phase flow through elbow bends is vital in mixing and separation system designs. This paper presents the computational fluid dynamics (CFD) simulations and experimental observations of gas-liquid two-phase flow pattern characteristic through a vertical to horizontal right angled (90°) elbow. Experimental observations were conducted in a transparent test section that consisted of a vertical pipe, elbow bend and horizontal pipe with an inside diameter of 0.036 m. The CFD simulations were performed by using a computer software package, FLUENT 6.2. Bubbly flow conditions were created in the vertical test section with the variation of superficial liquid Reynolds number from 13 497 to 49 488 and volumetric gas quality from 0.05 to 0.2. The CFD results showed a good agreement with experimental results in the following observations. The results showed that gas-liquid flow pattern inside and downstream of the elbow bend mainly depended on liquid velocity and it is also influenced by gas quality at high liquid velocities. At lower liquid velocities, gas-liquid separation began early in the elbow bend and gas-phase migrated to outer bend. Then, it smoothly transformed to stratified flow at elbow outlet. When the liquid velocity was further increased, the liquid phase occupied the outer bend rubbing the gas phase to the inner bend and delayed the formation of gas layer in the horizontal pipe. The increase of gas quality in higher liquid velocities promoted gas core formation at the elbow exit and caused wavy gas layers at the downstream of the elbow.

Key words: Gas-liquid; two-phase; flow pattern characteristic; vertical to horizontal; 90° elbow

The understanding of transport phenomena in multiphase flows plays a vital role in improving the performance of operating systems in boiling and condensing processes, hydrocarbon production and refining, minerals transport as well as power generation. Such transport phenomena are quite sensitive to the phase distribution in the flow termed as '*flow pattern*'. In turn, the flow pattern also mainly depends on the flow velocity and physical properties of each phase and pipe geometry (Akilli *et al*. 2001) In the case of pipe geometry, the correct usage of pipe bends is very critical in multi-phase flow piping system designs, since these can give strongly interference in phase distribution and consequently promote the vibration of the system (Abdulkadir *et al*. 2001) Hence, increasingly, attempts are being made to observe the effect of pipe bends (especially 90° elbows) on phase distribution and other flow related characteristics in multiphase flows.

Akilli (2001) conducted experimental measurements and CFD simulations of gas-solid concentration and velocity profile in horizontal pipe after vertical-to-horizontal 90°

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elbow bend. His distinct observation was that a strong rope was formed in the elbow bend and it disintegrated within 10*D* of the pipe after elbow exit. Huseyin (2004) has also performed investigations on gas–solid flow characteristics after a 90° vertical-to-horizontal elbow.

Yang and Kuan (2006) have measured the velocity fluctuation level of gas-solid flow inside a 90° elbow by using Laser Doppler Anemometer (LDA). They found that the level of velocity fluctuations in the solid phase was higher than that of the gas phase at the bend entrance because of particle-wall collisions. Kim *et al*. (2007) investigated the effect of 90° elbow on local void fraction distribution. According to their results, the elbow had more significant effect on phase distribution at further downstream (*L/D* = 43.9 after elbow) than immediate downstream (*L/D* = 18.1 after elbow). At the same time, Spedding and Benard (2007) performed pressured drop measurements through a vertical to horizontal 90° elbow bend. They proposed a general correlation for prediction of gas-liquid two-phase pressure drop for elbow bends. The same authors (2008) also reported the pressure drop characteristics of water-oil-air three-phase flow through a vertical to horizontal 90° elbow bend. Concerning the air-water two-phase flow pressure drop in vertical to horizontal internal wavy 90° elbow bends, Benbella *et al*. (2009) carried out a research. Their results demonstrated that wavy 90° wavy elbows had total pressure about 2–5 times greater than smooth bends. In the work of Abdulkadir *et al*. (2011), the effect of 90° bends on air-oil (silicon) flow pattern were observed using advanced instruments such as Electrical Capacitance Tomography (ECT), Wire Mesh Sensor Tomography (WMS) and high-speed video. Changing the flow velocities, they had discovered transitions of flow pattern before and after elbow bends. They concluded that horizontal bend has less effect on the flow patterns compared with the vertical bend.

Zhang *et al*. (2012) performed CFD simulations to observe erosive ware damages (puncture point locations) in elbow bends by varying slurry velocity, bend orientation and bend angle. They discovered that the location of the maximum erosive location moved to downstream (elbow exit) when slurry velocity increased. Liu *et al*. (2012) also experimentally studied air-water flow induced fluctuating force on a 90° elbow. They discussed force fluctuation phenomenon matching with the observations of flow pattern such as bubbly flow, slug flow and churn flow. For forgoing review, a summary of information of previous researches that focused on 90° elbow bend is shown in *Table 1*.

From the entire review, it is very obvious that multiphase flow phenomena before, inside and after elbow bend are very violent and the effects are undesirable. However, the existence of bended pipes is very common in conveying process of oil and gas mixture from downhole to separator because they are absolutely necessary for flow directional changes. Thus, in designing such bended pipes, a clear understanding of phase distribution (or flow pattern) before, inside and after the bend is very critical since these can negatively affect the performance of operating system.

However, available information in literature is mostly based on probe measurement. Indeed, experiences in visual observations of multiphase flow phenomena without any intrusive measuring tool are still lack and needed to obtain a better understanding on the phase distribution through bended pipes. Even though some visual observations can be found in Abdulkadir (2011), the focus point was only inside the elbow bend. Without having visual information at the downstream of the elbow bend, a sharp imagination still cannot be made in designing a bend pipe. Moreover, long elbow bends are also becoming attractive in industrial applications and related information is in demand.

Table 1. Summary of previous researches that focused on effect of elbow.

Research (Reference no.)	Multiple-phase classification	Elbow geometry	Methodology
Huseyin *et al.* 2004	Gas-solid (Two-phase)	R/D = 1.5,3 (vertical to horizontal upward flow)	Experiment, CFD simulation
Kim *et al*. 2007	Gas-solid (Two-phase)	R/D = 1.5,3 (vertical to horizontal upward flow)	Experiment
Liu *at al*. 2012	Gas-solid (Two-phase)	R/D = 1.5 (horizontal to vertical upward flow	Experiment
Mahvash & Ross 2008	Gas-liquid (Two-phase)	R/D = 1.515 (horizontal to horizontal, the same plane flow)	Experiment
Mahmoud *et al*. 2012	Gas-liquid (Two-phase)	R/D = 0.654 (vertical to horizontal upward flow	Experiment
Margot *et al*. 2012	Gas-liquid-liquid (Three-phase)	R/D = 0.654 (vertical to horizontal upward flow	Experiment
Riverina *et al*. 2006	Gas-liquid (Two-phase)	R/D = 4,6,8,10 (vertical to horizontal upward flow	Experiment and correlations
Singhal *et al*. 2002	Gas-liquid (Two-phase)	R/D = 2.3 (vertical to horizontal upward flow)	Experiment
Spedding & Benard 2007	Gas-solid (Two-phase)	R/D = 3.255 (various orientations)	CFD simulation
Spedding *et al*. 2008	Gas-liquid (Two-phase)	R/D = 1.451 (vertical to horizontal upward flow)	Experiment

In this regard, the aim of this work is to experimentally and numerically observe phase distribution (the flow pattern) characteristic of gas-liquid two-phase bubbly flow through a vertical to horizontal right angled (90°) long elbow bend with a wide focusing view and to provide more descriptive information.

NUMERICAL SIMULATION

Advanced computational methods in fluid dynamics are becoming powerful and capable of modeling multiphase flows and the acceptable simulated results are at proven stage in many researches (Akilli 2001; Zhang 2012; Singhal 2002). Thus, the CFD simulations of gas-liquid two-phase flow through a vertical to horizontal elbow bend are also performed to confirm the experimental observations in this work.

Governing Equations

Flow governing equations for a two-phase mixture are obtained from the ensemble averaging of the Navier–Stokes equations and the following mass and momentum continuity equations are solved in computation.

Continuity equation:

$$\frac{\partial(\rho_m)}{\partial t} + \nabla\cdot(\rho_m \vec{u}_m) = 0 \tag{1}$$

Momentum transfer equation:

$$\frac{\partial(\rho_m \varepsilon)}{\partial t} + \nabla\cdot(\rho_m \vec{u}_m \vec{u}_m) = -\nabla p + \nabla\cdot[\mu_m(\nabla\vec{u}_m + \vec{u}_m^T)] + \rho_m \vec{g} + \vec{F} + \nabla\cdot\left(\sum_{k=1}^{n} \alpha_k \rho_k \vec{u}_{dr,k} \vec{u}_{dr,k}\right) \tag{2}$$

where, the subscript m, k, dr describe the mixture, secondary phase and drift respectively, ρ is density, t is time, μ is viscosity, $\vec{u}$ is velocity, p is pressure, $\vec{F}$ is body force vector, α is volume fraction.

For having reasonable accuracy (Singhal 2002; Margot 2002; Mahmoud 2012) the standard k-ε model is adopted to solve the set of turbulent kinetic energy and dissipation rate equations. The equations for mixture turbulent kinetic energy and its dissipation rate are obtained by the summation of mixture as a single phase.

Turbulent kinetic energy equation:

$$\frac{\partial(\rho_m k)}{\partial t} + \nabla\bullet(\rho_m \vec{u}_m k) = \nabla\bullet\left(\frac{\mu_{t,m}}{\sigma_k}\nabla k\right) + G_{k,m} - \rho_m \varepsilon \qquad (3)$$

Turbulent dissipation equation:

$$\frac{\partial(\rho_m \varepsilon)}{\partial t} + \nabla\bullet(\rho_m \vec{u}_m \varepsilon) = \nabla\bullet\left(\frac{\mu_{t,m}}{\sigma_k}\nabla \varepsilon\right) + \frac{\varepsilon}{k}(C_{1\varepsilon} G_{k,m} - C_{2\varepsilon}\rho_m \varepsilon) \qquad (4)$$

The turbulent viscosity of the mixture was calculated by:

$$\mu_{t,m} = C_\mu \rho_m \frac{k^2}{\in} \qquad (5)$$

where the subscript t represents for turbulent, k is turbulent kinetic energy, ε is turbulent dissipation rate, $C_{1\varepsilon}$, $C_{2\varepsilon}$, C_μ, σ_k and σ_ε are the standard k–ε model constants and G is the turbulence production term. This standard model is used without any further modifications taking $C_{1\varepsilon}$ =1.44, $C_{2\varepsilon}$ =1.92, C_μ =0.09, σ_k =1 and σ_ε =1.3 since they have been accepted in a wide range of wall-bounded air and water turbulent flows.

Computational Domain

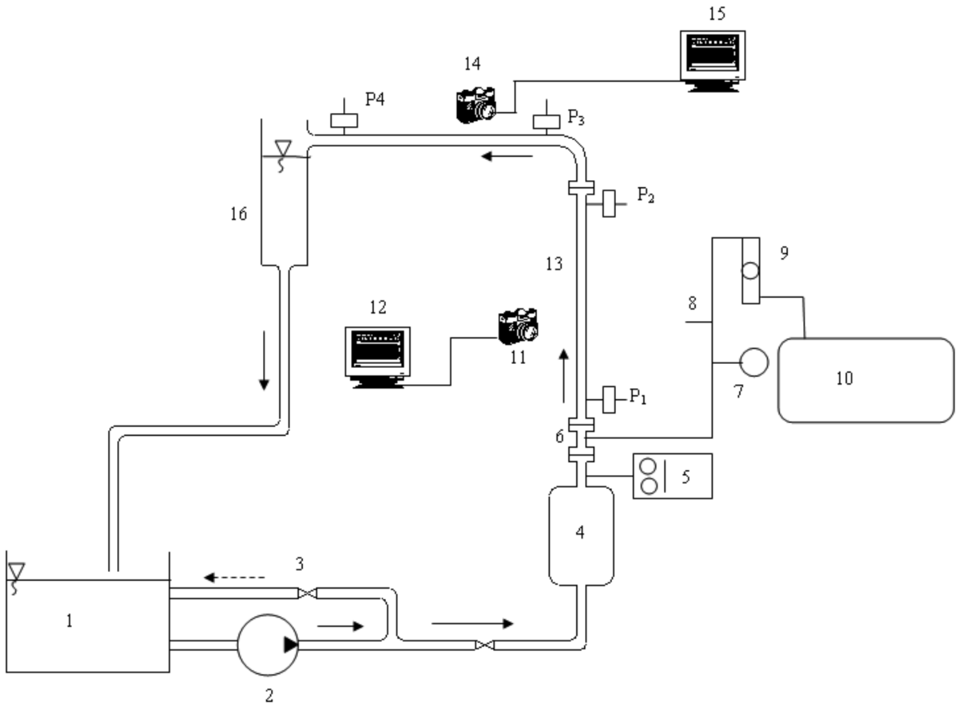
The whole test section is considered as the computational domain and constructed in GAMBIT 2.2. It is shown in *Figure 3a–3b.* The mesh has 240 000 *hexahedral elements*. The base of vertical pipe is set as velocity inlet condition and the end of horizontal as outflow condition. No slip condition is considered at the walls.

Solving Strategies

A commercial CFD package FLUENT 6.2 is used to solve the set of governing equations; continuity, momentum, and turbulent k-ε equations. Velocities of phases and gas phase fraction are set as known inlet boundary condition for every run. The SIMPLEC algorithm is used for coupling between velocity and pressure. The second-order upwind discretization scheme is used for the momentum equations while first-order upwind discretization is used for volume fraction and k-ε equations. The convergence criteria are third order residual value of each parameter and deviation of 10^{-5} between the inlet and outlet mass flow rates to satisfy the continuity law.

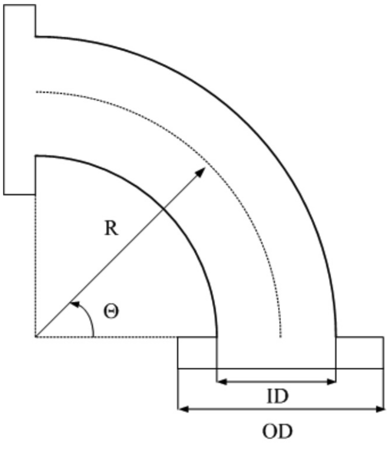
EXPERIMENTAL SETUP AND PROCEDURE

The schematic diagram of constructed air-water two-phase flow loop is shown in *Figure 1.* The test section consists of 2.1 m long vertical pipe, elbow bend and a horizontal pipe that runs 1 m after the elbow exit. The vertical and horizontal pipes are acrylic pipes which have inside diameter of 0.036 m. The elbow bend is also made of acrylic material and has the same inside diameter. The detail structure of the elbow bend is shown in *Figure 2*. Aiming to create bubbly flow in vertical test section, the air is injected at the base of vertical test section by using radial injectors. There are 32 ports of 710 µm diameter along the periphery of the injector. The liquid flow rate is measured by using "*Doppler*" flow meter and the gas flow rate is measured by using float type (*Dwyer Rate-Master*) gas flow meter. Two high speed digital cameras are used to capture the visual observation of flow patterns in vertical pipe, inside elbow bend and at its downstream. Experiments are conducted



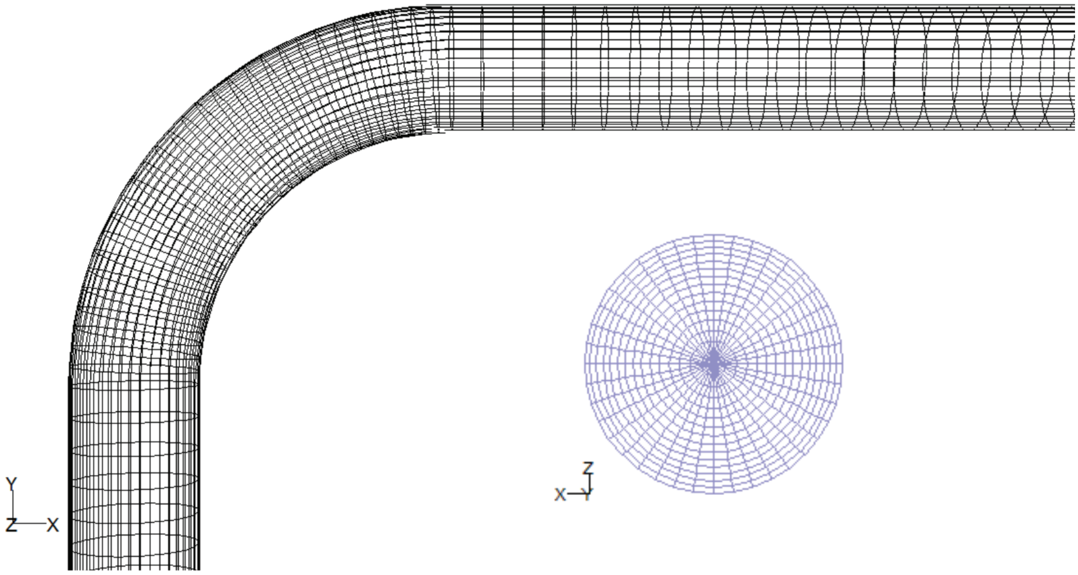
1. Water tank
2. Pump
3. Regulation system (Bypass)
4. Accumulator
5. Doppler flow meter
6. Annular air injector
7. Pressure gauge
8. Thermocouple
9. Rotameter
10. Compressor
11. Digital camera 1
12. Photo editing system 1
13. Test section
14. Digital camera 2
15. Photo editing system 2
16. Gas-liquid separator

Figure 1. Schematic diagram of experimental test loop.



Curvature radius, R = 0.09 m
Inside diameter, ID = 0.036 m
Outside diameter, ID = 0.04 m

Figure 2. Detail structure of elbow bend.



(a)

(b)

Figure 3. Computational grid structure of test section: (a) Side view (x-y plane) (b) Cross-sectional view (x-z plane and y-z plane).

by varying superficial liquid velocity and volumetric gas quality. The liquid superficial velocity (in terms of Reynolds number) is varied in the range of Re_{SL}=13497 to 49488 and the gas volumetric quality (β) is varied in the range of 0.05–0.2. These initial parameters are calculated from measureable parameters by using equations expressed in Appendix.

RESULTS AND DISCUSSION

Flow Pattern in Vertical Test Section

In this work, for all flow conditions, bubbly flow regimes are created in the vertical test section at the upstream of elbow bend. The visual observations of bubbly flow are captured with a high speed digital camera at a height of 0.35 m above the air injector. Some sample observations of the bubbly flows in vertical test section are shown in *Figures 4a–4c*. The observed bubbly flows can be classified into three categories.

Clustered bubbly flow. This kind of flow occurs at high superficial liquid velocities (Re_{SL} = 40490, Re_{SL} = 49488) with low volumetric gas qualities (β = 0.05, β = 0.07) as shown in *Figure 4a*. The gas phase (bubbles) are cluttered in liquid medium forming bubble-clusters. In other word, the bubble distribution is not symmetric to the vertical axis of the pipe at any elevation and a series of bubble-clusters is formed along the pipe.

Homogeneous bubbly flow. This flow condition is observed at every flow condition with medium range of volumetric gas qualities (β= 0.09–0.13) as shown in *Figure 4b*. The bubble size becomes larger and it seems that the bubbles occupy homogeneously over the entire cross-section of the pipe at any elevation.

Dense bubbly flow. *Figure 4c* shows dense bubbly flow condition. It is clear that this kind of bubbly flow occurs at high gas qualities.

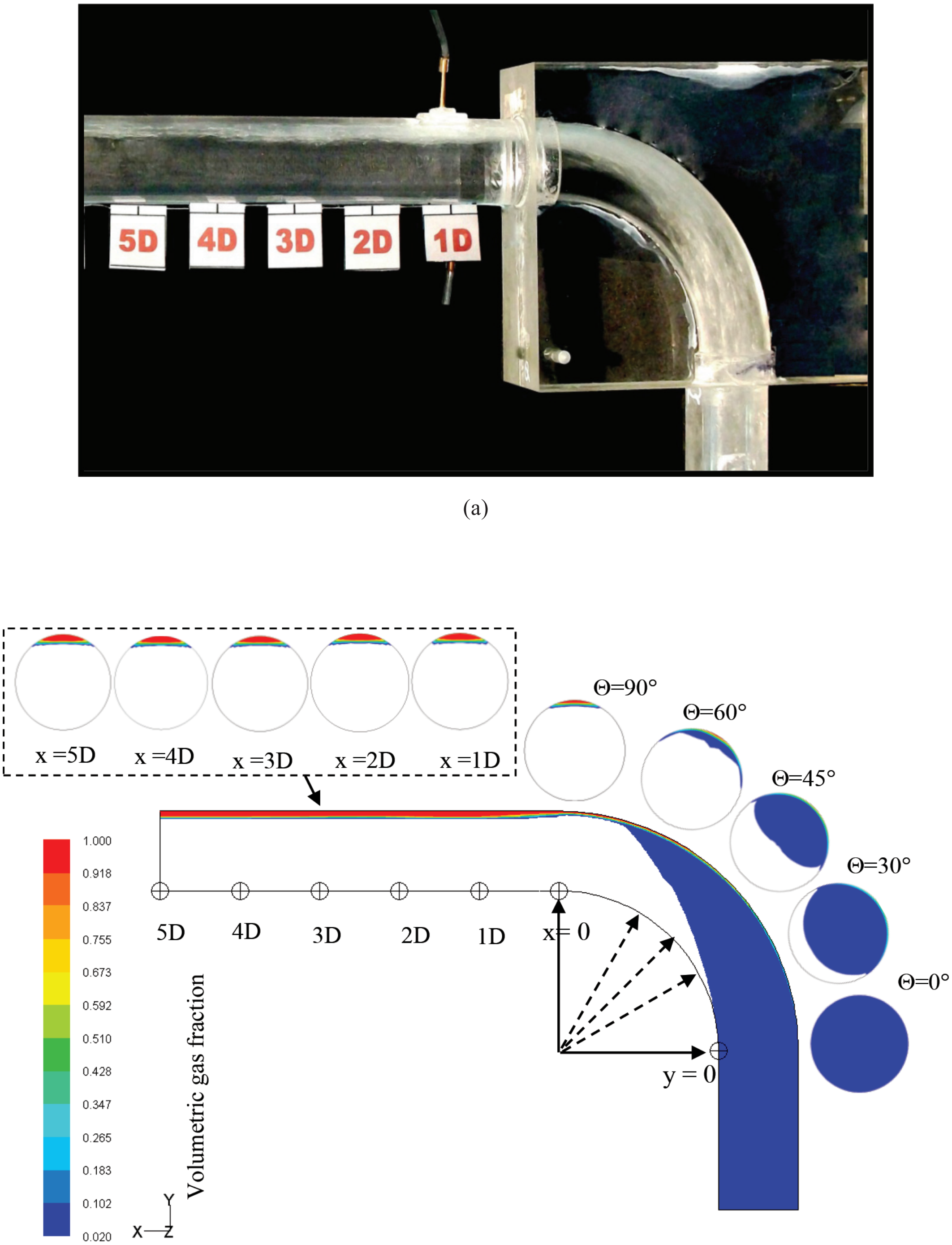
(a) (b) (c)

Figure 4. Visual observations of bubbly flow in vertical test section: (a) Re_{SL} *= 49488; β = 0.05; (b)* Re_{SL} *= 31492; β = 0.11; (c)* Re_{SL}*= 40490; β =0.2.*

The entire cross sectional area of the pipe is filled with bubbles, but formation of elongated bubbles is not yet observed.

Flow Pattern in Elbow Bend and Downstream

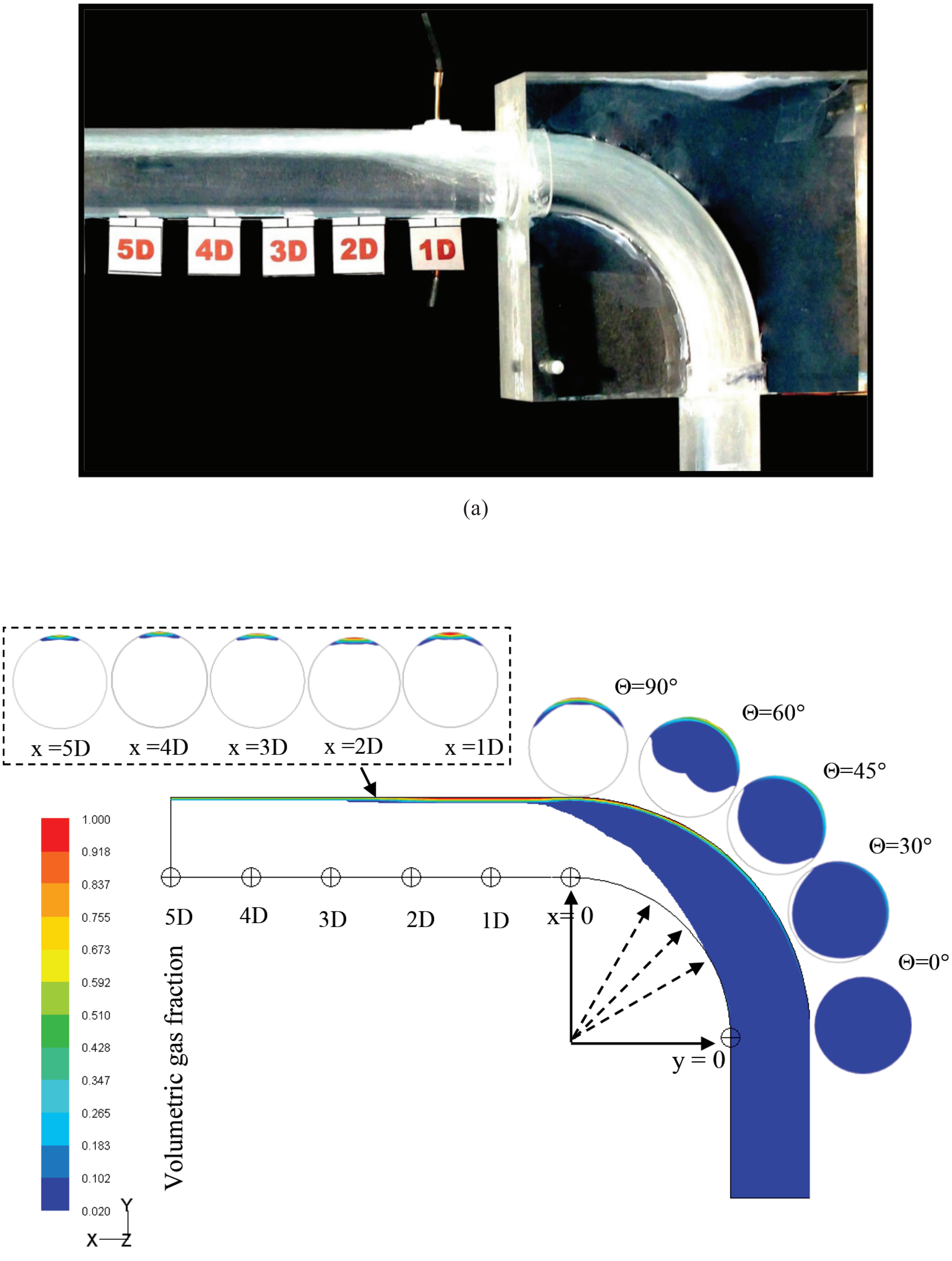
Figures 5–9 show the experimental observations and numerically simulated results of gas-liquid two-phase flow patterns through the elbow bend for flow conditions of Re_{SL} = 13497−49488 with constant volumetric gas quality β = 0.2. The numerical results are taken from both longitudinal mid-plane and transverse cross-sectional plane at various distances inside and after elbow bend. The experimental results clearly state that the two-phase flow pattern is mainly governed by liquid velocity. For flow conditions with low liquid velocities (*Figures 5−6*), the gas phase starts to separate from inner wall about bend angle (Θ) of 30°−45° and flows up to the outer wall. It can be explained that the momentum and buoyancy force attained by bubbles overcomes the pressure at the outer surface of the elbow and the bubbles try to migrate to the outer surface. Then, gas-liquid stratification begins before the exit of the elbow bend. Further increase of liquid velocity (Re_{SL} = 31492) delays the gas phase separation from inner surface of elbow bend showing uniformly distribution in the elbow bend. At the outlet of elbow bend, the gas phase moves up to upper surface of horizontal pipe. Formation of gas layer initiates about 2D−3D after elbow exit. *Figures 8−9* depict comparisons of recorded and computed results for flow conditions of Re_{SL} = 40490 and Re_{SL} = 49488 with constant gas quality. In contrast to the former observations, the gas phase leaves away from the outer surface of the elbow and totally concentrates on inner bend. The whole outer surface of the elbow bend is free from migration of gas bubbles and the entire inner surface is apparently covered by gas layer. This phenomenon is related to the increasing of pressure at the outer bend. When the mixture enters into the elbow bend with a high velocity, the liquid phase with higher



(a)

(b)

Figure 5 (a)–(b). Experimental and numerical observation of two-phase flow pattern through elbow bend at $\mathrm{Re}_{\mathrm{SL}}$ *= 13497,* β *=0.2.*



(a)

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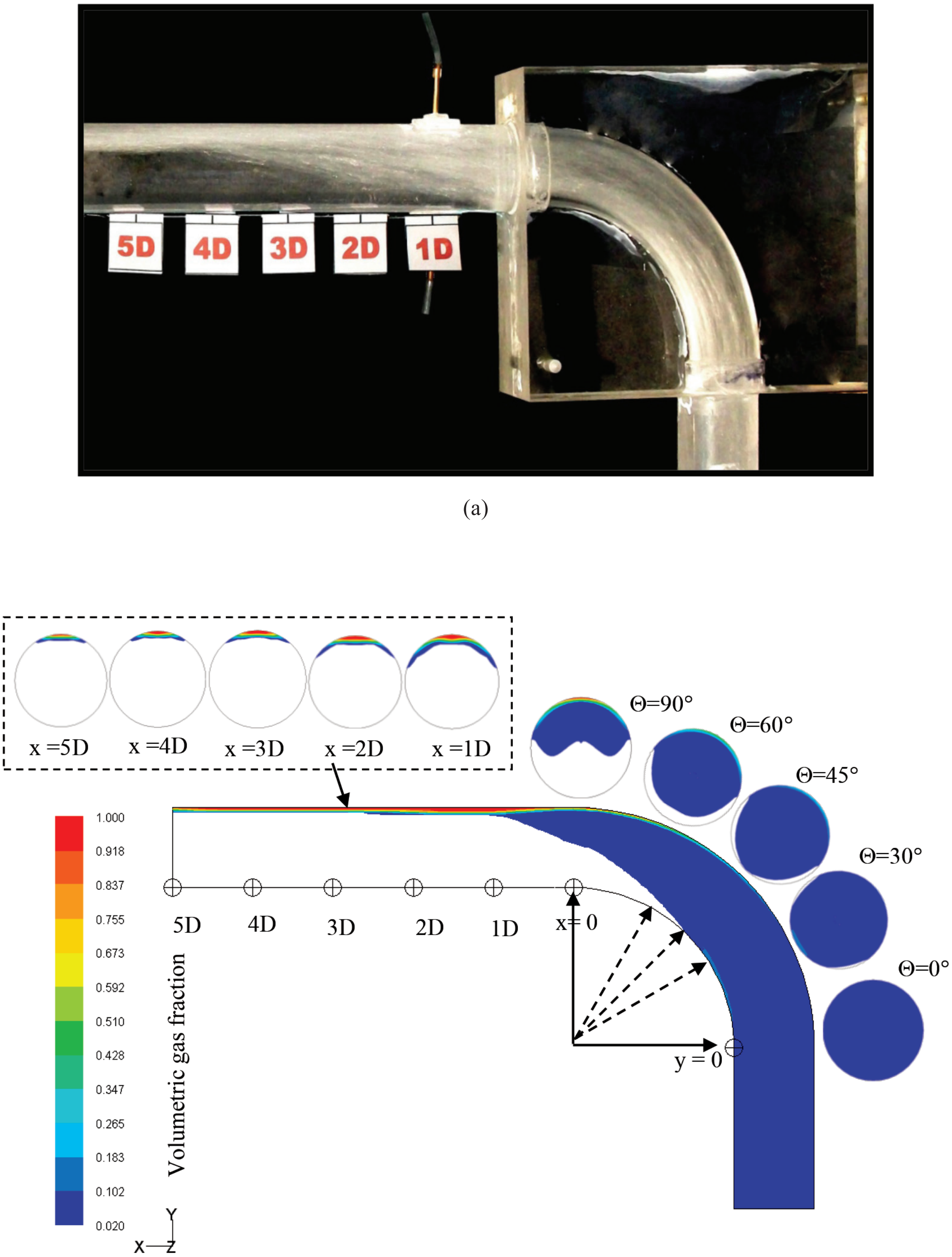
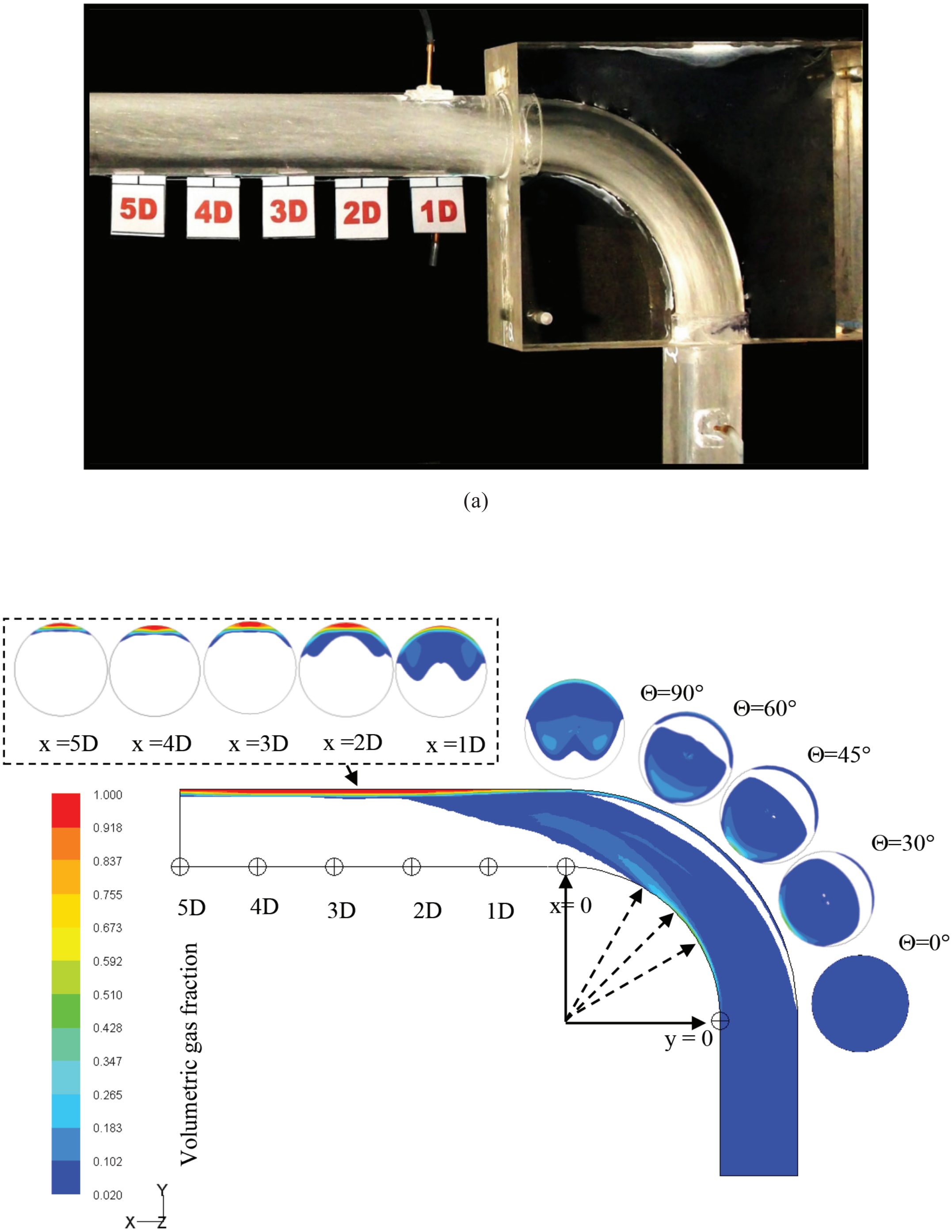
Figure 6 (a)–(b). Experimental and numerical observation of two-phase flow pattern through elbow bend at Re$_{SL}$ *= 22494,* β *=0.2.*



(a)

(b)

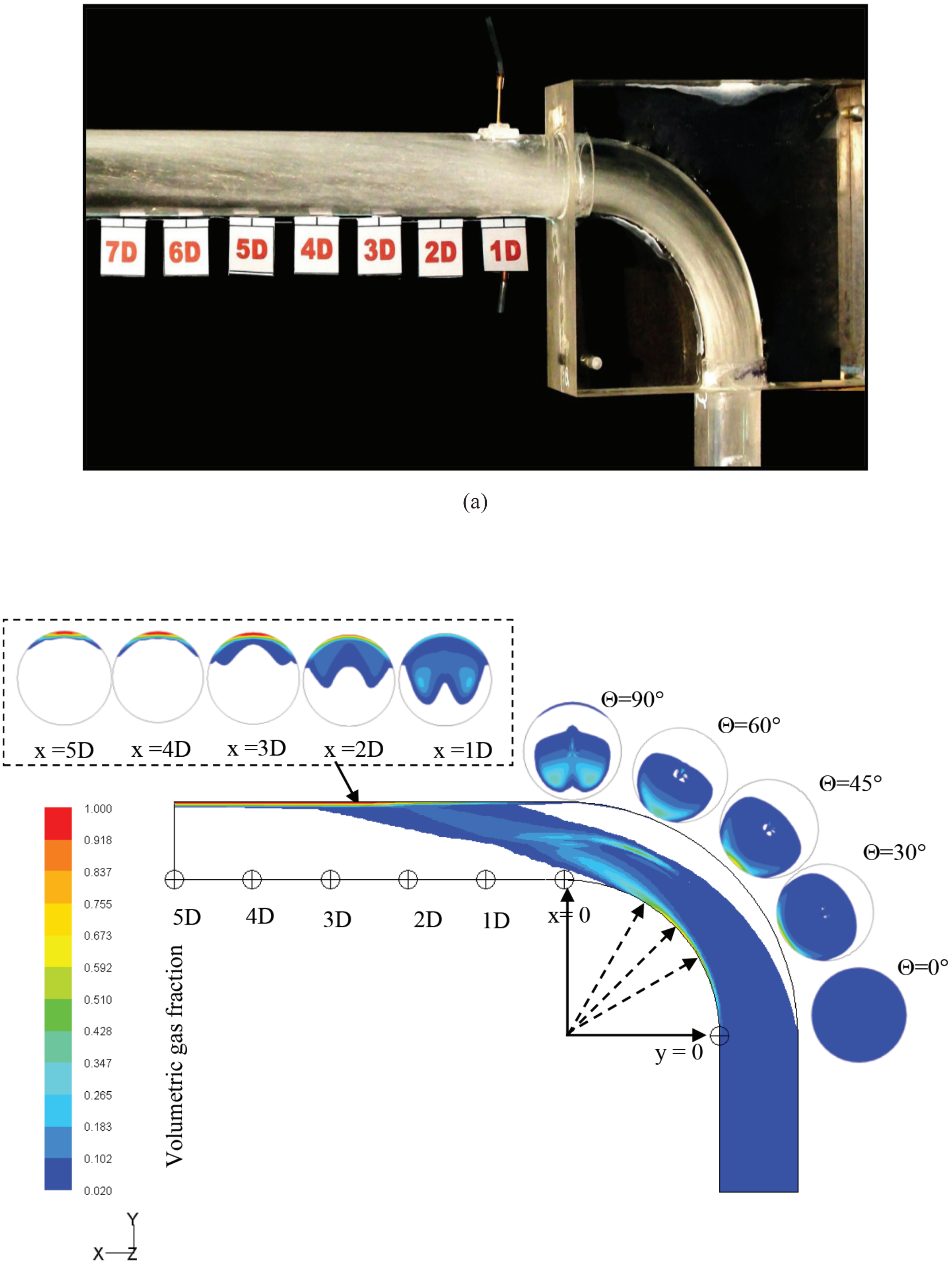
Figure 7 (a)–(b). Experimental and numerical observation of two-phase flow pattern through elbow bend at Re_{SL} *= 31492,* β *=0.2.*



(a)

(b)

Figure 8 (a)–(b). Experimental and numerical observation of two-phase flow pattern through elbow bend at Re_{SL} *= 40490,* β *=0.2.*



(a)

(b)

Figure 9 (a–b). Experimental and numerical observation of two-phase flow pattern through elbow bend at Re_{SL} *= 49488,* β *=0.2.*

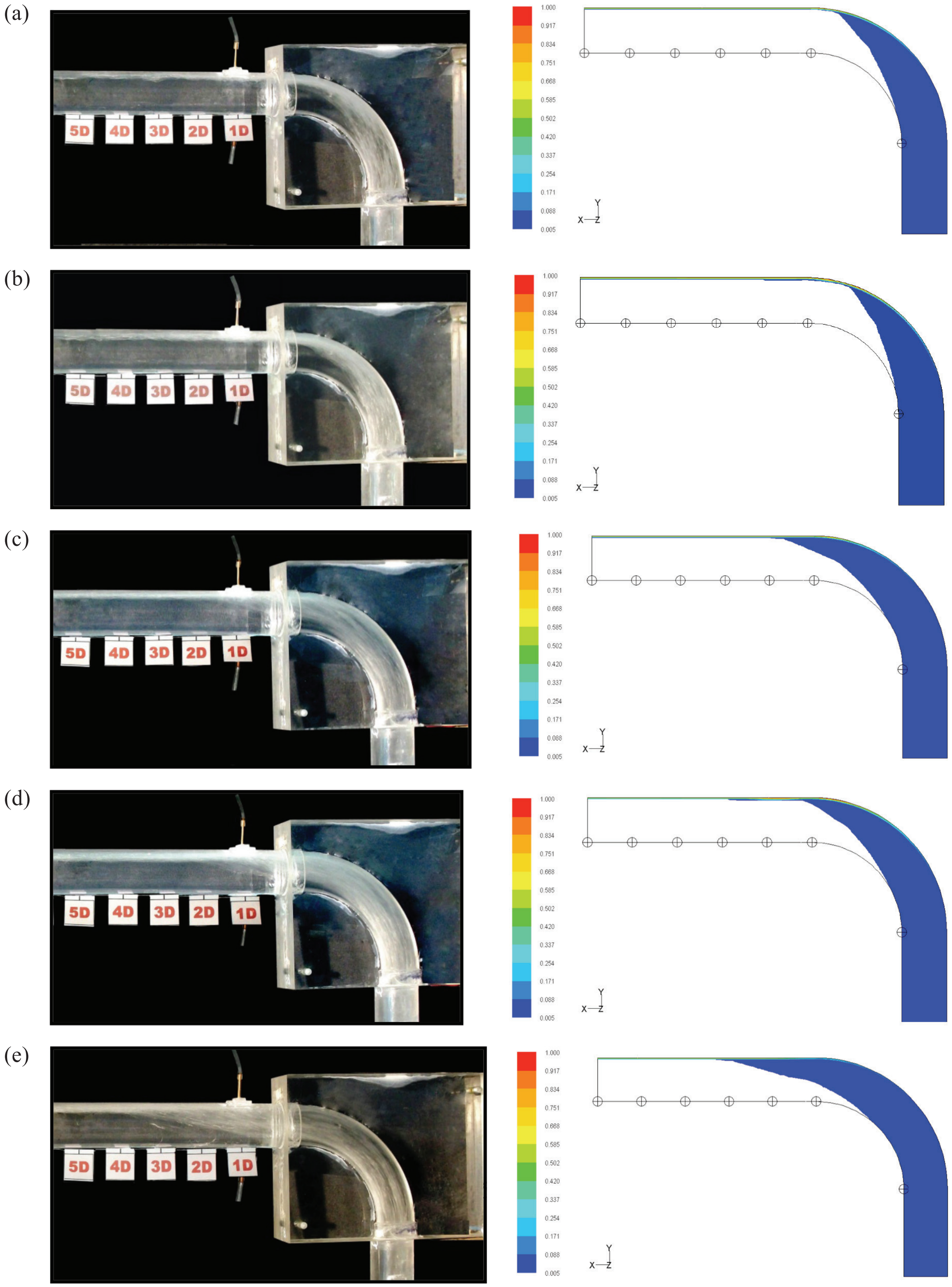


Figure 10(a)–(e). Experimental and numerical observation of two-phase flow pattern through elbow bend at: (a) Re_{SL} *= 13497, β = 0.11; (b) ReSL = 13497,* β *= 0.15; (c)* Re_{SL} *= 22494,* β *= 0.11; (d)* Re_{SL} *= 22494,* β *= 0.15; (e)* Re_{SL} *= 31493,* β *= 0.11.*

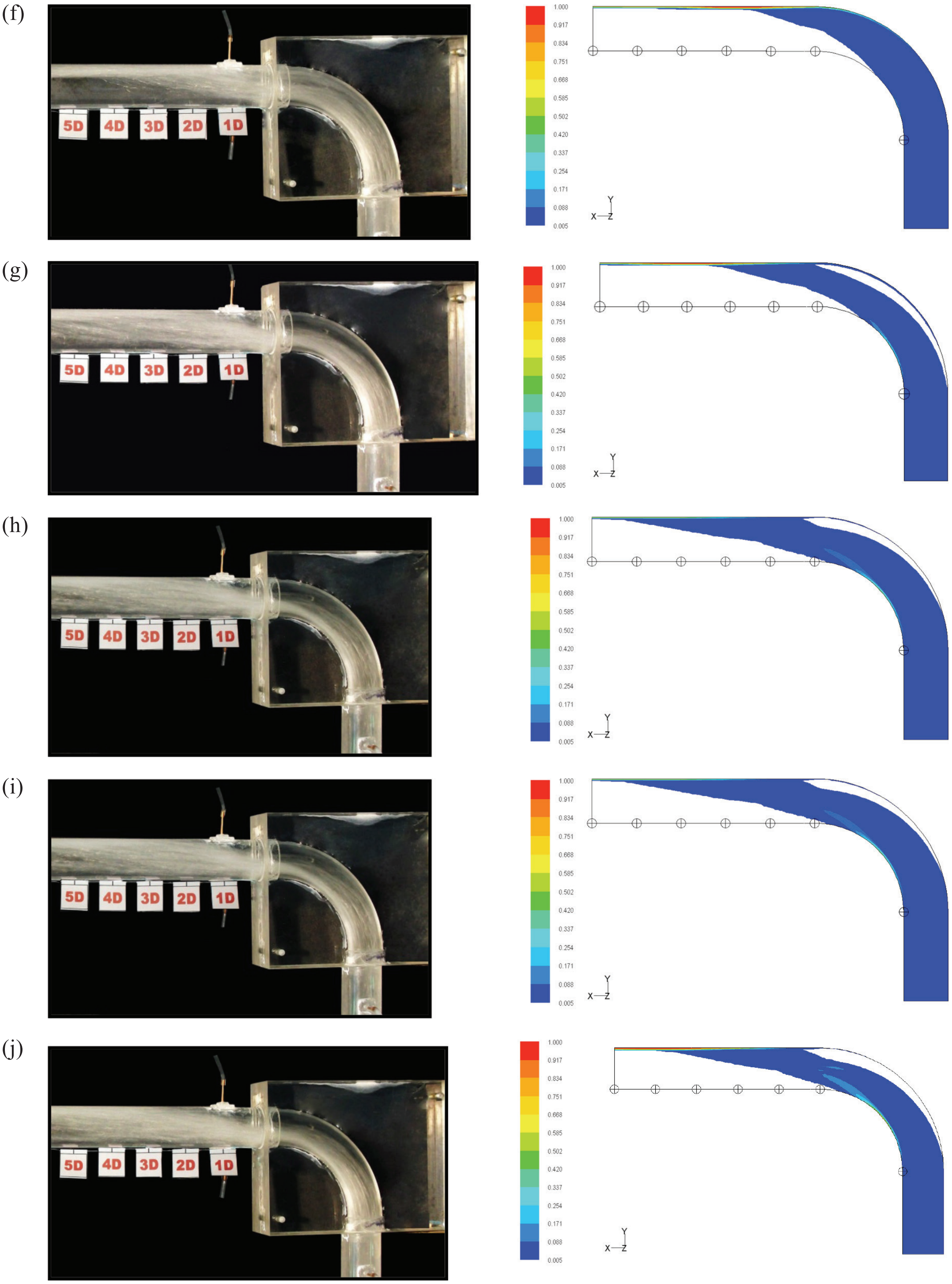


Figure 10(f)–(j). Experimental and numerical observation of two-phase flow pattern through elbow bend at: (f) Re_{SL} *= 31492,* β *= 0.15; (g)* Re_{SL} *= 40490,* β *= 0.11; (h)* Re_{SL} *= 40490,* β *= 0.15; (i)* Re_{SL} *= 49488, β = 0.11; (j)* Re_{SL} *= 49488,* β *= 0.15.*

momentum impinges the outer surface and increases the surface pressure by pushing the gas phase to the lower pressure region. However no coalescence of bubbles is found at the inner surface since centrifugal force accelerates the gas bubbles without giving a chance to merge each other. From numerical results (*Figures 8(b)−9(b)*), it is clearly seen that the formation of low pressure region at the inner bend creates transverse flow (or secondary flow) from elbow exit to about 1*D*. Therefore the bubbles cannot move up to the upper surface of horizontal pipe until a far distance after the elbow outlet. The bubbly flow is maintained until 5*D* to 6*D* after the elbow exit. Then, it starts to develop gas-liquid stratified flow pattern in horizontal pipe. It can be understood that elbow bend causes liquid velocity-dependent interference in flow pattern transition from vertical to horizontal.

Observed gas-liquid two-phase phenomena through elbow bend with the variation of gas quality are described in *Figures 10*. For lower constant liquid velocities (*Figures 10(a)−10(d)*), the increase of gas velocity enhances the gas layer formation near the elbow exit. However, the increase of gas quality promotes the interference at high liquid velocities forming a gas core in horizontal pipe (*Figure 10(g)−10(j)*). It postpones the formation of stratified flow pattern and creates wavy gas layer at downstream. Therefore, it can be concluded that the phase distribution after elbow bend also relates to gas quality in the mixture.

CONCLUSION

Designing a suitable elbow bend is a critical issue in separation and mixing processes. Since long elbow bends are becoming attractive in such applications, phase distribution (the flow pattern) characteristic of gas-liquid two-phase bubbly flow through a vertical to horizontal right angled (90°) long elbow bend is experimentally observed. Then numerical simulations are also performed by using CFD software, FLUENT 6.2 and qualitatively compared with experimental results. The constructed test section diameter was 0.036 m and the elbow curvature to diameter ratio (*R/D*) was 2.5. Different flow conditions were created by varying superficial liquid velocity and gas quality.

The experimental and numerical results from this work were of good agreement. These results provided a clear view and understanding of phase interaction and distribution phenomena before, inside and after long elbow bend. Based on recorded observations, it could be concluded that phase distribution inside and after elbow bend mainly depended on liquid velocity. At lower liquid velocities, the gas bubbles migrate at the upper surface of bend and smoothly changed to gas layer at the elbow exit. When the liquid velocity was further increased, it occupied the outer surface, suppressed the gas phase to inner region of the bend and delayed the formation of stratified flow pattern. The increase of gas quality in a high liquid velocity also caused wavy gas layer after the elbow bend.

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APPENDIX 1

Superficial liquid velocity, $U_{SL} = \frac{Q_L}{A}$ (6)

Superficial gas velocity, $U_{SG} = \frac{Q_G}{A}$ (7)

Volumetric quality, $\beta = \frac{Q_G}{Q_L + Q_L}$ (8)

Superficial liquid Reynolds number,

$$Re_{SL} = \frac{\rho_L U_{SL} D}{\mu_L} \tag{9}$$

Superficial gas Reynolds number,

$$Re_{SG} = \frac{\rho_G U_{SG} D}{\mu_G} \tag{10}$$

where, the subscripts *G, L, SG, SL* denotes gas, liquid, superficial gas, superficial liquid respectively, *U* is superficial velocity, *Q* is flow rate, *Re* is Reynolds number, *D* is pipe diameter.

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Performance Appraisal of Non-governmental Organizations: A Discussion on Pakistan 2010 Floods Response and Recovery

A. ALI[1]*, N. BAIG[1] AND A. KHAN[1]

The paper examines in detail the role of NGOs (Non-governmental Organizations) in response and recovery phase of a disaster. Donors, INGOs (International Non-governmental Organizations) and NGOs work for the betterment of affected community but due to certain shortfalls, the community did not benefit as much as intended. In this research those gray areas are highlighted in detail through field visits and interaction with the community devastated by 2010 floods. Lack of inter-agency co-operation, partnership with inexperienced local NGOs and lack of transparency and accountability mechanisms are those areas which reduce the effectiveness of response and recovery efforts. These gray areas cause loss of precious resources and duplication of efforts which results in increased vulnerability of that particular community.

Key words: Disaster; 2010 flood; response; recovery; non-governmental organizations; international non-governmental organizations; community; inter-agency co-operation

In the last week of July 2010, most of the Indus River basin in Pakistan submerged due to torrential rains. Resulting floods severely affected about 20 million people and their property, infrastructure and livelihood along with 1985 deaths and USD9.5 billion economic loss (Andrew *et al.* 2010).

Floods has destroyed 728 192 houses entirely and rendered more than 385 325 houses non-residential due to rigorous damages. These damages were mostly visible in districts of Muzafargarh and Rajenpur in Punjab, Nowshera and Dera Ismail Khan in Khyber-PukhtunKhwa, and Jafarabad, Jacobabad, Shikaarpur and Thatta in Sindh. The provision of critical facilities like schools and healthcare centres were severely affected by floods. More than 7600 schools were completely destroyed and needed to be reconstructed while 436 health units were either destroyed or severely damaged (Maryum *et al.* 2012). Electric power supply, foods and water supply were interrupted by floods in most parts of the country and destroyed most of the food stocks like wheat and other food stuffs across the country and created shortage of these commodities in the affected areas. Livestock and fisheries were destroyed and tube wells were contaminated and clogged with mud carried by flood water.

In district Nowshera of KPK, Nowshera Kalan is one of the most affected areas in 2010 floods as shown in *Figure 1* (Mateeul *et al.* 2012).

Nowshera Kalan was totally submerged in flood water which rendered more than 700 000 peoples homeless in Nowshera. Dead animals and dead bodies were lying in the streets, river and roads and it had a stinging smell in the air.

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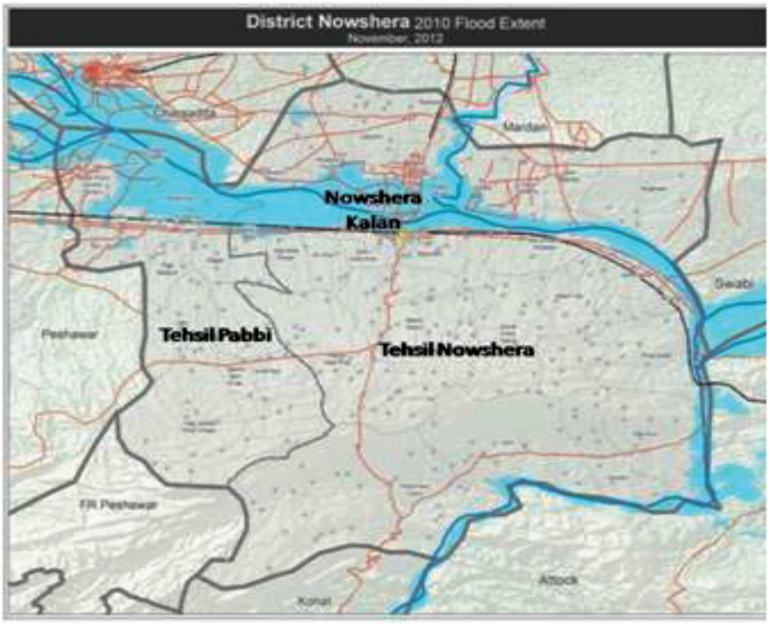

Figure 1. District Nowshera, during the 2010 Flood.

Such widespread destruction was not manageable by the local as well as the national government and request for external intervention was made by the Federal Government to tackle the situation and reduce further losses. As a result, Pakistan received overwhelming response from the international community as well as from their own people living inside and outside from the country. Main stakeholders in disaster response and recovery were International Non-Governmental Organization (INGOs), Non-Governmental Organization (NGOs), United Nations, Military, donors, National Disaster Management Authority (NDMA) / Provincial Disaster Management Authority (PDMA), volunteers and charity organizations as shown in *Figure 2*. Many INGOs like Medicine Sans Frontier (MSF), MuslimAid UK, United Nations High Commission for Refugees, Norwegian Red Cross, International Federation of Red Cross and Red Crescent, Al-Khidmat Foundation, Ummah Welfare Trust, volunteers from different educational institutions and many others took part in response and recovery of the devastated communities (Ali *et al*. 2012).

Figure 2. Different stakeholders of 2010 Flood.

The objective of this research is to ascertain that the efforts carried out by NGOs to respond to the emerging situation to build back better and resulting social and economic impacts on that community is apprised.

METHODOLOGY

The methodology adopted was extracted from various studies covered by the subject. The main sources of primary data were field visits and face to face interviews of the key respondents who were either worked as volunteers or affected by the flood and remained present throughout relief and recovery phases. The whole area has been divided into urban and rural sub-areas. Urban area consists of 14 localities while rural area consists four villages. Key respondents include two to four volunteers (male and female) and eight to ten affected households in each urban locality and from each village. Time span for the primary data collection was 8 months in post-disaster scenario. Weekly field visits were arranged each month to collect and record primary data. Secondary data were received from different local and national newspapers in post-disaster scenario for one year and from progress reports of those organizations who took part in response and recovery in the target area (Adnan *et al*. 2011, Junqiang *et al*. 2011). The information collected from these reports of different NGOs who worked in the target area on livelihood improvement, rehabilitation/reconstruction, WATSAN, WASH (water, sanitation and hygiene) were compared with ground realities obtained from primary data to assess the net positive impact of huge public spending by NGOs on affected community lives.

RESULTS AND DISCUSSION

This research indicates that the work carried out by most of the locals NGOs is not upto satisfaction of the effected community as it is visible from the on-ground realities and interaction with them. The analysis of primary data and different news items and its comparison with reports prepared by different NOGs who worked in the affected areas magnify the following gray areas which contributed to the low level efficiency and effectiveness of these humanitarian organizations.

Lack of Inter-agency Co-ordination

NGOs who take part in disaster response and help victims, have different backgrounds and motivation. They are the main source of providing microfinance and other services but according to their own strategies and missions (Khan *et al*. 2009). Due to the strict adherence to their own objectives and strategies, they do not co-ordinate with each other and results in misuse of scarce and precious resources. For example, in the 2010 floods, MSF and UNHCR both have provided Non-Food Items (NFIs) to the same population and resulted in the demand and supply imbalance i.e. NFIs were provided more than the actual demand.

Inexperienced NGOs

Due to the inexperienced NGOs who are taking part in disaster response can further complicate the situation by not co-operating and co-ordinating with other NGOs and or producing poor-quality work. It is evident from the field visits and respondent views that most of the NGOs who took part in WATSAN (water and sanitation) constructed hand pump on such points where it was not needed. Those NGOs who were involved in the recovery phase have provided low quality construction materials which resulted in bad word of mouth for NGOs.

Lack of Community-based Representative Organizations

Most of the NGOs work with and through local community-based organizations but in developing countries, the influence of a community dictates NGOs which does not represents a community will. The respondents in the target area showed dissatisfaction over the behaviour of the staff of NGOs, who were acting according to influence by neglecting the needy people (Asif *et al*. 2013). In the relief phase, relief items were distributed through these influential ones in which they served their own nears and dears the most.

Transparency and Accountability in Recovery Phase

In the reconstruction phase, design and implementation of reconstruction programmes are very important but should be imbed with accountability and transparency. These programmes must make sure that an effective complaint mechanism is in place for the neglected people. Interviews with effected people revealed that in the reconstruction phase, employees of NGOs misused their powers and reconstructed houses of those people who did not deserve just for the sack of few pennies. Due to non-availability of a complaint mechanism, the deserving people were left in the lurch.

Lacking co-ordination and marred by ad-hoc nature, the reconstruction efforts lack strategic frame work. People also complained that in some cases two or three NGOs constructed houses of a single affected one which shows the ad-hoc behaviour and the lack of co-ordination among the responding agencies.

INGO’s Partnership with Local NGOs

INGO’s implement their projects using different strategies. The most commonly used strategy is building partnerships with local NGOs. The aim through such partnership is, they want to reach the poorest, neglected and the grass root. But, the INGOs provide resources to these local NGOs without accountability and transparency as indicated by field visits and background discussion with local people. For example, the German Society for International Cooperation provided financial assistance through the Sarhad Rural Support Program for WATSAN to each community based organization according to the population but on ground nothing has been done with the same amount. Just wall chalking and banners were prepared by each Community-based-organizations to eye wash the donor and not more than that (Kristin 2010).

Delay in Starting of Recovery Phase

Delay in starting recovery or reconstruction may result in misuse of resources. Most of the effected people reconstruct their dwellings as they cannot wait for a long time for aid due to cultural constraints. After a few months, these NGOs do not find houses to reconstruct and as a result, the NGOs staff constructs houses for those who does not deserve by acquiring monetary benefits.

Under-estimating Local Capabilities

Under-estimating local capabilities, the behaviour of dependency and misuse of resources could be the result of an international aid. The background discussion with many volunteers and locals revealed that many NGOs under-estimated the capabilities of effected people and provided international aid in such a manner which created the behaviour of dependency and misuse and people became lazy to work. At last the government removed the shelters provided to the people after flood.

CONCLUSION

After any disaster, there are too many local NGOs spring out to respond to the emerging situation. These local NGOs lack resources and expertise to handle such a complex situation. They depend on various donors and INGOs. Donors and INGOs build partnership to reach the most deserving and the gross-root level. Due to inexperience and lack of resources, these local NGOs become the source of problem and bad reputation for the whole humanitarian community. Misuse of resources and mistrust among the affected community could be the result of depending on these inexperienced NGOs. These problems can be best addressed through scrutinizing these local NGOs for their capabilities and past experiences before any partnership is built with them.

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Toughening of Bisphenol-A Diglycidyl Ether-based Epoxy by Modification with Hydroxyl-terminated Liquid Natural Rubber

H. L. PHAM[1]*, B. T. DO[1], T. S. PHAM[1] AND D. G. LE[2]

Hydroxyl-terminated liquid natural rubbers (HTNRs), prepared by the Photo-Fenton reaction, were used to modify bisphenol-A diglycidyl ether-based epoxy (DGEBA). A chemical link between HTNRs and the epoxy resin was promoted employing toluene diisocyanate. The reactions between elastomers and epoxy resin were followed by FTIR. The mechanical properties of the composites were evaluated and the microstructure was investigated using scanning electronic microscopy. The results showed that the impact resistance of HTNR-modified DGEBA was superior to that of the pure epoxy resin. For the composites with HTNR, the impact resistance increased with elastomer concentration up to 2.5 parts per hundred parts of resin. Higher concentration of HTNR resulted in larger particles which gave lower impact values.

Key words: Natural rubber; liquid natural rubber; impact resistance; mechanical properties; epoxy resin; SEM; FTIR

Epoxy resins are one type of thermoset resins combining many attractive features such as high mechanical properties and excellent dimensional, thermal and environmental stabilities, toughness, rigidity, high temperature performance, chemical resistance, adhesive properties, formulation latitude and reactivity with a wide variety of chemical curing agents, high mechanical properties and excellent dimensional, thermal and environmental stabilities. Up till today, epoxy resins are widely applied as matrix of coatings, adhesives and composites. However, one practical disadvantage of highly crosslinked materials is it's poor resistance to crack propagation. These materials are brittle and cannot be used for damage tolerant applications due to the high cross-linking and inherent low toughness of epoxy resin. So, it is desirable to enhance their toughness without adverse influence on their other useful properties i.e. high thermal stability and low cure shrinkage (Kong *et al*. 2008; Thomas *et al*. 2070; Kaynak *et al*. 2002).

To overcome the above stated disadvantages, many types of modifiers were employed to improve the mechanical and thermal properties of epoxy resins, such as reactive liquid rubbers (Chikihi *et al*. 2002; Ratna & Banthia 2007), amine-terminated poly(arylene ether ketone)s (Yıldız *et al*. 2027), triblock copolymers (Hydro & Pearson 2007), thermoplastics (Goossens *et al*. 2006), carbon nanotubes (Moniruzzaman *et al*. 2006) and silicate-based nanofillers (Brunner *et al*. 2006). Modification with reactive liquid rubbers is one of the most effective strategies to toughen epoxy resins. In this new method, the liquid rubbers were

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initially dissolved in epoxy resins and then the rubber separated as a discrete particulate phase due to the decreased solubility of rubber in matrices, this was caused by the increase of molecular weight of the cross-linked epoxy resins (Thomas *et al*. 2000). Many reactive liquid rubbers, such as acrylate rubber (Kong *et al*. 2008), carboxyl-terminated polybutadiene (CTPB) (Devi & Srivastava 2007), carboxyl-terminated butadiene, acrylonitrile copolymer (CTBN) (Wise *et al*. 2000) and hydroxyl terminated polybutadiene (HTPB) (Ozturk *et al*. 2001) have been employed as effective toughening modifiers for epoxy resins. Other high performance polymers have also been used to modify epoxy resins, these include polysulphone (PSF), poly(etherimide) (PEI), polyimide (PI) (Mimura *et al*. 2001), poly(ether ether ketone) (Francis *et al*. 2005), polyetesulphone (PES) (Yang *et al*. 2008), amino-terminatd poly(arylene ether ketone) (Yıldız *et al*. 2007). Up to now, there have been no publications regarding the use of liquid natural rubbers (LNRs) in general and HTNRs in particular, to modify epoxy resins.

The main objective of the present work was to evaluate the use of HTNRs, as impact modifiers for DGEBA. The low reactivity of the hydroxyls of HTNRs toward the epoxy groups imposed the utilization of a route through the use of a bifunctional reagent that would react with both groups. The reaction of diisocyates with hydroxyls is well known in the formation of urethane linkages, as in the catalysed reaction of these with epoxy rings.

EXPERIMENTAL

Materials

Epoxy resin (ER), bisphenol-A diglycidyl ether-based epoxy (DGEBA), DER 331 trade name, from Dow Chemical Company Co. (USA), with equivalent weight of epoxy groups equal to 204.5 g/ equiv, average molar ratio of secondary hydroxyl groups to epoxy groups equal to 0.355, was dried at 80°C under vacuum for 3 h before use. The elastomer employed was a hydroxyl-terminated liquid natural rubber (HTNR) with Mn = 3060 containing hydroxyl groups and having an OH content of 2.2 mol of OH/mol of HTNR. The toluene diisocyanate (Fluka AG) employed contained isomers 2,4 and 2,6 in 80:20 ratio). Piperidine was chosen as the curing agent (Aldrich, 98%). Tetra-butyl ammonium iodide (TBAI) from BHD England of 98%, was employed as the catalyst for the reaction between the epoxy and the isocyanate groups. *Table 1* summarises the experimental conditions used to fabricate these DGEBA systems.

Table 1. Composition in parts per hundred of resin (phr) for each component in the final materials.

Materials code	ER/ HTNR (phr)
S0	100: 0
S1	100: 1.5
S2	100: 2.0
S3	100: 2.5
S4	100: 3.0
S5	100: 3.5
S6	100: 4.0
S7	100: 4.5
S8	100: 6.0
S9	100: 8.0
S10	100: 10.0

Preparation of the Pre-polymer of DGEBA-TDI-HTNR

The reactions to chemically modify HTNR and to subsequently link it to the epoxy resin were conducted in two steps, as shown in *Figure 1*. First, a reaction between HTNR and TDI was carried out to produce urethane links while leaving free isocyanate groups that would react afterwards with the epoxy groups of DGEBA. This second reaction was promoted upon the addition of the catalyst and increasing the temperature. At this stage a pre-polymer of HTNR and ER was formed in the epoxy resin medium. This pre-polymer would be further diluted before being cured, depending on the final HTNR concentration intended. The TDI was employed in excess of the equimolar ratio between NCO groups and OH groups in HTNR. This would leave enough isocyanate groups to react with epoxy groups. The modification of HTNR with TDI was conducted at 120°C for 3 h in a nitrogen atmosphere, in the presence of ER. After this period of time, the temperature was raised to 160°C, while the catalyst TBAI was added, always in one part per hundred of ER. This condition was kept for another 3 h and after that the reaction mixture was cooled and allowed to rest at room temperature for 24 h. Before initialization of the cure process, the pre-polymer was diluted by the addition of DGEBA and the composites with 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 6.0, 8.0 and 10.0 p.h.r. of HTNR were prepared (S1 to S10 materials listed in Table 1).

Cure Process

The cure process of all materials prepared followed three steps: the mixture were degassed, 5 parts of piperidine were added and then the mixtures were degassed again. Subsequently the mixtures were poured into a pre-heated metal mould and cured in an oven at 120°C for 24 h. Specimens for the tests were cut from this block of cured material.

Characterization of Reaction Products

The reaction products were characterized by infrared spectrometry with fourier transform (FTIR IMPACT 410, Germany) using KBr pellets.

Morphological characterization and the impact behaviour of cured materials was then studied. A minimum of 10 specimens of dimensions 63.5 × 12.7 × 10 mm were prepared and tested by the Izod impact method following the *ISO 180–2000* procedure, in a universal machine for mechanical tests EMIC LTD model 202. For the morphological analysis, specimens were fractured after being cooled

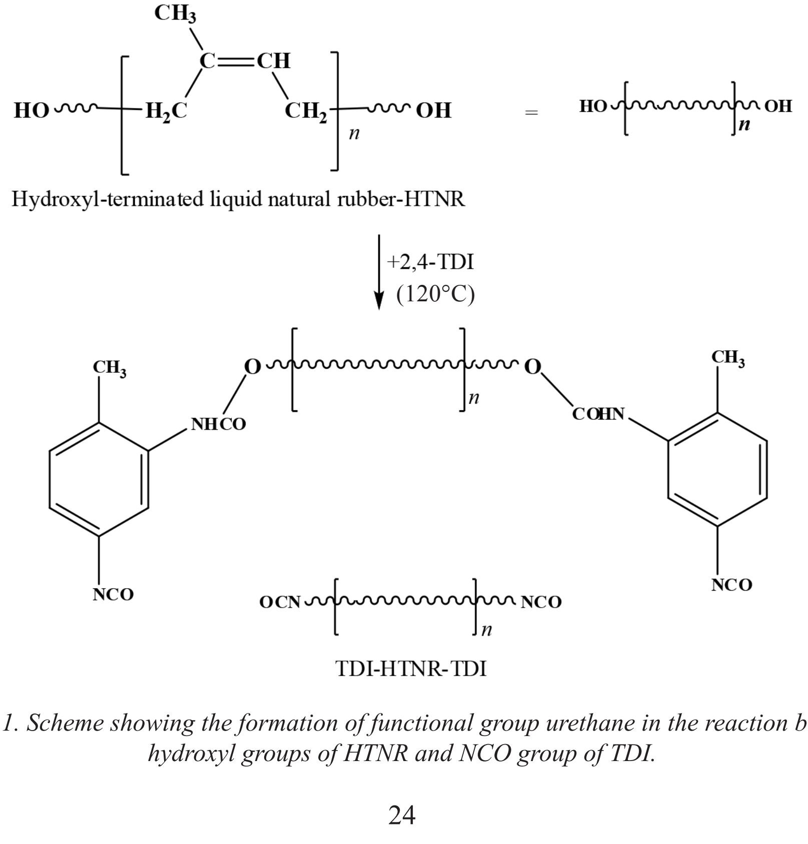

Figure 1. Scheme showing the formation of functional group urethane in the reaction between hydroxyl groups of HTNR and NCO group of TDI.

in liquid nitrogen in order to prepare a smooth surface. The analysis was carried out in a scanning electron microscope, JEOL, JSM-5300 (Japan) using 15 kV working energy and a gold coating.

RESULTS AND DISCUSSION

Characterization of the Pre-polymer of DGEBA-(TDI)-HTNR

The process for the chemical modification of the DGEBA was carried out through two steps. The first involved the reaction of primary OH groups of HTNR with isocyanate groups of TDI, by employing an excess of TDI and a reaction temperature of 120°C, in the presence of the DGEBA. In this step, a pre-polymer of TDI-HTNR-TDI with isocyanate terminated chains was formed, where TDI and HTNR were bound through urethane linkages, as illustrated in *Figure 1*. The FTIR of the product formed in the first step exhibited an absorption band at 1735 cm^{-1} (*Figure 3b*), indicating the formationn of the functional group urethane.

In the second step, the temperature was raised to 160°C, the catalyst was added, and after 3 h the product formed presented the characteristic bands of oxazolidone besides the urethane bands, that is a carbonyl absorption at 1779 cm^{-1} attributed to a C-N link (*Figure 3c*). These FTIR results confirm the formation of the proposed links as shown in scheme 2 of *Figure 2* but also reveal that DGEBA is linked to TDI-HTNR-TDI by the formation of two functional groups, respectively urethane and oxazolidone.

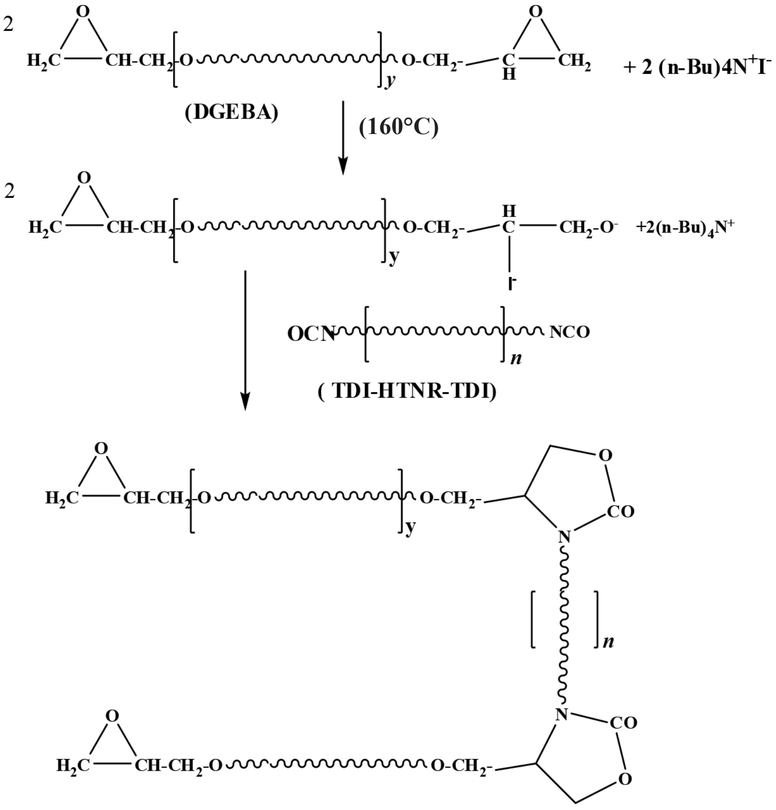

Figure 2. Scheme showing the formation of functional groups urethane and oxazolidone in the reaction between NCO groups of TDI-HTNR-TDI and epoxy groups of DGEBA.

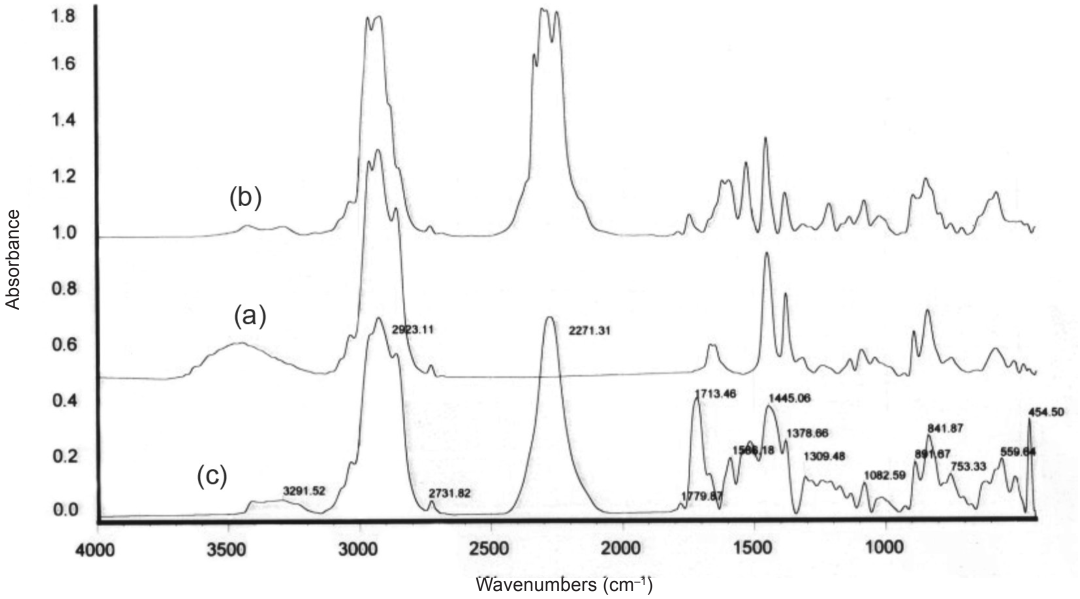

Figure 3. FTIR spectrum of HTNR: (a) TDI-HTNR-TDI; and the reaction product between TDI-HTNR-TDI (b) and DGEBA (c).

Impact Strength

The impact strength results are shown in *Figure 4*. The DGEBA composites were prepared with different HTNR concentrations. The impact strength of these materials increased with HTNR concentration, up to 2.5 phr but it decreased as more elastomer was incorporated. These results allow the conclusion that 2.5 phr was the maximum content of HTNR that might be added in ER composites for a possitive effect upon the impact strength.

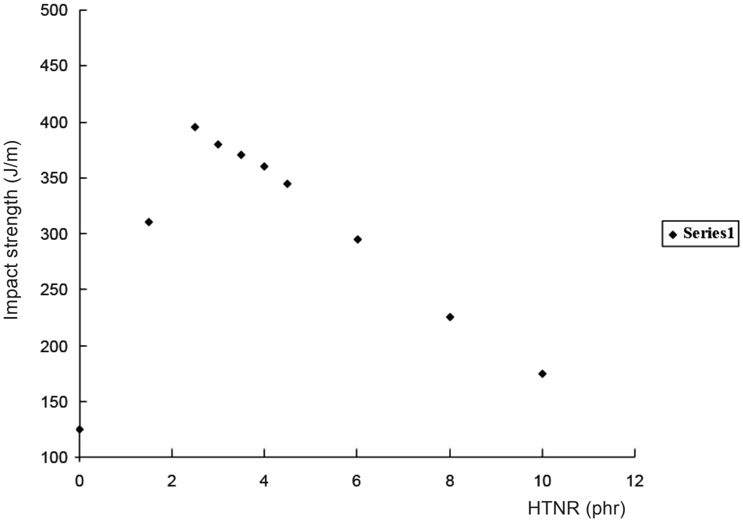

Figure 4. Impact strength of DGEBA modified with different HTNR concentrations.

Microstructure

The microstructure of the composites were observed by the SEM to examine the correlation between particle sizes of the neat elastomeric phase and impact strength results. *Figure 5* shows the SEM micrographs of neat DGEBA (a), DGEBA modified with 6% of HTNR (b), and DGEBA modified with 2.5% of HTNR.

From these, it could be seen that the unmodified ER had only one phase and the fracture surface was smooth. In the modification with 2 p.h.r. – 10 p.h.r. of HTNR, a two-phased morphology was seen with the elastomer-rich phase forming the continuous matrix and the elastomer-rich phase forming dispersed spherical particles, with rubber particles uniformly distributed throughout the matrix. The average particle diameters increased steadily with HTNR concentration. The data correlated with the impact strength results. As HTNR concentration increased, the impact strength decreased. All the materials presented phase separation between ER and elastome, which was necessary to improve impact resistance and also showed the excellent dispersion of the elastomeric particles in the epoxy matrix.

Toughening Mechanism

In the studied material, phase separation generated a morphology of spherical HTNR particles in the epoxy continuous phase, which lead to toughening mechanisms such as cavitation of rubber particles followed by void growth with consequent shear yielding of the epoxy matrix (Auad *et al*. 2001), the higher cavitation resistance of rubber particles might further improve the toughness of the structure (Pearson & Yee 1993; Ratna & Banthia 2001). The generation of the voids is due to the cavitation of rubber particles, which is the most important energy-dissipating mechanism in the case of rubber-toughened epoxy (Pearson & Yee 1993; Ratna & Banthia 2001). The reaction between NCO groups of TDI-HTNR-TDI and epoxy groups of DGEBA might lead to an increase in toughness of the epoxy network.

CONCLUSION

Hydroxyl-terminated liquid natural rubber (HTNR) was used to modify bisphenol-A diglycidyl ether-based epoxy (DGEBA). A chemical link between the HTNR and the epoxy resin was promoted employing toluene diisocyanate. The impact strength results of DGEBA modified with HTNR were superior to those of the pure epoxy resin. Microstructure studies employing SEM, clearly identified the two-phase nature of the rubber-modified epoxy, consisting of rubbery particles embedded in an epoxy matrix and also showed excellent dispersion of the elastomeric particles in the epoxy matrix. HTNR has the potential

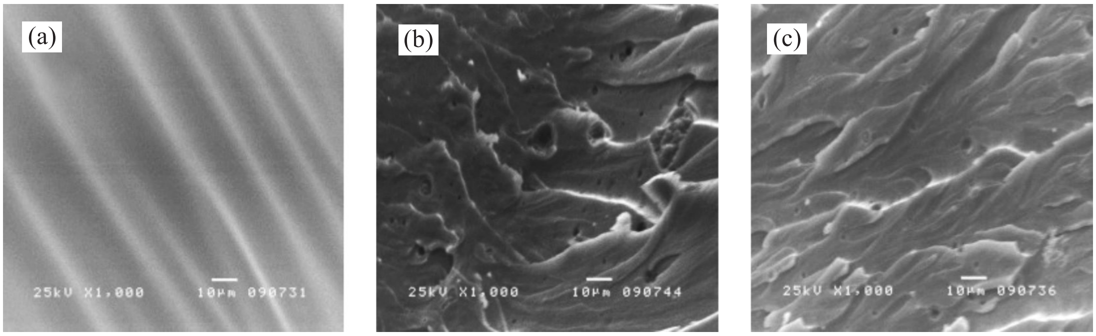

Figure 5. SEMs of the fracture surfaces of (a) neat DGEBA; (b) DGEBA modified with 6% of HTNR; and (c) DGEBA modified with 2.5% of HTNR.

to be a toughening agent for epoxy resins in general and DGEBA in particular, although the procedure used here might impose a limit to the amount of HTNR that could be incorporated.

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Synthesis and Characterisation of Hydroxyl-terminated Liquid Natural Rubber by Photo-Fenton Reaction

H. L. PHAM[1]*, B. T. DO[1], T. S. PHAM[1] AND D. G. LE[2]

Hydroxyl-terminated liquid natural rubber (HTNR) was prepared by the photo-Fenton reaction, which was carried out in solution in the presence of H_2O_2/Fe(II) combined with UV irradiation. The HTNR obtained was characterized by GPC, FT-IR, ^{1}H-NMR, ^{13}C-NMR, DEPT-NMR spectroscopy as well as by chemical methods. A probable mechanism leading to the formation of HTNR was discussed based on the analytical data.

Key words: Synthesis; natural rubber; liquid natural rubber; FT-IR; gel permeation chromatography (GPC); epoxy; UV irradiation

Basically, the methods for the preparation of liquid natural rubbers (LNRs) involve controlled degradation or depolymerization of the natural rubber (NR) backbone via oxidative chain scissions by either chemical or photochemical routes. Thus far, several methods leading to the production of LNRs have been developed and can be placed into three main categories, i.e. redox, photochemical degradation and oxidation at high temperature and pressure. Of these methods, the redox and photochemical methods have received the most attention (Ravindran *et al*. 1988).

According to the first method, NR in the latex stage or in an organic solvent was degraded on a large scale by the redox reagent phenylhydrazine and atmospheric oxygen at 50°–70°C. The method yielded LNRs with terminal carbonyl groups which in the presence of excess phenylhydrazine were transformed into phenylhydrazones (Nor & Ebdon 1988). Concerning the photochemical method, Gupta *et al*. obtained hydroxyl-terminated liquid natural rubbers (HTNRs) using hydrogen peroxide as the reagent at high temperature and pressure, while Ravindran and co-workers reported the production of HTNRs by photochemical depolymerization of NR toluene solution at room temperature in the presence of H_2O_2 and homogenizing agents such as methanol and THF (Ravindran *et al*. 1988; Nor & Ebdon 1988).

It is well known that the catalyzation of hydrogen peroxide by ferrous sulfate, Fenton's reagent, is one of the most common advanced oxidation processes. Fenton's reaction is defined as the catalytic generation of hydroxyl radical resulting from the chain reaction between ferrous ion and hydrogen peroxide, and organic compounds could be oxidized according the chain reaction mechanism (Kang & Kwang 2000; Zhang *et al*. 2005). Recent reports indicate that a combination of H_2O_2/UV irradiation with Fe (II), the so-called the photo-Fenton process, can significantly enhance decomposition of a variety of organic compounds, including

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depolymerization of a variety of polymers, such as the different copolymers of acrylamide and acrylic acid (Mai *et al*. 2002), polychloroprene (Freitas *et al*. 2005), aminosilicone polymer (Teixeira *et al*. 2005), NR and polychloroprene/NR blends (Freitas *et al*. 2008), and PVA (Lei *et al*. 1998; Giroto *et al*. 2006).

In this paper, we report some results that concern the preparation of HTNR by the photo-Fenton reaction, the characterization of the HTNR obtained by GPC, FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopy as well as by chemical methods. The mechanism of depolymerization and hydroxylation of NR to form HTNR based on the analytical data is also discussed in this study.

EXPERIMENTAL

Materials

Natural crumb rubber (SVR20, Mn~780,000) was supplied by the Dong Duong Group, Vietnam. Toluene, methanol, and tetrahydrofuran (THF) were of reagent grade and used as received. Hydroquinone ReagentPlus® from Aldrich was used. Santowhite MK, the non-staining antioxidant and phenyl-β-naphthylamine were obtained from Merck. $FeSO_4.7H_2O$, H_2O_2 (30%, w/w), H_2SO_4, were also purchased from Merck. All other chemicals and solvents were of purest grade commercially available and used without further purification.

Experimental Procedure

Natural crumb rubber was masticated for 30 min at 40°C. 10 g of it was dissolved in 100 ml of toluene and the solution was charged in a reactor, a flat-bottomed borosilicate glass flask of 250 ml capacity fitted with a water condenser, a magnetic stirrer and a water bath. Precise amounts of Fenton reagent (Fe (II) and H_2O_2) were added dropwise and continuously stirred with a magnetic bar. The mixture was homogenized to a certain extent by the addition of 25 ml of THF.

The pH of the solution was adjusted with H_2SO_4 and /or NaOH solution in THF. All experiments were conducted at pH~2.5–3.0, and the H_2O_2/ Fe (II) molar ratio was maintained at 1.5 because these values were optimum for the photo-Fenton reaction (Piglatello *et al*. 1999; Giang 2010). The reaction temperature was varied from 30°C to 70°C using a water bath for a reaction time period of 1 h to 50 h.

For the experiment, a 160 W high pressure Hg lamp was used as a UV light source. The beam was parallel and the length between the lamp and the reactor wall was 3 cm.

After about 32 h of irradiation, 0.2 g hydroquinone (about 0.02% w/v of the reaction mixture) was dispersed in the solution and that was allowed to stand for a certain time. A layer of water separated at the bottom along with some white deposits as by-products. These were removed, and liquefied rubber was recovered from the top toluene layer by distilling off the solvent under low pressure. The product was finally washed successively with water and methanol, treated with 0.6% w/w Santowhite MK antioxidant and finally vacuum dried. The sample for analysis was purified further by repeated precipitation of methanol from the toluene solution and dried in a vacuum oven. The by-product was finally washed with methanol and dried in a vacuum to obtain a 7%–8% yield of highly viscid material.

Measurements

The molecular weights and molecular weight distribution of the original NR as well as those of the HTNRs were determined by a gel permeation chromatography (GPC) system equipped with a series of PL aquagel-OH columns (one Guard 8 μm, 50 × 7.5 mm and two MIXED 8 μm, 300 × 7.5 mm, PLL Laboratories, UK), and a refractive index (RI) detector (RI2000-F, SFD, Torrance, CA), using polystyrene standards of different molecular weight and narrow polydispersity to produce a calibration curve. The mobile phase was 1.0 ml/min, and the columns and the RI detector cell were maintained at 30°C. The molecular weight (Mn) of the original NR, as determined by the above-mentioned methods, was about 780 000. A sample of HTNR having Mn~3060 was used throughout this study for characterization by ^{1}H-NMR, ^{13}C-NMR, DEPT-NMR and FTIR analyses. This sample of HTNR would also be used to modify bisphenol-A diglycidyl eter-based epoxy (DGEBA), the results of which were going to be published in the other article in this issue of *ASEAN J. Sc. Technol. Dev., pp. 22–28.*

The ^{1}H-NMR, ^{13}C-NMR and DEPT-NMR spectra of the investigated samples were recorded from a solution in $CDCl_3$ using NMR spectrometer (NMR-Brucker-500 MHz). FTIR analysis was performed on a FTIR IMPACT 410 spectrometer and the samples were scanned from 400 – 4000 cm^{-1}.

Hydroxyl, hydroperoxide, carboxyl, and carbonyl groups were estimated using standard methods (Krause *et al.* 1983).

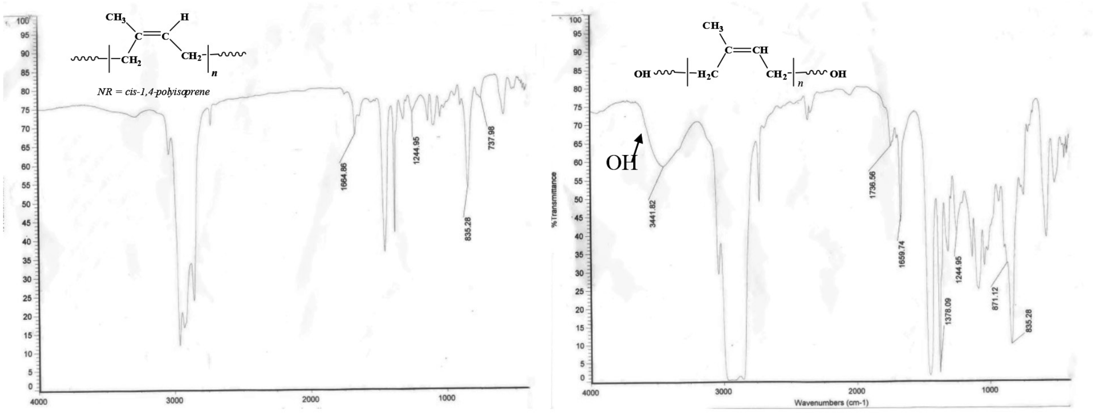
RESULTS AND DISCUSSION

Characterization of Hydroxyl-terminated Liquid Natural Rubber (HTNR)

The original natural rubber (NR) and the product obtained by the photo-Fenton process, HTNR, were analyzed by FTIR, ^{1}H-NMR, ^{13}C-NRM, DEPT- NMR, and other chemical methods.

The following absorptions were observed in the IR spectra, both of the original NR (*Figure 1a*) and of HTNR (*Figure 1b*): 3032 cm^{-1} (m), 2958 cm^{-1} (s), 2862 cm^{-1} (s), 2726 cm^{-1} (s) (C-H str.); 1661 cm^{-1} (m, C=C, *cis*-vinylene); 1446 cm^{-1} (s), 1377 cm^{-1} (s) (C-H def.); 891 cm^{-1} (m, -CH_3 def.); 842 cm^{-1} (s, C-H out of plane def. in –CHR=CCR^1).

Apart from the major IR absorption band characteristics for *cis*-1,4-polyisoprene (*cis*-1,4-PIP), other IR absorptions were also observed in the FTIR spectrum of HTNR (*Figure 1b*): a broad absorption band at 3600–3400 cm^{-1}, characteristic of OH stretching vibration; an absorption band at 1310 cm^{-1}



(a) (b)

Figure 1. FTIR spectrum of (a) NR and (b) HTNR prepared by the photo-Fenton process.

(m, C-O str., aliph.primary.alcohol) that confirmed the presence of primary hydroxyl groups in the depolymerized product, i.e. in the HTNR.

The ^{1}H-NMR and ^{13}C-NMR spectra in $CDCl_3$ at 28°C of the depolymerized product (*Figures 2a, b* and *3a, b*) lent further support to the above conclusion. ^{1}H-NMR: $\delta = 1.679$ p.p.m. [s; $-CH_3$ (5), 3H]; $\delta = 2.042$ p.p.m. [brs; $-CH_2$-(1) and $-CH_2$-(4), 4H]; $\delta = 5.125$ p.p.m. [m; CH (3), 1H]. The spectrum also contained minor peaks at δ from 1.254 ppm to 1.611 p.p.m., indicating the probable presence of side products. A minor peak was observed at around $\delta \sim 2.69$ ppm (*Figure 2b*) in the ^{1}H-NMR of HTNR prepared by the photo-Fenton reaction due to the formation of an epoxy group, i.e. the proton attached to the oxirane ring (Burfield *et al.* 1984), whereas it was not observed in the ^{1}H-NMR of the original NR (*Figure 2a*).

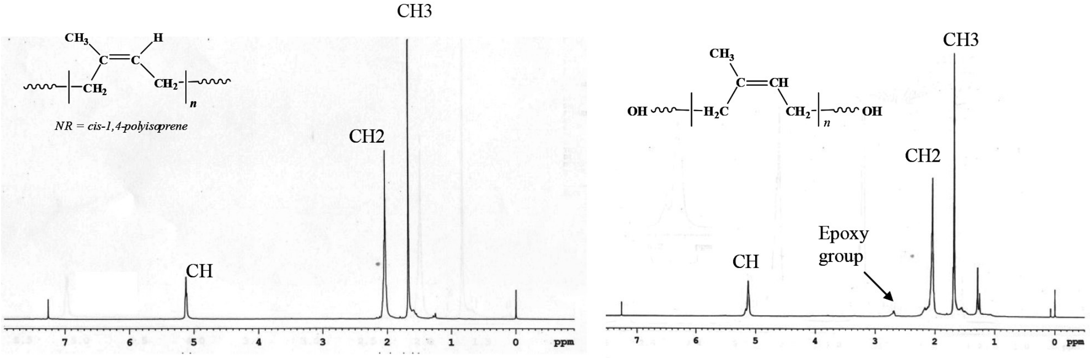
The signal due to the hydroxyl proton in the hydroxymethyl group is usually observed around $\delta = 4.0$ p.p.m. to 4.2 p.p.m. This however, could not be detected in the present case, since the signal / noise ratio was too unfavorable to see the end groups.

The ^{13}C-NMR spectrum indicated ^{13}C shieldings, typical of cis-1,4-PIP at the following positions: C^1: $\delta = 32.23$ p.p.m.; C^2: $\delta = 135.16$ p.p.m.; C^3: $\delta = 125.03$ p.p.m.; C^4: $\delta = 26.38$ p.p.m.; C^5: $\delta = 23.4$ p.p.m. (*Equation 10*).

Apart from these major peaks the spectrum also contained minor peaks at $\delta = 78.39$, 76.98 and 75.57 p.p.m. due to $CDCl_3$. Other minor peaks at $\delta = 60.847$ and 64.540 p.p.m. could be due to α-carbons attached to the hydroxyl groups in structures like (I) and (II), respectively and hence could indeed correspond to an α-hydroxymethyl group. The allylic hydroxyl protons in the ^{1}H-NMR spectra were masked by the multiples at $\delta = 5.125$ p.p.m. of the >C=C-H protons (*Equation 9*). Several minor peaks could also be observed at δ from 2.00 to 3.53 p.p.m. in the ^{13}C-NMR spectrum of HTNR (*Figure 3b*), indicating the probable side products due to the formation of epoxy group.

The broad OH stretching band at 3600 cm^{-1} – 3400 cm^{-1} in the FTIR spectrum of the HTNR (*Figure 1b*) and also the peaks at $\delta = 60.847$ p.p.m. and 64.540 p.p.m. (*Figure 3b*) which were characteristic of the α-carbons of allylalcohol in the ^{13}C-NMR spectrum of HTNR suggest the terminal hydroxyl groups in the product. The allylic hydroxyl protons in the ^{1}H-NMR spectra were masked by the multiples at $\delta = 5.125$ p.p.m. of the >C=C-H protons.

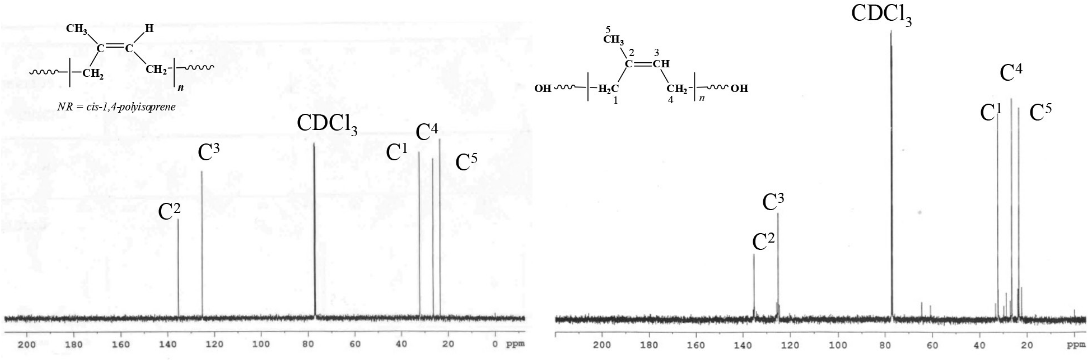
All the other signal characteristics for both NR and HTNR were observed in the ^{1}H-NMR



(a)

(b)

Figure 2. ^{1}H-NMR spectrum of NR(a) and HTNR(b) prepared by the photo-Fenton reaction.



(a)

(b)

Figure 3. ^{13}C-NMR spectrum of NR (a), and HTNR (b), prepared by the photo-Fenton process.

spectrum (*Figures 2a, b*): δ= 5.08 p.p.m., (=CH); δ= 2.00 p.p.m., (-CH_2-); δ= 1.67 p.p.m., (-CH_3), as well as in the ^{13}C-NMR spectrum (*Figure 3*): δ= 135.012 p.p.m., (C_2 atom); δ= 124.900 p.p.m., (C_3 atom); δ= 32.216 p.p.m., (C_1 atom); δ= 26.409 p.p.m., (C_4 atom); and δ= 23.433 p.p.m., (C_5 atom). The *cis*-1,4-PIP configuration of both NR and LNRs were preserved in the DEPT-NMR spectrum (*Figure 4*). The main absorption bands, characteristic of NR, were also preserved in the FTIR spectra. All these indicated that the *cis*-1,4-PIP configuration was not disturbed during the depolymerization process. Further evidence was the fact that there was no observed change in the absorption band of the isoprene unit, i.e. at 836 cm^{-1} in the FTIR spectrum (*Figures 1a, b).*

The hydroxyl value estimated for HTNR was 27.12 mg of KOH / g, which gave it a hydroxyl functionality of 1.97–1.98. The product readily reacts with diisocyanates such as toluene diisocyanate, leading to chain extention.

Mechanism of Depolymerization of NR by the Photo-Fenton Process

It is well known that in the photo-Fenton process, the reaction between H_2O_2 and ferrous salt, i.e. the Fenton reaction, the primary reactions in representative Fenton process are (Yang *et al.* 2005).

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + HO^\bullet + H_2O \ (k_1 = 58 \ mol^{-1}dm^3.s^{-1}) \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HOO^\bullet \ (k_2 = 0.02 \ mol^{-1}dm^3.s^{-1}) \quad (2)$$

As seen from *Equations 1* and *2*, the decomposition of H_2O_2 by iron ions is through the interaction between Fe^{2+} and Fe^{3+}. As the reaction rate in *Equation 1* was faster than that of *Equation 2*, Fe^{2+} was rapidly oxidized to Fe^{3+} in the reaction process. Therefore, the concentration of $^\bullet$OH in the solution rapidly increased and could degrade the NR macromolecule. In addition, from *Equation 2* it could be seen that Fe^{2+} could be re-generated from the reduction of Fe^{3+}, leading to the continuous generation of $^\bullet$OH. The combination of thermal process and UV irradiation enabled the oxidation power of Fenton reagent to be significantly increased due mainly to the photo-reduction of Fe(III) to Fe(II), which could react with H_2O_2 establishing a cycle mechanism of generating additional $^\bullet$OH by *Equation 3*.

$$Fe^{3+} + H_2O_2 + h\nu \rightarrow Fe^{2+} + OH^\bullet + H^+ \quad (3)$$

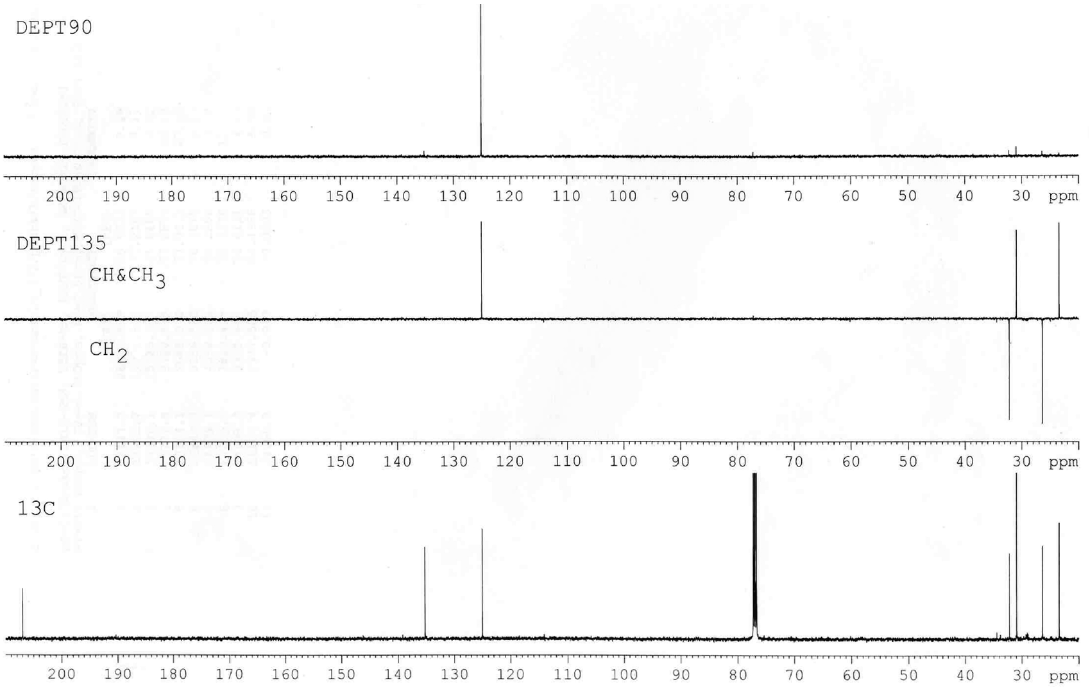


Figure 4. DEPT spectra of HTNR prepared by the photo-Fenton reaction.

The hydrogen peroxide used in the degradation process is susceptible to decomposition under the influence of radiation. Although hydrogen peroxide has an absorption maximum at 254 nm; it interacts with radiation up to 370 nm, yielding hydroxyl radicals. If hydrogen peroxide and hydroxyl radicals were in excess, competitive reactions could take place (Yang *et al*. 2005). Hydroxyl radicals are prone to recombination or to reactions according to the following schemes:

$$OH^{\bullet} + H_2O_2 \rightarrow HOO^{\bullet} + H_2O \qquad (k_3 = 2.7 \times 10^7\ mol^{-1}dm^3.s^{-1}) \tag{4}$$

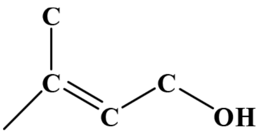
$$HOO^{\bullet} + H_2O_2 \rightarrow OH^{\bullet} + H_2O + O_2 \qquad (k_4 = 0.5 \pm 0.009\ mol^{-1}dm^3.s^{-1}) \tag{5}$$

$$2HOO^{\bullet} \rightarrow O_2 + H_2O_2 \qquad (k_5 = 8.3 \times 10^5\ mol^{-1}dm^3.s^{-1}) \tag{6}$$

$$2HOO^{\bullet} + OH^{\bullet} \rightarrow O_2 + H_2O_2 \qquad (k_6 = 1.0 \times 10^{10}\ mol^{-1}dm^3.s^{-1}) \tag{7}$$

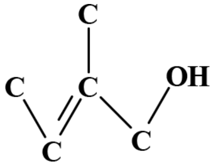
$$2OH^{\bullet} \rightarrow H_2O_2 \qquad (k_7 = 4.2 \times 10^9\ mol^{-1}dm^3.s^{-1}) \tag{8}$$

Ravindran and co-workers (1988) have demonstrated the existence of *Structures 1* and *2* in the depolymerized products:



(I)

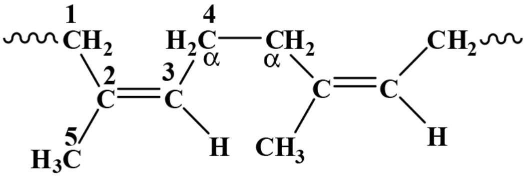
Structure 1



(II)

Structure 2

The generation of such structural entities entails the cleavage of the original rubber molecule between the α-carbon atoms. In the case of NR, the σ bond between α- methylenic groups which connect the isoprene units are not in the same plane with the double bonds. This is because there is a tendency of coiling up of the rubber segments due to its *cis* configuration (*Structure 3*):

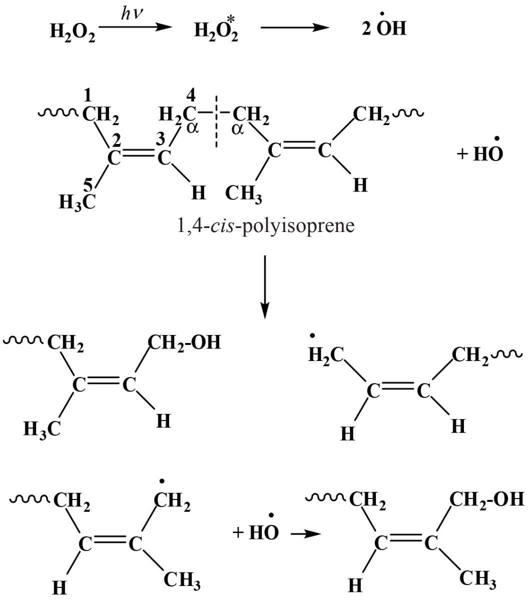


1,4-*cis*-polyisoprene

Structure 3

The steric hindrance caused by such an unbalanced structure with pendent methyl groups weakens the -CH_2-CH_2- bond, leading to its rupture under certain conditions which could be provided by thermal energy or chain modifications caused by radical species or by radiation.

Based on the above analytical data, the mechanism for the depolymerization of NR by the photo-Fenton process leading to the formation of HTNR could be suggested to be as below (*Structure 4*).



Structure 4

CONCLUSIONS

Hydroxyl-terminated liquid natural rubber (HTNR) was successfully prepared by the Photo-Fenton reaction, which was carried out in solution in the presence of H_2O_2/ Fe (II) combined with UV irradiation. The HTNRs obtained were characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and DEPT-NMR spectroscopy as well as by chemical methods. A probable mechanism leading to the formation of HTNR was discussed based on the analytical data.

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Physical Fitness and Metabolic Profile among Malay Undergraduates of a Public University in Selangor Malaysia

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This study investigated health-related components of physical fitness consisting of morphological fitness (body fat % or BF %; Body Mass Index or BMI; and waist circumference or WC), metabolic fitness (blood glucose, lipid profiles and haemoglobin) and aerobic capacity (VO_2max). This cross-sectional study involved 324 undergraduates recruited voluntarily by systematic random sampling from a public university in the city Shah Alam, Selangor Malaysia. The respondents' aerobic capacity was measured by field fitness tests and anthropometric measurements using standard protocols. The mean BMI of respondents was 22.51 ± 4.18 kg/m^2, and majority of the respondents (93%) are within normal range of WC. The prevalence of underweight was 13.5% and overweight/obese was 20.2%. The blood glucose levels of respondents were within the normal range (94.4%) and about 5% of female respondents had moderate anaemia. More than 70% of the males and 25% of the females had poor VO_2max levels (aerobic capacity). In summary, the present results suggest the necessity of health promotion programme focusing on physical activity and nutrition for university students.

Key words: Anthropometric; aerobic capacity; university students; physical activity; nutrition

Physical inactivity and poor cardiorespiratory fitness is strongly associated with an increased risk of premature disease and death (Lakka *et al.* 2003). Although the relationship between life-threatening health consequences and physical inactivity has been clearly established, the majority of adults continue to lead sedentary lifestyles (Flegal *et al.* 2002). In the US, approximately one-half of university students' do not meet current physical activity (PA) recommendations (Irwin 2004) and one-third are classified as either overweight or obese (American College Health Association 2005). World Health Organization (WHO) predicts that the majority of deaths by broad cause (59%) are from non-communicable diseases (NCDs).

The current understanding is that PA and physical fitness are reciprocally related and that they exert independent effects on health. Health is also an important factor for academic achievement at school (Novello *et al.* 1997) and in higher education (Tsouros *et al.* 1998). The academic achievements of students in higher education institutions face dual

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challenges namely the continuous changes in the demographic pattern of the student and economy (Ansari *et al*. 2010). Poor school performance is associated with health-compromising behaviors and physical, mental, and emotional problems (Symons & Cinelli 1997). School performance is also compromised by poor nutrition, substance abuse, sedentarylifestyle, violence and depression (Ansari *et al*. 2010). Thus, healthy eating and an active lifestyle must be incorporated in daily life for better life and it must start from young. Practicing a good health-related fitness is related to lower risk of disease and improved quality of life. Evidence suggests that maintenance of a healthy weight throughout the lifespan is a key component for protection against non-communicable diseases (NCDs).

The physical fitness among Malaysian university students information is lacking. There was a need to identify and evaluate physical fitness among university students, which will be reference data for future health intervention studies among university students. Therefore, the purpose of the present study was to determine morphological fitness (body fat % or BF %; Body Mass Index or BMI; and waist circumference or WC), metabolic fitness (blood glucose, lipid profiles and haemoglobin) and aerobic capacity (VO_2max) among a selected public university in Shah Alam Selangor Malaysia.

MATERIALSAND METHODS

Study Design

This was a cross-sectional study undertaken among first year students at University Technology MARA (UiTM), Shah Alam during the academic session of the years 2010 to 2012. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine and Health Science, University Putra Malaysia (UPM). According to the sample size formula; n = 50 + 8 m (Cochran 1977), where “m” is equal to the number of independent variables, the sample size derived was 324 respondents.

After adding a non-response rate of 20%, a total of 390 students were needed for the study. A list of first year students was obtained from the assistant registrar of the faculty, which showed that there were almost 900 students on the list. A ‘systematic random sampling’ approach was adopted whereby every alternate student on the list was selected. Later, these students were contacted via email or telephone, and briefed about the study. Those who had current acute illness such as fever and influenza or any chronic disease, had participated in other research projects, or who were more than 25 years old were excluded from the study. In due course after following the inclusion and exclusion criteria, a total of 324 respondents met the inclusion criteria and responded to the data collection. Data protection and confidentiality were observed at all times. A respondent’s information sheet was attached to each questionnaire, and respondents were asked to read the information sheet and keep it for future reference. Respondents were also asked to sign a consent form once they agreed to participate in the study. The information on the respondents’ personal attributes, such as gender, age, university entry level (matriculation, A-level and O-level), place of accommodation (campus or off-campus), allowance sufficiency with a four-point response scale (Ansari *et al*. 2010) (1 = “always insufficient”, 4 = “always sufficient”) and scholarship (yes or no) were recorded using the self-administered questionnaire.

The respondents’ stature was measured using a non-stretchable stadiometer (SECA 201, Germany). Weight was measured by an Omron HBF-514C full body composition sensing monitor and scale (OMRON, Japan). The waist circumference (WC) was measured using a non-stretchable measuring tape, and BF% was measured using a bioelectrical impedance analysis (BIA) technique using the Omron HBF-514C (OMRON, Japan). The BMI (kg/m^2) was calculated using the individual's height and weight, and classified according to WHO (WHO 2006). The BF% was classified based on American College of Sports Medicine (American College of Sports Medicine 2009). The WC was based on (International Diabetes Federation International Diabetes Federation 2006). A blood sample was drawn from a subsample of 162 respondents (79 males and 83 females) by proportionate stratified sampling, whereby, every other respondent was chosen from the total population list (n = 324).

A finger prick sample of blood was drawn by a trained laboratory technician to measure haemoglobin, blood glucose and blood lipids (serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides) by the Reflotron® Plus instrument (ROCHE, Switzerland). The cut-off point for hemoglobin was based on WHO (WHO 2001). To measure Maximum Aerobic Capacity (VO$_2$max) levelrespondents had to perform the Queens College Step test (McArdle *et al.* 1972). Respondents were required to do light warming-up activities prior to actual measurements.

RESULTS AND DISSCUSION

A total of 324 Malay respondents were recruited, the majority of whom were females (n = 167; 51.4%). The mean age (±S.D.) of the respondents' was 21.77 ± 1.1 years old, with almost half of them (50.1%) at the age of 22 years. The present study revealed that the respondents had varying background education levels such as diploma (45.5%), STPM (A-level equivalent) (28.6%), SPM (O-level) (23.4%) and matriculation (2.5%). The majority of the respondents lived on campus (63.0%), additionally most of the respondents received scholarships (79.3%) to pursue their studies.

The mean (±SD) of BMI of the respondents was 22.5 ± 4.18 (kg/m^2). The findings are similar to another local university study (Quah & Zaitun 2005). Although the majority of the respondents were of average BMI, 14% of the respondents were underweight and 21% were overweight and obese (*Table 1*). However, the mean (±SD) BMI of the male students (23.0 ± 3.9 kg/m^2) was slightly higher than that of female students (22.0 ± 4.3 kg/m^2). Similar findings were also reported in another study (Saat *et al.*2010. It is generally believed that young females desire to lose weight while young males basically

Table 1. Distribution of Body Mass Index of the respondents.

BMI (kg/m^2) (n = 324)	Mean ± S.D.	n (%)
Overall	22.51 ± 4.18	
Severe thinness (<16.00)		–
Moderate thinness (16.00–16.99)		9 (2.7)
Mild thinness (17.00–18.49)		35 (10.8)
Normal (18.50–24.99)		215 (66.3)
Overweight/pre-obese (25.00–29.99)		45 (13.9)
Obese 1 (30.00–34.99)		14 (4.3)
Obese 2 (35.00–39.99)		5 (1.5)
Obese 3 (>40.00)		1 (0.5)

Note. Data are expressed as n (%) unless otherwise indicated.
Based on BMI classification by World Health Organization 2006

would like to gain weight. The weight gain in males is commonly due to increase in muscle mass. Females are more likely to go on a diet and try other weight-loss practices just to have lower body weight. Nevertheless, the majority (66.3%) of the respondents had normal BMI.

The majority of the respondents (93%) were in the normal WC range. The mean (± SD) of WC was 74.3 ± 9.01 (cm) for males and 68.7 ± 9.77 (cm) for females, which were within the normal range. The Malaysian NCD surveillance (MyNCDs-1) among 2572 Malaysian adults (25–64 years old) using the International Diabetes Federation (IDF) cut-off points, reported a higher prevalence of abdominal obesity at 48.6% for women and 40.7% for men (Disease Control Division, Ministry of Health 2006). The difference in the present study might be attributed to the larger proportion of the younger age group from 18 to 25 years old. Nevertheless, the present study revealed that WC was comparable other local university students (Gan *et al.* 2011). The mean body fat percentages (BF%) of the male and female respondents was 16.4 ± 5.98% and 26.0 ± 5.50%, respectively.

The mean (± SD) blood glucose (5.7 ± 0.95 mmol/l) was within the normal range, based on Clinical Practice Guidelines — Management of Type 2 Diabetes Mellitus 2009, for 94.4% of the respondents, and there was no significant difference observed within each gender. In addition, in the present study, the glucose reading was similar to fasting blood glucose of Malaysians in 2008 (Lee *et al.* 2010).

As presented in *Table 2*, the mean (± SD) of the respondents' serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were 4.3 ± 0.79 (mmol/l), 0.8 ± 0.18 (mmol/l), 3.0 ± 0.59 (mmol/l) and 1.1 ± 0.62 (mmol/l), respectively. The current findings reveal that total cholesterol levels were lower when compared with data on Malaysian lipid profiles in 2008 (Lee *et al.* 2010), which may be due to the younger age group (18–25 years old) in the present study. While, in haemoglobin measurement (*Table 3*), mild or moderate anaemia was reported in the current study (34.5%), which is slightly higher when compared to another study conducted in Tuaran District of Sabah (Leng *et al.* 2004).

Table 2. Lipid profile of the respondents (n = 162).

Lipid (mmol/l)	Mean ± S.D
Serum total cholesterol	4.29 ± 0.79
HDL-cholesterol	0.75 ± 0.18
LDL-cholesterol	2.98 ± 0.59
Triglycerides	1.10 ± 0.62

Note: Data are expressed as n (%) unless otherwise indicated.
Based on cut-offs by International Diabetics Federation 2006

The majority of the respondents had poor aerobic capacity (70.7% males and 25.7% females) (*Table 4*). It is also noted that the male respondents generally had a higher VO_2max than their female counterparts. The VO_2max values of the present study (51.17 ± 3.93 ml/kg/min) were lower when compared with another study (56.8 ± 3.5 ml/kg/min) (Singh *et al.* 1989).

Although our study relied on the inclusion of a large, convenient sample, a major limitation still exists, data were self-reported and cross-sectional (does not infer causal relationships). The limited blood samples are obtained that may not be able to give comprehensive metabolic profiles. The aerobic capacity measurement may need to include lab protocol using treadmill to obtain actual aerobic capacity. Food consumption should have been obtained which will able to portray the eating habits as well as energy consumption. Due to the lack of direct physical activity and food consumption assessment in our current study, concrete evidence to support this finding requires further investigation.

Table 3. Distribution of haemoglobin of the respondents by gender.

Haemoglobin (g/l) (n = 162)	Mean ± S.D	n (%)
Male (n = 79)	147.4 ± 14.9	
Severe anemia (< 80)		–
Moderate anemia (80–109)		–
Mild anemia (110–129)		19 (24.0)
Non-anemia (≥ 130)		60 (76.0)
Female (n = 83)	122.9 ± 13.7	
Severe anemia (< 80)		-
Moderate anemia (80–109)		3 (4.5)
Mild anemia (110–119)		34 (40.1)
Non-anemia (≥ 120)		46 (55.4)

Note. Data are expressed as n (%) unless otherwise indicated.
Based on cut-offs by WHO, UNICEF, UNU2001

Table 4. Distribution of VO_2max of the respondents by gender.

VO_2max (ml/kg/min) (n = 324)	Mean ± S.D	n (%)
Male (n = 157)	51.17 ± 3.93	
Excellent (≥48.20)		16 (10.2)
Good (44.23–48.19)		16 (10.2)
Fair (40.98–44.22)		14 (8.9)
Poor (<40.98)		111 (70.7)
Female (n =167)	38.82 ± 3.93	
Excellent (≥40.98)		13 (7.8)
Good (36.65–40.97)		29 (17.4)
Fair (33.76–36.64)		82 (49.1)
Poor (<33.76)		43 (25.7)

Note. Data are expressed as n (%) unless otherwise indicated.
Based on cut-offs American College of Sports Medicine 2006

The metabolic risk factors for cardiovascular disease and Type 2 diabetes are increasingly apparent in young adults (Ramachandran *et al.* 2012). There is no data available on physical fitness among younger age group in Malaysia. Physical fitness and physical activity are strong determinants for health outcomes. In fit individuals, confer a lower metabolic risk profile. It is noted from recent National Health Morbidity Survey (NHMS), Malaysian are generally inactive and non-communicable disease increasingly apparent in adults (National Health and Morbidity Survey 2011). The respondents' blood profiles were good; BF% and WC are within normal range. Conversely, the poor aerobic capacity, which means they are not physically fit, may predispose to non-communicable disease in future.

CONCLUSION

The current study provides important insight into the level of physically active lifestyles and metabolic profile in the university-aged population, revealed that the respondents in this study have low physical fitness level, and there is evidence of under- as well as over-nutrition (overweight and obese) issues among undergraduates. Despite knowing the health benefits associated with an active lifestyle, the majority of young adults do not engage in sufficient levels of physical activity. Increasing physical activity and obesity prevention has been identified as the top priorities in the national health agenda, which may require additional focus among university students. PA needs to be increased and emphasized during university life because it is likely that the undergraduates may persist in this low level of PA, or even decrease it further in the years following graduation. Establishing healthy lifestyle from the time they are in university would ensure that they are in better health when they enter the workforce where stress levels and time demands will be even greater. Additionally, it is also important that varsity-aged students are educated concerning how important is the simple height and weight measurement to calculate the BMI and understand that this measure is abasic health-screening tool. On the other hand, the university should also incorporate a health promotion programme propounding PA and nutrition into the university's general education requirements.

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A Preliminary Study: Comparative Toxicity of Extracts from *Tinospora tuberculata* Beumee and *Lumnitzera racemosa* Willd on *Aedes aegypti* Linnaeus Larvae (Diptera: Culicidae)

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Research has been widely made on the potential use of herbal plants as effective agents for mosquito vector control. However, only a few studies have been carried out to investigate the potential insecticidal activity of mangrove plants against these dengue vectors. This study was carried out to examine the comparative toxicity of two mangrove plants, *Tinospora tuberculata* and *Lumnitzera racemosa* against the *Aedes aegypti* larvae. The leaf and stem of *T. tuberculata* and *L. racemosa,* were extracted using 95% methanol and these extracts bioassayed against the 3rd instar *larvae of aegypti* under laboratory conditions. The LC_{50} values of the crude extract of *T. tuberculata* stem and leaf were 0.5778 mg/ml and 0.7213 mg/ml, respectively. For *L. racemosa*, the LC_{50} value for stem was 1.2833 mg/ml and 1.1957 mg/ml for leaf. However, no significant differences in toxicity were detected for the stem and leaf of both plants. Both plants, especially *T. tuberculata* might contain insecticidal components toxic to larvae of *Ae aegypti*.

Key words: Toxicity; *Tinospora tuberculata*; *Lumnitzera racemosa*; *Aedes aegypti;* herbal plants; mosquito vector; dengue vectors

Some mosquitoes are known to transmit many serious diseases to humans. Among these mosquitoes, the commonest genus that bites humans are *Anopheles, Culex, Aedes, Mansonia, Haemogogus, Sebethes* and *Psorophora.* In Malaysia, there are 381 species in 20 genera of mosquitoes, some of which are medically important vectors of arboviruses (Ken *et al*. 2002).

The principal mosquito vector that transmits the arbovirus of dengue is *Aedes aegypti*. The dengue (Flaviviridae) virus is arguably the most important arbovirus affecting humans (Curtis 1986). Dengue fever and dengue hemorrhagic fever are caused by one of four closely related but antigenically distinct virus serotypes, DEN-1 DEN-2 DEN-3 and DEN-4. Infection by one of these serotypes provides immunity to only that serotype for life, so people living in a dengue-endemic area could have more than one dengue infection during their lifetime (Glubber 1989).

The incidence of dengue has attained levels that are of considerable concern to local authorities. Until today, there are no effective dengue vaccines available. The only way of decreasing the incidence of the disease is the suppression of *Ae. aegypti*. Experience has shown that spraying of chemical insecticides against this mosquito was not effective, since

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it was highly domesticated and many adults rested indoors in hidden places such as closets. One of the possible ways of reducing mosquito densities to a level where dengue epidemics did not occur was to control the immature stage. Therefore, systematic treatment of the breeding places using larvicide could be effective.

In recent years, the use of environmentally friendly and biodegradable agents of plant origin has received renewed interest for disease vector control (Alkofahi *et al*. 1989). They are potentially suitable for use in integrated vector management programmes as the selective pressure imposed by conventional insecticides was enhancing resistance in mosquito populations. Today, over 2000 species of plants that possess some insecticidal activity are known (Jacobson 1989). The demand for new products that are environmentally safe, target-specific, and degradable enhance interest for natural products. Considerable effort has been focused on plant derived material for potentially suitable useful products such as commercial insecticides. The first compound that was used extensively against adult mosquitoes was the flower extract of *Chrysanthemum cinerariaefolium* (Senthil *et al*. 2005). Plant derived chemicals also show some potential in reducing the biological activities of insects rather than synthetic insecticides. For example, the oil extracts from *Cinnamomun pubescens*, *C. micropyllum* and *Litsea elliptice* (Family: Lauraceae) show effective larvicidal activity (Wong 2001). Other plants such as *Bougainvillea glabra* (Family: Nyctaginaceae) also have the potential as pesticides (Thangam & Kathiresan 1990).

Considering the large number of plants that are reputed to possess some form of insecticidal activity, only a few local plants have been scientifically evaluated. In this current study, we were interested in examining the potential insecticidal activity of the herbal plant, *Tinospora tuberculata* (Ranunculales: Menispermaceae) and the mangrove plant, *Lumnitzera racemosa* (Angiospermacea: Combretaceae) on 3rd instar *Ae. aegypti* larvae. There have been very few studies focused on *T. tuberculata* and *L. racemosa* as insecticides or pesticides (Thangam & Kathiresan 1990; Lee & Hishamudin 1990). We chose *L. racemosa* as this mangrove plant was abundantly found in Terengganu. Furthermore, some studies have reported that the fruit, bark and flower of *L. racemosa* have been used as treatment for asthma, diabetes and snake bite (Premanathan *et al*. 1992). Attempts also have been made to compare the different toxicity effects of crude extract from the leaf and stem of both plants. The outcome of the study could hopefully contribute to the discovery of an alternative bio-insecticide for the control of *Ae. aegypti* larvae.

MATERIALS AND METHODS

Sample Collection and Extraction

T. tuberculata was collected from an area around Wakaf Tembusu in Terengganu, whereas *L. racemosa* was obtained from a mangrove area near the nursery of Universiti Malaysia Terengganu (UMT). The plant material according to the part needed, were crushed into small particles and dried at room temperature (~27°C). Dried plant particles were ground into powder separately. 100g of the powder from each needed part of the plant were mixed with 1000 ml of methanol (95%) and stirred for 3 days in a stirrer machine. Filtration was done on the suspension using Whatman No.1 filter paper and filter funnel into a beaker. The filtered solution was placed into a flask and concentrated by evaporation using a low pressure rotary evaporator at 40°C–45°C. Extractions were done until all the solvent was evaporated leaving the solid crude. The crude extract was stored in a beaker covered with aluminum foil at 20°C for future bioassay. The same process was done for all needed parts of both plants.

Preparation of Stock Solution and Test Concentrations

Stock solutions of extracts from both plants were prepared with concentrations of 50 mg/ml. These were obtained by diluting 5 g of crude extract in 100 ml of solvent (1% of DMSO). The stock solution was then serially diluted, in triplicate, with distilled water to prepare test solutions of 2 mg/ml, 1 mg/ml, 0.500 mg/ml, 0.250 mg/ml and 0.125 mg/ml crude extract. Sterile water was used to dilute the crude extract and preparation of the dilutions of leaf and stem for each plant species were the same using the method as above.

Aedes aegypti Larvae

Eggs of *Ae. aegypti* were obtained from the insectarium of the Unit of Medical Entomology, Institute for Medical Research, Malaysia. Larvae of *Ae. aegypti* were obtained by placing a filter paper with dried mosquito eggs into a tray filled with water. The eggs were left for 1 h to 48 h to hatch. One teaspoon of dried ox liver powder was added into the container as larval food once the eggs had hatched. The larvae were maintained at room temperature (27°C ± 2°C) and at a relative humidity of between 75%–85%. The water in the container that was used to rear the larvae was changed frequently to ensure that it was clean and clear. The container was covered with muslin cloth to ensure that other mosquitoes did not lay eggs into the tray.

Bioassay Procedure

Bioassays were performed on the late 3rd or early 4th instar larvae of *Ae. aegypti*. One hundred ml of test solutions of five different concentrations were prepared. At each concentration, 10 larvae were tested. In control runs, 100 ml of distilled water without the extract was prepared. The treatments were replicated three times and each replicate set contained one control. Experiments were conducted at room temperature (~27°C), photoperiods of 12 h light followed by 12 h dark (12L : 12D) and at a relative humidity of 75%–85%. Mortality counts were made after 24 h of exposure. In the analysis, both dead and moribund larvae were considered as dead, whereas numbers of live larvae were counted separately.

Statistical Analysis

Data obtained from the bioassay toxicity test were analysed by probit analysis (Finney 1971). Probit analysis was used to calculate the confidence intervals for dose-response quantiles (LC_{50}). One-way ANOVA was used to compare the toxicity of leaf and stem crude extract for each part of plants studied. The data was $\log(x+1)$ transformed to ensure normality in calculations of means and ANOVAs. If the control mortality was below 20%, Abbott's formula was used (World Health Organization 1981). However, in tests where the control mortality was above 20%, the experiment was discarded.

RESULTS

The LC_{50} of the leaf extract of *T. tuberculata* was 0.7213 mg/ml against third instar *Ae. aegypti* larvae. In comparison, the LC_{50} of the stem was 0.5778 mg/ml against *Ae. aegypti* larvae. There was no mortality observed in the controls. In this experiment, there was no significant difference detected between the leaf and stem of the herbal plant, *T. tuberculata* ($p = 0.2396$).

The LC_{50} value for stem and leaf of *L. racemosa* was 1.2833 mg/ml (for stem) and 1.1957 mg/ml (for leaf). There was no mortality observed in the controls and no significant difference between the leaf and stem of the mangrove plant, *L. racemosa* was detected ($p = 0.2430$).

Table 1 summarizes the lethal concentration of extract (LC50) for each part of plants against the 3rd instar *Ae. aegypti* larvae. The insecticidal activity of stem and leaf of *T. tuberculata* was higher (0.5778 mg/ml and 0.7213 mg/ml) than the stem and leaf extraction of *L. racemosa* (1.2833 mg/ml and 1.1957 mg/ml). However, the variance between leaf and stem of *T. tuberculata* showed no significant difference (p = 0.2370). Similarly, no significant difference between the leaf and stem of *L. racemosa* was detected (p = 0.243).

DISCUSSION

Even though there were no significant differences between the leaf and stem for each plant, both plants showed the presence of certain insecticidal chemical compounds. The study suggests that both *T. tuberculata* and *L. racemosa* did have compounds which exhibited insecticidal activity on the target insect. Both *T. tuberculata* and *L. racemosa* plants exhibited different levels of toxic effect on third instar *Ae. aegypti* larvae. *T. tuberculata* exhibited higher toxicity compared to *L. racemosa* against the 3rd instar *Ae. aegypti* larvae. However, no significant differences of LC_{50} values were detected between leaf and stem of *T. tuberculata*. For *L. racemosa*, both stem and leaf exhibited LC_{50} values of 1.2833 mg/ml and 1.1957 mg/ml, respectively. Similarly, both LC_{50} values of leaf and stem of *L. racemosa* showed no significant differences.

The results showed that extracts of *T. tuberculata* stem greatly affected the growth of *Ae. aegypti* larvae compared to extracts from the leaf. Generally, higher concentrations of each part of *T. tuberculata* affected the larvae development and did have a significant impact on the mortality of the larvae. We found most of the larvae exhibited abnormal and erratic movements, dying before reaching the pupal stage when they were exposed to higher concentrations of *T. tuberculata*. The bioactivity of phytochemicals from plants against mosquito larvae could vary significantly depending on the plant species, plant part, age of plant part, solvent used in extraction and mosquito species (Essam *et al.* 2004). Presumably, the toxicity effect exhibited by both plants indicated possible bioactive compounds that were engaged in insecticidal response. Plants from family Menispermaceae had been identified to exhibit pharmaceutical properties and also as insecticides (Essam *et al.* 2004). Previous studies using plants from family Menispermaceae had obtained very good toxicity effects on *Ae. aegypti* larvae. For example, *Stephania japonica,* which was distributed widely in Ulu Anap, Sarawak secondary forest, had been identified to have antimalarial properties (Ibuka *et al.* 1967). Although the toxicity effect possessed by *T. tuberculata wa*s much lower than other plants from family Menispermacea, the experiment had proved that the plant exhibited insecticidal activity. *Tinospora tuberculata* contained insecticidal phytochemicals that

Table 1. LC_{50} values for crude extract of stem and leaf of *Tinospora Tuberculata* and *Lumnitzera Racemosa* against *Aedes Aegypti* larvae after 24 h.

Plant species	Part of plant	Regression equation	Chi-square value	LC_{50} (mg/ml)
Tinospora tuberculata	Leaves	$y = -0.3623x + 5.6422$	0.8957	0.5778
	Stem	$y = -0.4287x + 5.9133$	0.3968	0.7213
Lumnitzera racemosa	Leaves	$y = -1.1381x + 4.6715$	1.3587	1.2833
	Stem	$y = -1.4967x + 4.6275$	1.1192	1.1957

were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects (Ibuka *et al.* 1967).

Pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, *d*-limonene camphor and turpentine were some of the important phytochemical insecticides which were widely used in many countries before the introduction of synthetic organic insecticides (Thangam & Kathiresan 1990; Bandara 2000). *T. tuberculata* also contained alkaloid as its secondary metabolite. Secondary metabolites or phytochemicals could be extracted from either the whole plant or from specific parts of the plant that were known to contain a concentration of the desired active chemical. Alkaloids are known as an important secondary metabolites which impart resistance against many insect pests. The resistance to feeding insects by a number of well known alkaloids, the plant sources from which they had come and the insect species deterred, have been uncovered (Essam *et al*. 2004; Ibuka *et al.* 1967; Bandara 2000).

Lumnitzera racemosa was considerably less explored compared with other plants, especially herbal plants which were well known for their medicinal properties. That was possibly because mangrove plants were presumed to have no value for medicinal usage and thus very few studies had been conducted to investigate the potential of mangrove plants as an alternative product in medicinal usage. Due to that reason, we would like to explore and understand whether mangrove plants can possibly be used as an alternative to conventional synthetic insecticides, besides the herbal plants. In this study, we have found that the leaf of the *L. racemosa* was more effective that the stem. *L. racemosa* did have a toxic effect on *Ae. aegypti* larvae, with leaf extract more toxic compared with the stem extract. However, the active chemical compounds of *L. racemosa* were unknown and for the future research, we plan to investigate the chemical properties of *L. racemosa* and their role as potential insecticides for controlling the population of *Ae. aegypti*.

Different mosquito species display different susceptibilities to similar phytochemicals. Thus, this toxicity study might have been much more promising if it had been carried out against different species of mosquitoes. The results of this study however, could be useful in the research for newer, more selective and biodegradable larvicidal compounds. The extracts of these plants can also be combined with synthetic insecticides to obtain more suitable results. For example, leaf and flower extracts of *Bougainvillea glabra*, exhibit higher insecticidal activity when combined with DDT, Benzene hexachloride and malathion (Thangam & Kathiresan 1990). It would be interesting to investigate whether the insecticidal and insect repellent compounds in both mangrove and herbal plants contain tannins and alkaloids or other classes of compounds. Further investigations needed are to isolate these compounds using combinations of liquid chromatography, nuclear magnetic resonance spectroscopy and high-pressure liquid chromatography. These techniques have been used by natural product chemists to isolate and determine the structures of interesting molecules.

CONCLUSION

The results of this study shows promising insecticidal activity from both herbal (*T. tuberculata*) and mangrove (*L. racemosa*) plants towards the 3rd instar *Ae. aegypti* larvae. *T. tuberculata* exhibited higher larvicidal toxicity compared to *L. racemosa*. Both plants, *T. tuberculata* and *L. racemosa* had the potential to produce alternative insecticides effectively against the *Ae. aegypti* larvae. Further research would have to be done to determine the active compounds from both plants that could be used as larvicides.

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Biodegradation of NR Latex-based Materials via a Carbon Dioxide Evolution Method†

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NR as a natural polymer has biodegradable characteristics and their existence was examined using CO_2 evolution methods. The CO_2 molecule produced by micro-organism metabolisms in the degradation system was quantified using a conventional acidimetric method. An aerobic system was determined as most suitable condition to be examined under this method. The presence of O_2 in the system would help micro-organisms to destabilize the natural polymer. The material of LATZ, HA film and NR gloves showed significant weight loss and were able to produce CO_2 evolution curves after 45 days in the biodegradation system compared to synthetic polyisoprene films. Gel permeation chromatography, fourier transform infrared spectroscopy and scanning electron micrograph were used to characterize the degraded sample at molecular and physical levels.

Key words: Natural rubber latex; biodegradation; CO_2 evolution; aerobic system; micro-organisms

The increasing usage of natural rubber (NR) latex as the major ingredient of material especially for medical usage such as gloves and catheters in the last decade has created a vast market for NR latex. During the last decade, rubber consumed for latex products had increased from 348 907 tonne (2002) to about 563 192 tonne (2012) (*Figure 1*) (Malaysian Rubber Board 2008). This increasing trend has also become anissue of concern among environmentalists and other concerned parties. The growing amount of waste generated through this industry in landfills has had caused great concern among the environmentalists. The management of this waste has thus become a much debated issue among our society nowadays. Therefore, material possessing biodegradable characteristics have greater potential as the more sought out material by the product technologists and the latex-based product industry.

Degradation is defined as an irreversible process which leads to a significant change in the material structure such as the loss of its original properties (Pagga 1997a). Examples of these properties are the integrity, molecular weight or structure of the materials and the process is usually affected by environmental conditions. Biodegradation is a part of the degradation process which involves the presence of micro-organisms in the degradation process. *ISO 14855* (International Organization for Standardization 1999) describes ultimate aerobic biodegradation as a polymer converted into a new biomass (remaining fragment), carbon dioxide and water molecules (*Equation 1*) as a result of polymer organic compound breakdown by micro-organisms in the presence of oxygen.

$$\text{Natural rubber polymer} + O_2 \xrightarrow{\text{Micro-organism}} \text{Biomass} + CO_2 + H_2O \tag{1}$$

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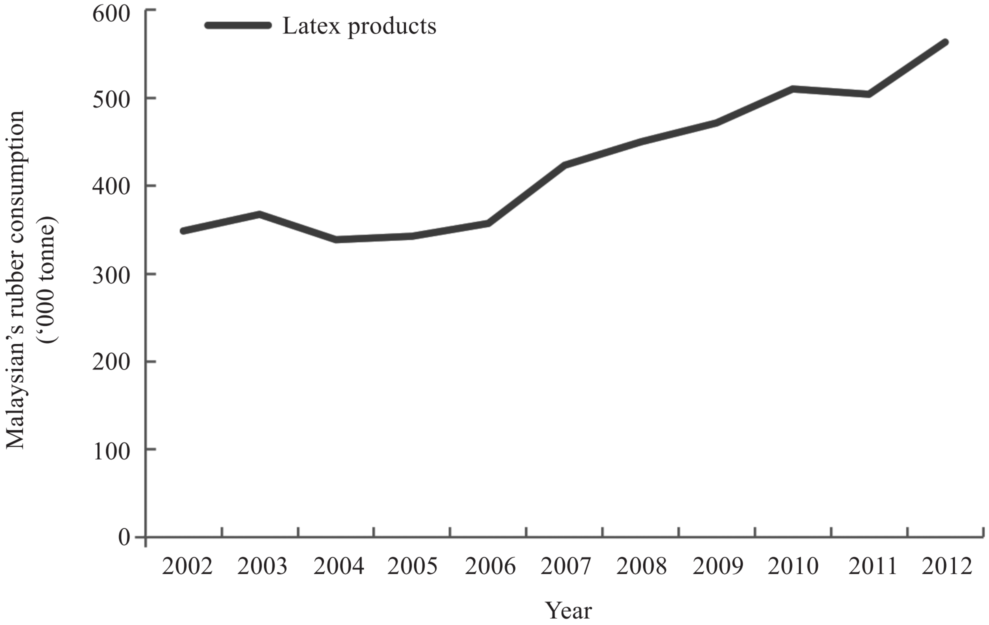
† Part of this data was presented at the *Malaysian Science and Technology Congress 2010*.



Source: Department of Statistics, Malaysia and Malaysian Rubber Statistics 2013

Figure 1. Malaysia's rubber consumption for latex product sector.

A brief description of numerous methods to assess the biodegradability of polymeric material is described elsewhere (Pagga 1997b). The objective of the present study was to investigate the aerobic biodegradability of NR and synthetic polymers using modified standard test methods designed specifically for plastics (International Organization for Standardization 1999). The biodegradability of these polymers was evaluated using indirect measurements such as carbon dioxide production. Numerous reports describe different methods of measuring the carbon dioxide production of polymer materials directly such as by infrared spectroscopy (Ellis & Schurin 1969), gas chromatography (Mohr *et al*. 1093; Kamiński *et al*. 2003) or manometric pressure (Umbreit 1946) or indirectly such as by gravimetric methods (Koltoff 1936) and acidimetric methods (Freijer & Bouten 1991). However, some of these direct measurements were found to be either not suitable for the analysis of small gaseous samples or to be used for inexpensive portable carbon dioxide assessment (Satienperakul *et al*. 2004). Therefore a simple, fast and economical method was selected in this experiment which was the acidimetric method. A much better understanding of the degradability of the sample could be determined by assessing its degradability physically and chemically. That could be investigated by determining the molecular distribution and molecular structure of the degraded samples. The biodegradability of NR was assessed by the principle of determining the CO_2 gas evolution andthe disintegration of the test material in the system as produced by microbial activityas the sole source of carbon and energy. It is hoped that this information would provide greater potential to NR as a sustainable green material to the industry and society.

MATERIALS AND METHODS

Preparation of Samples

Test samples of about 0.5 mm thickness were prepared from High Ammonia (HA) and Low Ammonia (LATZ) latices. The samples were subjected to 40% dilution and the volume of amount to be cast calculated before the preparation of the latex film samples. The samples were then cast on leveled glass plates (18 mm × 25 mm) and left to dry for about 2 to 3 days. The cast film was then peeled and powdered using cornstarch. The samples were each cut into 2 cm by 2 cm pieces for degradation testing.

Additional test samples were also selected and cut from NR powder-free glove and synthetic polyisoprene film. Cellulose paper was the positive reference for this experiment.

Preparation of Composting Soil

A controlled aerobic composting condition was designed to simulate the conditions of an aerobic composting environment as recommended in *ISO 14855*. The test material was mixed with innoculum which consisted of stabilized and mature material composted for at least three months prior to use. The tests were conducted in darkness under room temperature. Characterization of commercial and composted soil has been reported elsewhere (Shabinah & Amir 2010).

Assembled Apparatus

The apparatuswas assembled as illustrated in the schematic diagram *Figure 2*. Three sets of complete degradation systems were assembled in a single experiment to ensure that the experiment would be conducted simultaneously. Of the three sets, two were conducted for test samples and other was for the blank test.

Procedure

Aqueous solutions of 0.25 M KOH and 0.25 M NaOH were prepared. These solutions were used as CO_2 absorption solutions because of their ability to accurately assess the recovery of CO_2. NaOH functioned as a CO_2 gas trap at the introductory stage and KOH functioned as a CO_2 absorption trap at the end of the degradation process.

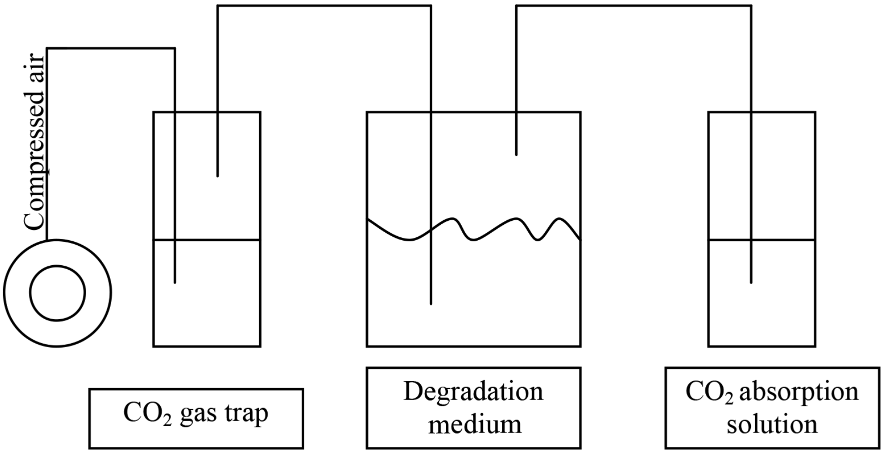


Figure 2. Schematic diagram of CO_2 evolution degradation method.

The samples were buried in separate composting vessels and the degradation process was conducted for 45 days. About 100 ml of the 0.25 M KOH with absorbed CO_2 gas was collected and titration conducted every alternate day.

Methods for Determining CO_2

Conventional method: The CO_2 produced were retained in an alkaline solution (NaOH) and the quantity was determined through acidimetric titration. A CO_2 evolution graph was plotted as CO_2 concentration (measured valuesin the sample flask subtracted with blank values to obtain exact values of the degradation activity) versus time (day) of degradation process. *Table 2* summarises the different batches conducted according to the test method.

Table 1. Batches of experiment.

Batch	Sample 1	Sample 2
1	NR powder free (PF) glove	Cellulose paper
2	HA film	LATZ film
3	NR film	Polyisoprene film

Characterization of Degraded Samples

Weight loss. The retrieved samples were further characterized by determining the percentages of initial weight remaining and weight loss. The percentages of initial weight remaining and weight loss were calculated following *Equations 2* and *3*:

$$\%\ \text{initial weight remaining} = W2/W1 \times 100\% \tag{2}$$

$$\%\ \text{weight loss} = \frac{W1 - W2}{W1} \times 100\% \tag{3}$$

where, $W1$ = Initial weight; $W2$= Final weight.

Gel permeation chromatography. Molecular weight distributions were obtained by gel permeation chromatography using GMHHR columns on a Viscotek Triple or Tetra Detector Array (TDA) Max with a Refractive Index (RI) Detector. Molecular weights of all the latex film samples were determined by using standard samples in polystyrene.

Fourier transform infrared (FTIR) spectroscopy. FTIR spectrums of the samples were recorded with a Varian Spectrum 2000 FTIR spectrophotometer using a horizontal 45° Ge-ATR accessory. Sixty four scans were completed with a resolution of 4 cm^{-1}.

SEM micrograph. The surface morphology of sampleswere observed under JOEL JSM-6701 F operated under 2.0 kV with 15 mm working distance.

RESULTS AND DISCUSSION

An aerobic condition which utilized O_2 in the system became a vital source for microbial activity in the degradation medium. An aerobic composting condition was found to be able to construct a much reliable CO_2 evolution curve than an anaerobic composting condition. As described elsewhere (Imal *et al*. 2007), the serrated O_2 course in the biodegradation system had dependencies corresponding towards aeration and gradual respiration of microbial activity. The gradual reduction of O_2 in the system might be due by consumption by micro-organismswhich would ultimately create the accumulation of CO_2 in the system. The CO_2 could be detected by acidimetric methodology. Therefore in this study, it was postulated that constant aeration and O_2 input into the system would continue the respiration of these micro-organisms in assisting assimilation of the test samples.

The resulting curves could be divided into three regions namely lag phase, biodegradation phase and plateau phase (*Figure 3*). Lag phase described the adaptation of microbial inoculums to the test substance in the system (Massardier-Nageotte *et al*. 2006). Thus, the lag phase periodwas identified as the time required for micro-organisms to condition to the degradation medium environment.

The degradation phase would take place after the lag phase ended and micro-organisms in the test vessel sourced available materials in the vessel such as organic materials as a food source. It was noted in the study that the biodegradation phase commenced after approximately the 10th day. By that time, it was assumed that the microorganisms had consumed the test material and produced CO_2 which caused the disintegration of the sample and evolution of CO_2. All three NR based samples were able to produce a constant increment of CO_2 in the biodegradation phase and began easing off only after the 38th day. Micro-organisms in the system might able to assimilate almost all NR-based film especially for HA and LATZ.

The synthetic polyisoprene film's CO_2 evolution curve showed lower CO_2 evolution compared to NR. That indicated that natural micro-organism did not degrade synthetic

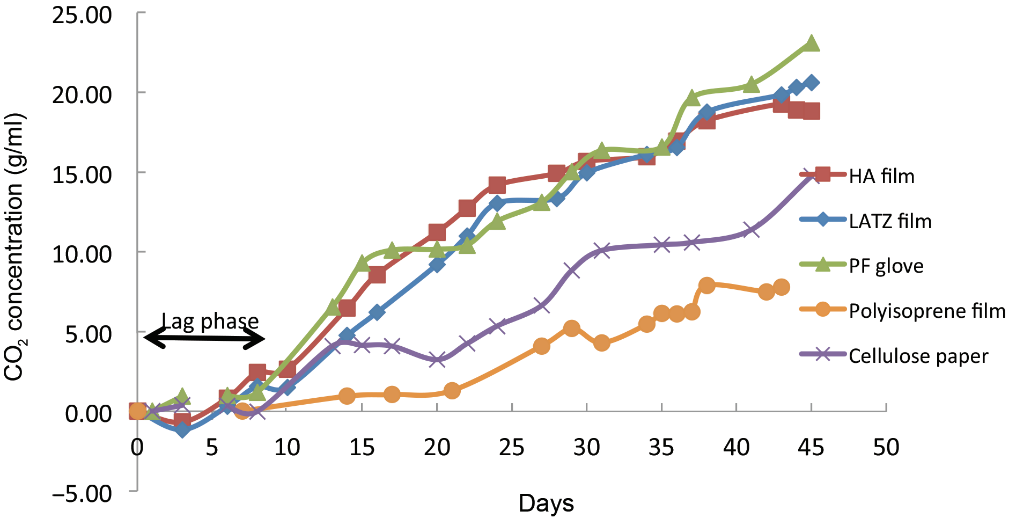


Figure 3. CO_2 evolution curves of LATZ and HA film, NR, synthetic polyisoprene film and cellulose paper in a 45 day aerobic composting environment.

material much, although the polymer structure of the synthetic sample was very much similar to NR. Synthetic polyisoprene as petrochemical-based plastic materials were not easily degradable in the environment because of their hydrophobic characteristics and dimensional structure (Singh & Sharma 2008). In our case, we observed that NR which hada similar molecular structure as synthetic polyisoprene could be easily degraded compared to synthetic polyisoprenes. On the contrary, natural based material such as cellulose paper material disintegrated at a much faster rate compared to the others. As shown in *Table 2*, cellulose paper resulted in a proportionately greater reduction in weight compared to the others.

The higher weight loss by LATZ and HA film than the NR glove was expected indicating that the rubber chemical additives present in latex compounding had hindered the degradation process. LATZ film had the highest weight loss (42.8%) followed by HA film (31.3%), NR glove (7.59%) and polyisoprene film (0.96%) as tabulated in *Table 2*. Synthetic polyisoprene film had the lowest weight loss among all the other samples indicating it's inability to biodegrade as the NR film.

The molecular weights discussed in this study were obtained from tests performed on monodispersed polystyrene standards and these values should only be considered as relative molecular weights and not absolute values (*Table 3*). A more direct and absolute method for determination of scission is to follow the M_n value reductions (Barnard & Lewis 1988) and this can be identified using GPC. Besides, determination of molecular mass distribution may provide additional information on the deterioration of the main chain (Flemming 1997). The degraded HA film showed tremendous reduction in molecular weight ranging from 14.42 × 10^5 Da to 7.97 × 10^5 Da with a polydispersity of 1.54 compared to the other samples. The molecular weight difference in percentage after the degradation process isillustrated in *Figure 4*. HA degraded film had the highest molecular weight difference (39.04%) in comparison to the other degraded

Table 2. Weight loss of degraded samples.

Test material	Initial weight remaining (%)	Weight loss (%)
HA	68.7	31.3
LATZ	57.2	42.8
PF glove	92.4	7.6
Polyisoprene	99.0	1.0
Cellulose paper	95.0	5.0

Table 3. Weight-average (M_W), Number-average (M_n) molecular weights and polydispersity (M_W/M_n) of the degraded samples.

	Initial samples				Degraded samples			
	HA film	LATZ film	PF glove	Polyisoprene film	HA film	LATZ film	PF glove	Polyisoprene film
M_w (x10^5 Da)	14.42	14.06	ND	8.810	7.97	9.93	ND	8.43
M_n (x10^5 Da)	9.37	10.34	ND	3.120	3.55	5.04	ND	4.05
M_w / M_n	1.54	1.36	ND	2.818	2.48	1.97	ND	2.08

ND = Not detectable

samples. This was followed by the degraded LATZ and polyisoprene film which had values of 29.37% and 4.31%, respectively. The higher molecular weight difference indicates that a more comprehensive cleavage of the molecular bond had taken place. The reduction in molecular weight of natural rubber implied an endo-cleavage mechanism had occurred proceeding in the biodegradation of natural rubber (Jendrossek *et al*. 1997). Similar observations were reported elsewhere (Jendrossek *et al*. 1997; Tsuchii & Tokiwa 1999) where it was observed that a degraded natural rubber sample had lower molecular weight after being disintegrated by micro-organisms.

HA and LATZ degraded film showed similar FTIR spectra as shown in *Figures 5* and *6*. Strong absorbance occurred at 2960 cm^{-1} and 2854 cm^{-1} on the HA spectrum which indicated the presence of asymmetric and symmetric stretching in C-H. A more distinct disappearance of 2960 cm^{-1} was observed in both samples indicating the disappearance of saturated CH stretching which meant that the sp^3 C-H stretching molecule had broken down after the film had undergo the degradation process. The observation was consistent with NR destruction due to degradation process.

The weaker intensity observed at 1647 cm^{-1} showed that the C=C molecule had reduced and it further supported the breakdown happening in the film. The appearance of a medium intensity peak at 1236 cm^{-1} and a sharper intensity peak at 1030 cm^{-1} on degraded film indicated the C-O mixed ether molecule form as the film degraded. Peak shifts from 843 cm^{-1} to 912 cm^{-1} were caused by the transformation of C-H tri-substituted alkenes to terminal alkenes. *Tables 5* and *6* summarize the spectra while describing the wavelength bands (cm^{-1}) and the possible alignments observed on the HA and LATZ film.

LATZ film spectra (*Figure 6*) have the same appearance as HA film spectra (*Figure 5*) and it is obvious that the peaks appear at the same position as HA film. The disappearance of three peaks namely 2960 cm^{-1}, 2853 cm^{-1} and 1446 cm^{-1} suggest that disintegration had occuredin the polymer as a result of the degradation process. *Table 6* describes the assignment peak of the LATZ and its degraded film.

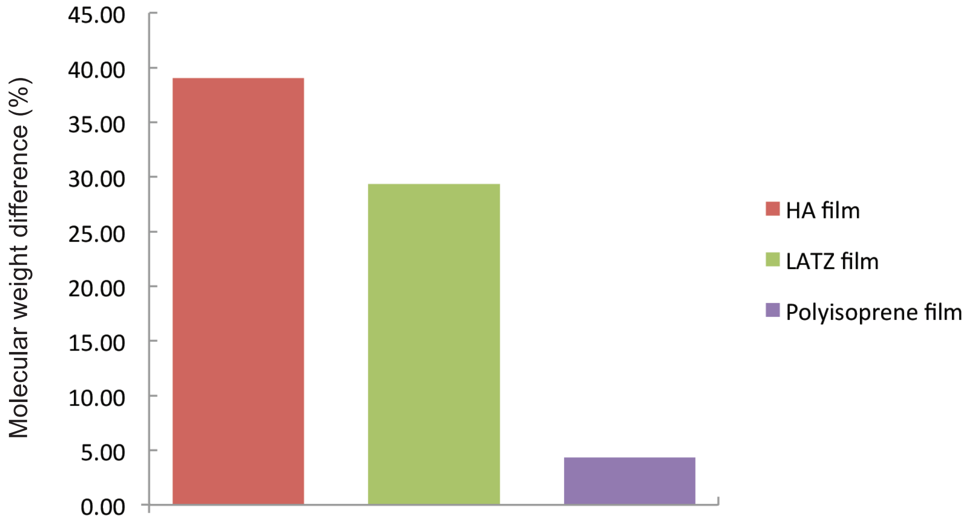


Figure 4. Molecular weight difference in percentage after degradation process.

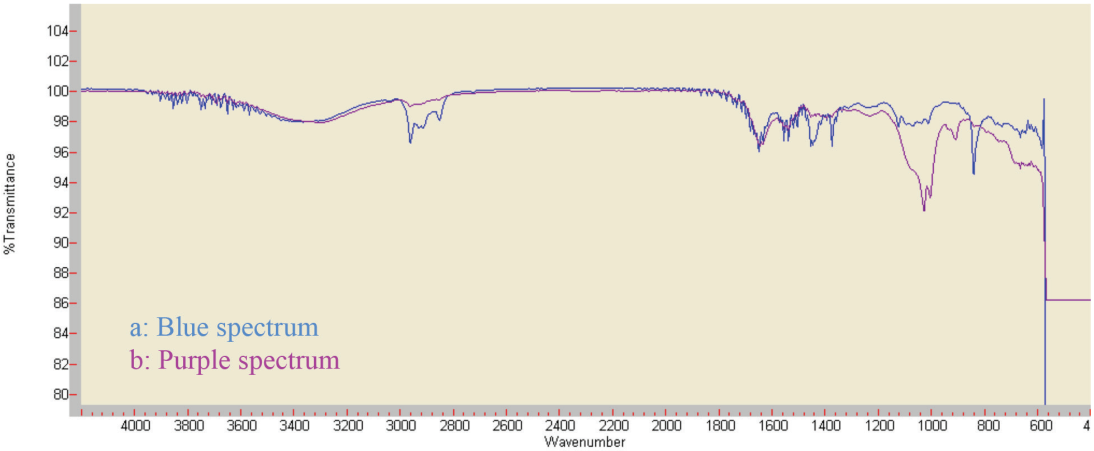


Figure 5. Spectra of HA (a) and degraded HA (b) film.

Table 4. Wavelength bands (cm^{-1}) observed on ha and degraded ha film with their possible peaks.

Wavelength band (cm^{-1})		Assignment
HA film	Degraded HA film	
Strong sharp intensity peak at 2960 cm^{-1}	ND	Evidence of saturated CH asymmetric stretching
Sharp intensity peak at 2854 cm^{-1}	ND	Evidence of saturated CH symmetric stretching
Sharp intensity peak at 1647 cm^{-1}	Less intensity peak at 1647 cm^{-1}	Evidence of alkenyl C=C stretching
Medium intensity peak at 1447 cm^{-1}	ND	Evidence of $–CH_2$- bending
ND	Appearance of peak at 1236 cm^{-1}	Evidence of C-O streching
Weak intensity peak at 1014 cm^{-1}	Sharp intensity peak at 1030 cm^{-1}	Evidence of O-C-C stretching
Sharp intensity peak at 843 cm^{-1}	Shifted medium intensity peak at 912 cm^{-1}	Evidence of alkenes C-H tri-substituted to terminal alkenes C-H

ND = Not detectable

PF and degraded PF gloves spectra are illustrated in *Figure 7*. A strong sharp intensity peak detected at 2954 cm^{-1} decreased its intensity as the sample degraded. That might contribute to saturated CH stretching molecule which reduced after the sample degraded. A more obvious peak was detected at 1030 cm^{-1} which assigned to S=O molecule and the intensity increased in degraded spectrum which suggested the sample had broken down and formed more S=O molecule side molecules. The carboxyl molecule indicated at 750 cm^{-1} for the PF gloves spectrum shifted to 912 cm^{-1} as the sample degraded. Detailed alignments of the spectra are described in *Table 6*.

Medium intensity was observed at 2960 cm^{-1} on both polyisoprene and its degraded

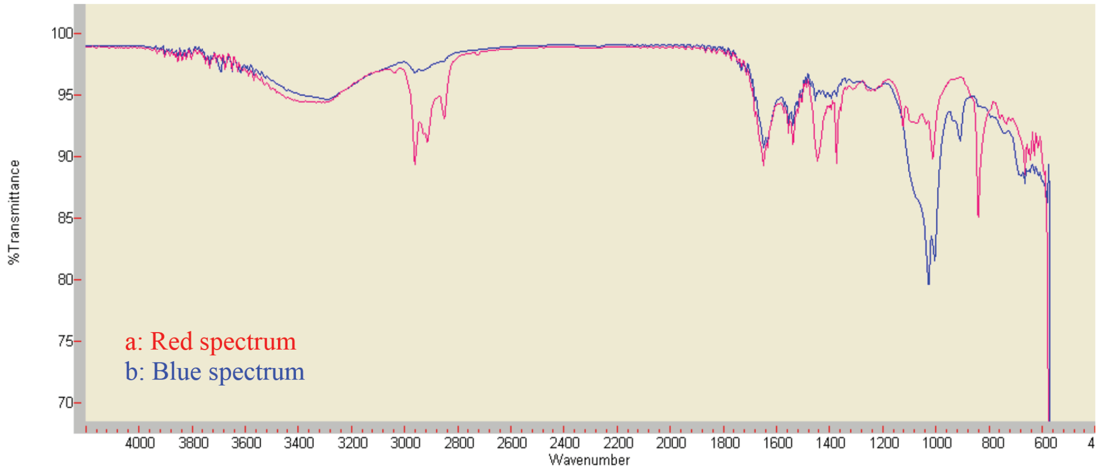


Figure 6. Spectra LATZ (a) and degraded LATZ (b) film.

Table 5. Wavelength bands (cm^{-1}) observed on latz and degraded latz film with its possible peaks.

Wavelength band (cm^{-1})		Assignment
LATZ film	Degraded LATZ film	
Strong sharp intensity peak at 2960 cm^{-1}	ND	Evidence of saturated CH asymmetric stretching
Strong sharp intensity peak at 2853 cm^{-1}	ND	Evidence of saturated CH symmetric stretching
Sharp intensity peak at 1653 cm^{-1}	Less intensity peak at 1647 cm^{-1}	Evidence of C=C molecule
Medium intensity peak at 1446 cm^{-1}	ND	Evidence of –CH_2- bending
Weak intensity peak at 1015 cm^{-1}	Sharp intensity peak at 1030 cm^{-1}	Evidence of low C-O stretching
Sharp intensity peak at 843 cm^{-1}	Shifted medium intensity peak at 912 cm^{-1}	Evidence of alkenes C-H tri-substituted to terminal alkenes C-H

ND = Not detectable

film suggesting the presence of saturated C-H asymmetric stretching which slightly decreased afterthe degradation process. However, other peaks such as saturated C-H symmetric (2856 cm^{-1}), alkenyl C=C stretching (1647 cm^{-1}) and sulphuoxide S=O (1030 cm^{-1}) remained at the same intensity after the degradation process. This indicated that polyisoprene film do not degrade and have the same molecular level as before the degradation process. The peak at 915 cm^{-1} showed the presence of vinyl C-H out of plane bend and it impliedthat polyisoprene film remained in the degraded sample as the peak shifted at 913 cm^{-1}.

SEM Micrograph

The presence of microbial colonization on the surface of the film (*Figure 9*) was evident in the micrograph. It was seen as small inoculums and the colonization was observed near a film void (*Figure 9b*). This further displayed that the

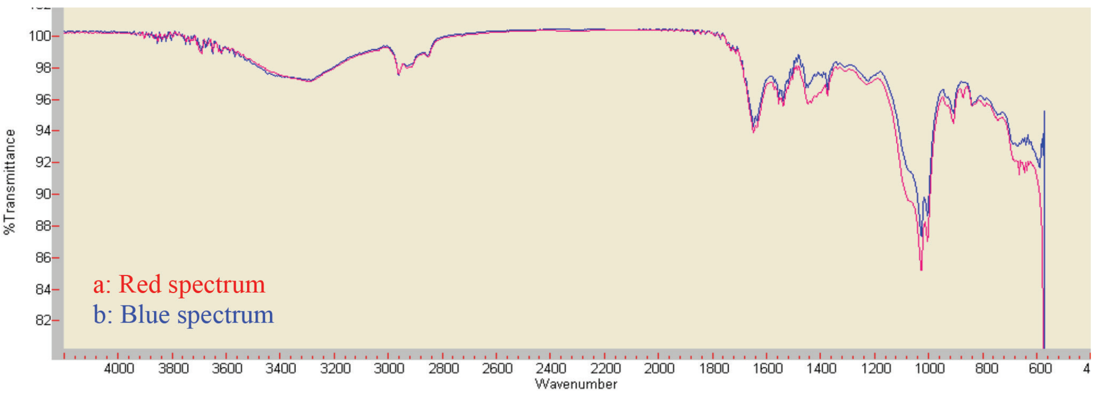


Figure 7. *Spectra of PF (a) and degraded PF (b) gloves.*

Table 6. Wavelength bands (cm^{-1}) observed on PF glove and degraded PF glove with its possible peaks.

Wavelength band (cm^{-1})		Possible peaks
PF glove	Degraded PF glove	
Strong sharp intensity peak at 2954 cm^{-1}	Weak intensity peak at 2923 cm^{-1}	Evidence of saturated CH stretching
Strong sharp intensity peak at 1646 cm^{-1}	Strong sharp intensity peak at 1646 cm^{-1}	Evidence of C=C molecule
Strong sharp intensity peak at 1030 cm^{-1}	Sharper intensity peak at 1030 cm^{-1}	Evidence of S=O molecule
Weak intensity peak at 750 cm^{-1}	Shifted weak intensity peak at 912 cm^{-1}	Evidence of low C-O stretching

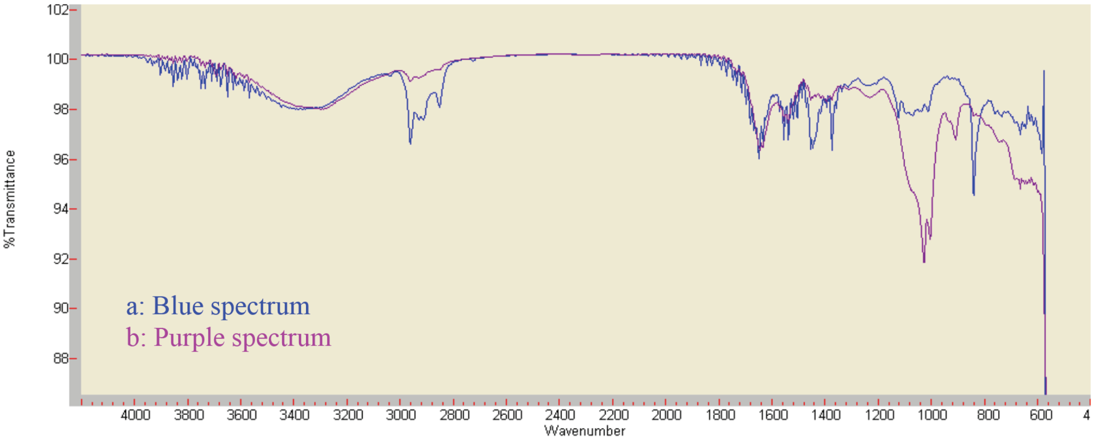


Figure 8. *Spectra of synthetic polyisoprene (a) and degraded synthetic polyisoprene film (b).*

Table 7. Wavelength bands (cm^{-1}) observed on polyisoprene and degraded polyisoprene film with its possible peaks

Wavelength band (cm^{-1})		Possible peaks
Polyisoprene film	Degraded polyisoprene film	
Medium intensity peak at 2960 cm^{-1}	Weak intensity peak at 2924 cm^{-1}	Evidence of saturated CH stretching
Weak intensity peak at 2856 cm^{-1}	Weak intensity peak at 2856 cm^{-1}	Evidence of saturated CH symmetric
Strong sharp intensity peak at 1647 cm^{-1}	Strong sharp intensity peak at 1647 cm^{-1}	Evidence of C=C molecule
ND	Strong intensity peak at 1450 cm^{-1}	Evidence of saturated methyl C-H asymmetric bending
ND	Strong intensity peak at 1380 cm^{-1}	Evidence of saturated methyl C-H symmetric bending
Strong sharp intensity peak at 1031 cm^{-1}	Sharper intensity peak at 1030 cm^{-1}	Evidence of S=O molecule
Weak intensity peak at 915 cm^{-1}	Shifted weak intensity peak at 913 cm^{-1}	Evidence of vinyl C-H out of plane bend

ND = Not detectable

film degradations were facilitated by presence of micro-organisms in the compost mediums. The SEM micrograph revealed cracks and voids formed on PF glove surface. The extensive cracks might due to chlorination on the surface of the glove and the void was due to the biodegradation. However, the surface treatment on PF glove might prevent the micro-organism from colonizing on its surface. The clumps of voids on the sample were probably due to micro-organism utilization of the test piece as carbon and energy sources. However, synthetic polyisoprene illustrated only a few strands of cracks on the sample surface. This indicated that synthetic polyisoprene does not degraded as extensively as the other NR based samples.

CONCLUSION

Biodegradation study conducted on latex using an adopted modified *ISO 14855* test method was able to result in carbon dioxide evolution plots which could be used to assess biodegradability properties in an aerobic compost condition. Additional information on the degraded samples was also gathered including properties such as percentage weight loss, molecular distribution, molecular structure and surface morphology. This study further confirms that latex based material such as HA and LATZ film degraded at a faster rate than processed product such as PF glove. The results were also compared to synthetic material such as synthetic polyisoprene latex film whose material remains intact throughout the test duration. In conclusion, the biodegradability of NR as assessed using the technique proposed for plastic material (*ISO 14855*) confirmed that NR latex based material is much superior as a green material.

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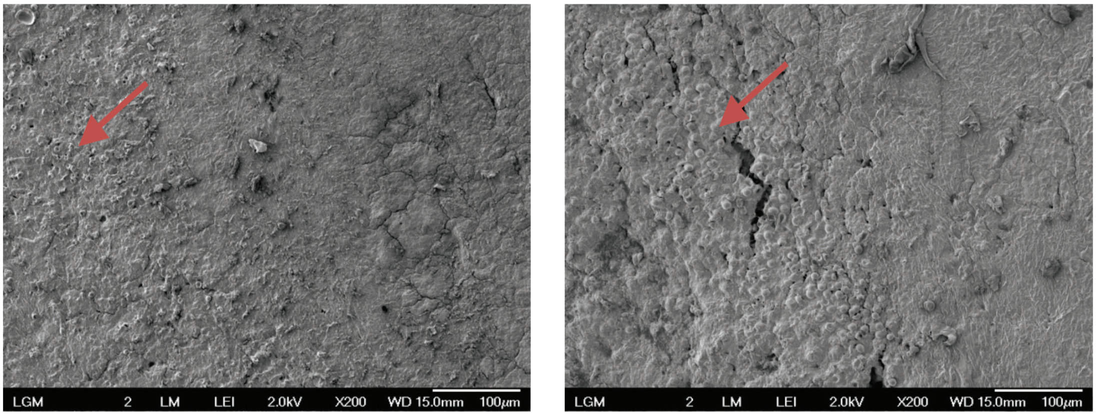
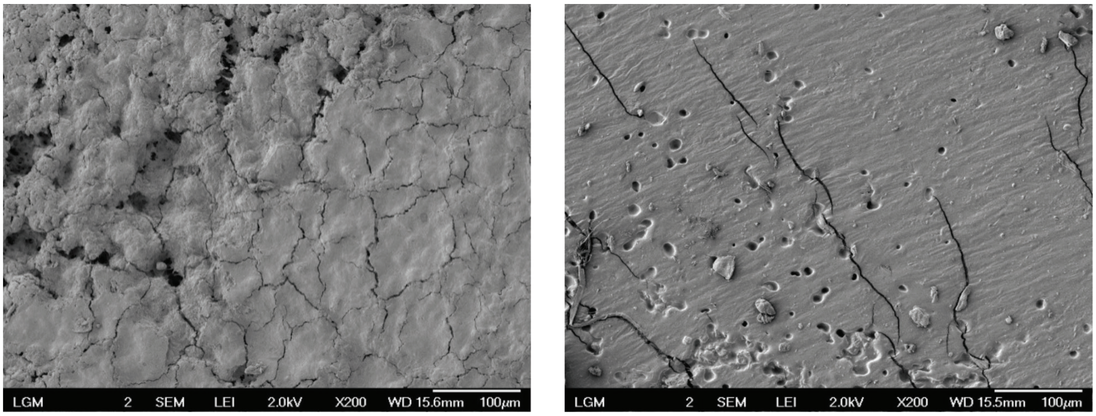


(a) Degraded HA film (b) Degraded LATZ film



(c) Degraded NR PF glove (d) Degraded synthetic polyisoprene film

Figure 9. SEM micrographs of (a): degraded HA film; (b): degraded LATZ film; (c): degraded NR powder free glove; and (d): degraded polyisoprene (Red Arrow: Microbial colonization).

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Effects of Chocolates Using Low Calorie Cocoa Butter Substitutes on Rat's Plasma Profile and Determination of *Sn*-1,3 Position

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The present study sought to determine the effectiveness of newly developed low calorie chocolate using modified fat through enzymatic interesterification process as cocoa butter substitute of male Sprague Dawley rats (n = 35) compared to cocoa butter as control. Body weight gains did not differ significantly ($p>0.05$) in all the test groups fed with chocolates which were using different types of fats. Triacylglycerol and cholesterol test showed no significant difference ($p>0.05$) for all treatment samples. In this study, a pancreatic lipase hydrolysis was performed to determine the fatty acid composition of the *sn*-2 position of the structure lipids.

Key words: Low calorie; chocolates; *sn*-1,3; body weight; plasma profile; fatty acid composition; pancreatic lipase hydrolysis

Cardiovascular disease accounted for approximately 29% of all deaths worldwide in 2004 and reduction in the consumption of high-fat foods is recommended in order to reduce cardiovascular disease burden (WHO 2010). Despite known health risks, it is extremely difficult for people to eat less fat. There have been considerable efforts to reformulate foods to have less energy from fat yet provide comparable sensory qualities by replacing the naturally occurring triacylglycerols with fat substitutes.

Medium-chain TAG (MCT) provides the advantage of being rapidly digested and thus rapidly providing energy. Therefore, they have been used in clinical nutrition for patients suffering from malabsorption. However, these patients also suffer from a lack of essential FA, a problem that is not solved by using MCT.

Human and animal studies conducted during the past 40–50 years have established that different categories of dietary fatty acids have different effects on blood lipid and lipoprotein levels (Hegsted *et al.* 1993; Katan *et al.* 1994). Unsaturated fatty acids found primarily in fats derived from vegetable sources tend to lower total and low density lipoprotein (LDL) cholesterol when compared with saturated fatty acids. On the other hand, saturated fatty acids found primarily in animal fats tend to raise total and LDL-cholesterol when compared with unsaturated fatty acids. For example, linoleic acid, which is abundant in most liquid vegetable oils (e.g. soybean, corn, and safflower), has been associated with blood cholesterol lowering, whereas lauric, myristic, and palmitic acids, common in animal fats and certain vegetable oils (palm and coconut), have been associated with blood cholesterol raising. The saturated fatty acid stearic acid has been reported to have a neutral or lowering effect on total and LDL-cholesterol compared with other saturated fatty acids (Bonanome & Grundy 1988). The monounsaturated fatty

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acid oleic acid, compared with saturated fatty acids, appears to lower LDL-cholesterol level but to retain high density lipoprotein (HDL) cholesterol (Mattson & Grundy 1985).

Studies focusing on feeding high levels of specific saturated or unsaturated fatty acids can be difficult to interpret, because fats having similar ratios of unsaturated to saturated fatty acids may have dramatically different fatty acid compositions (Hunter 2001). For example, cocoa butter and milk fat have similar levels of saturation; however, the saturated fatty acids of cocoa butter (expressed as a percentage of total saturated fatty acids) are primarily palmitic (42.5%) and stearic (55%) acids, whereas milk fat contains a wider variety of saturated fatty acids including stearic (19%), palmitic (42%), myristic (16%), and medium- (MCFA) and short-chain fatty acids (SCFA) (≤12:0 = 23%). As for palm oil which contain about 51% saturated fatty acids. As a percentage of total saturated fatty acids, palm oil contains about 9% stearic and 88% palmitic acids (Decker 1996).

In addition to overall fatty acid composition, the stereospecific distribution of fatty acids in a particular fat also should be considered when fatty acid effects are examined. Fatty acids can occupy any of three positions on the glycerol backbone, designated as *sn*-1, *sn*-2, and *sn*-3 (*"sn"* stands for "stereospecific numbering"). Oils and fats of plant origin, such as soybean oil and cocoa butter, contain unsaturated fatty acids in the *sn*-2 position and saturated fatty acids in the *sn*-1 and *sn*-3 positions. The stereospecific position of fatty acids on triglycerides plays a major role in the functionality of fats in food products. Cocoa butter, the unique positioning of palmitic, oleic, and stearic acids in two predominant triglyceride forms gives cocoa butter a sharp melting point just below body temperature. The way cocoa butter melts is one of the reasons for the pleasant eating quality of chocolate. The unique functionalities attributed to the location of specific fatty acids in fats, such as cocoa butter, is difficult to duplicate in fat substitutes (Hunter 2001).

The stereospecific position of fatty acids is important because it determines how triglycerides are digested. These investigators showed that during digestion in the gastrointestinal tract, pancreatic lipase, an enzyme highly specific for the *sn*-1 and *sn*-3 esters, catalyzes the formation of *sn*-2 monoglycerides and free fatty acids that are absorbed in the small intestine. The 2-monoglycerides are reacylated into new triglycerides that enter the lymph chylomicrons. Fatty acids released from the *sn*-1 and *sn*-3 positions often have different metabolic fates than fatty acids retained in the *sn*-2 position. These metabolic fates depend on the fatty acid chainlength and stereospecific location on the triglyceride. SCFA and MCFA (≤10 carbon atoms) can be solubilized in the aqueous phase of the intestinal contents, where they are absorbed, bound to albumin, and transported to the liver by the portal vein. Longer-chain fatty acids, such as palmitic and stearic, have low coefficients of absorption because of melting points above body temperature and because of their ability to form calcium soaps.

The role of stereospecific location of dietary fatty acids can be studied using a process called interesterification (sometimes referred to as randomization), in which the positions of the fatty acids on the glycerol backbone are rearranged. Interesterification is accomplished by catalytic methods at relatively low temperature and permits the exchange of fatty acids both within and between triacylglycerols. The process may be enzymatic (i.e. highly controlled) or chemical (i.e. relatively uncontrolled). The food industry uses interesterification (largely chemical) to modify the melting and crystallization behaviour of fats. Also, the hardening of a liquid oil by interesterifying it with a solid fat offers an alternative to the use of partial hydrogenation in the manufacture of margarines and spreads. This exchange of fatty acids results in their randomization among all three stereospecific positions. Thus, a naturally occurring fat having oleic acid primarily in the *sn*-2 position after interesterification could have oleic acid roughly equally distributed among the *sn*-1, *sn*-2, and *sn*-3 positions (Hunter 2001). The positional distribution of MCFA in the dietary fat had no significant effect on lymph flow, triglyceride output, or lipid composition of the chylomicrons. The positional distribution of *sn*-2 fatty acids in the synthetic triglycerides was largely maintained in the chylomicron triglycerides. These results indicated that fatty acids with chain lengths less than 10 in the dietary triglycerides are transported by the lymphatic system, and the positional distribution is preserved in chylomicron triglycerides. The interesterification (randomization) process does not change the degree of unsaturation or the isomeric state of the fatty acids, as they transfer in their entirety from one position to another. Interesterification thus allows investigators to compare nutritional effects of diets that are identical in fatty acid composition but different in triacylglycerol composition. Randomization of naturally occurring fats could have implications regarding cardiovascular health. Early events in the metabolic processing of dietary triglycerides may have an important impact on the risk of chronic diseases such as coronary heart disease. By using structured triglycerides containing predominantly stearic and oleic acids, Summers *et al*. assessed the possibility that the initial removal of fatty acids from chylomicron triglycerides might be fatty acid-specific. The hypothesis was that a stearoyl chain at the *sn*-2 position of chylomicron triglyceride might hinder lipoprotein lipase action and thus clearance of stearic acid.

Our aim in this study was to determine the position of fatty acids at *sn*-1 and *sn*-3 in of low calorie CBS and the effects to the experimental animals (rats). The nutritional value of the chocolate to the rats was also observed through their body weight gain and plasma cholesterol level.

MATERIALS AND METHODS

Source of Chocolates

Newly developed chocolates using five types of fats (CBS1 = PMF, CBS5 = PKS, CBS2 = PMF:PKS, SCBS2 = EIE PMF:PKS:MCT, CB) were prepared using cocoa powder, fats, skim milk powder, emulsifier, vanillin and sucrose. Cocoa powder purchased from Pastry Pro Sdn. Bhd. at Kepong, Malaysia, skimmed milk from Promac Enterprises Sdn. Bhd., cocoa butter (CB) from Malaysian Cocoa Board, Bangi, Malaysia.

Rats and Experimental Design

Male Sprague Dawley rats (n = 35, 2 months of age, 200 g – 250 g of weight) were obtained from Animal House, Universiti Kebangsaan Malaysia, Bangi. All treatments and diets were reviewed and approved by the Animal House of University Kebangsaan Malaysia, Bangi, Selangor. After arrival, rats were quarantined for a week (wk) during which they were fed with rodent chow (control) diet (Barastoc Rat & Mouse Cubes, Ridley Agri Products).

Rats were then divided randomly into five experimental groups (n = 7) and were housed individually under controlled temperature (25°C) with a 12 h light:dark cycle and fed about 15 g control diet followed by experimental diets for four weeks (oral feed). Rats had free access to water and were weighed weekly throughout the study. Treatments given to experimental rats were as follows.

Sampling and Analytical Procedures

The body weight of each rat was recorded weekly. On the final day of the experimental period, wk–1(7d), –2(14d), –3(21d) and 4–(28d), rats were fasted overnight and killed by chloroform inhalation.

Blood samples of fasting rats were collected immediately in sterile tubes with EDTA-K3 by heart vena puncture (before rats were killed).

Table 1. Diet treatment for Wistar rats

Group	n of rats	Control diet + experimental diet
A	7	15 g control diet + 5 g CBS1
B	7	15 g control diet + 5 g CBS5
C	7	15 g control diet + 5 g CBS2
D	7	15 g control diet + 5 g SCBS2
E	7	15 g control diet + 5 g CB

5% w/w doses in rats were equivalent to approximately 5 g/day in human diet by Rao (2001) & Bouhnik *et al*. (1999).

Tube with blood samples were kept in ice box immediately to avoid from heamolysis occurred. Samples were centrifuged for 15 min at 3000 × g, at 4°C using Centrifuge Micro 22R (Hettich, Germany) to get the blood plasma. The plasma samples were transferred to tube adapter and kept it in low ultra freezer at –70°C. The plasma samples were defrosted at ±20°C for biochemistry analysis. The plasma samples were measured for total cholesterol, triacylglycerols, HDL and LDL using Blood Analyzer Clinic Chem-Vitalab Selectra E Analyzer.

Determination of Fatty Acid Composition at the *Sn*-1,2 and 3 of Triacylglycerols of Fats (AOCS Ch 3-91)

(a) Neutralization and purification especially for enzymatic interesterification fat before lipase hydrolysis.

(b) Lipase hydrolysis: 0.1 ml oil was pipette into a flask. 0.2 ml calcium chloride and 0.5 ml bile salt solution were added and capped. Mixture was incubated while shaking at 40°C for 5 min. 2 ml lipase solution was added. The reaction was stopped and 1 ml HCL was added. 3 ml × 2 ml chloroform was added. All rinsed chloroform was transferred into a separating funnel. Mixture was kept for several hours before filtered and evaporated. The neat lipid mixture was ready for thin layer chromatography (TLC).

(c) TLC: Merck multipurpose 20 × 20 plate (TLC silica gel 60) was dried at 100°C for several hours. 100 µl chloroform was added to the neat lipid extract obtained above with syringe and blunt-tip needle. It was transferred onto the plate as a straight line about 2 cm to 2.5 cm above the lower edge of the plate. The plate was developed in hexane:diethyl ether:formic acid 70:30:1 v/v/v for 1.5 h to 2 h. The plate was dyed with dicholofluorescein spray and the bands were visualized under UV light. The MAG band was isolated by scraping the silica coating off the plate.

(d) Analysis of FAME

(e) Calculation of results.

A modified version of Luddy *et al.* (Decker 1996) was used to perform pancreatic lipase hydrolysis to determine the fatty acids at the *sn*-2 position of the structured lipids (SL) products.

Statistical Analyses

All data obtained were analyzed using SPSS Inc. software (version 10.0.1). One-way ANOVA was used to determine a significant difference between means of the dietary groups and sampling sites with a significance level of $p<0.05$.

RESULTS AND DISCUSSION

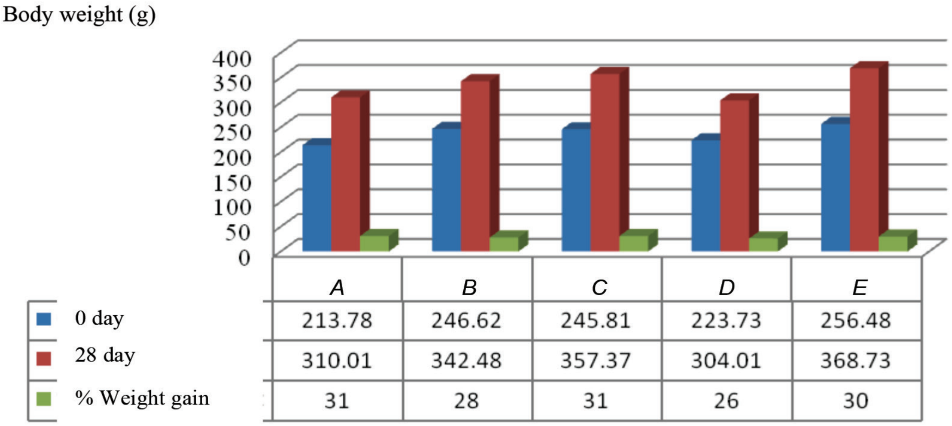
Rat's Body Weight

During the 4 week experiment, body weight (BW) gains did not differ significantly (p>0.05) among the rats fed with control and the different experimental diets as shown in *Figure 1*.

However, rats fed with *A* and *C* showed highest BW gain (31%) compared to *B*, *D* and *E* group. All rats were generally healthy throughout the feeding trial period. While group *D* [low calorie cocoa butter substitute (CBS)] showed the lowest weight gain with only 26%.

Plasma Profile

The plasma profile of treated and control rats, which comprised of cholesterol, triacylglycerols, HDL and LDL contents at the beginning and the end of each treatment period is shown in *Table 2*. No significant difference (p>0.05) in plasma cholesterols, triacylglycerols, HDL and LDL contents of rats fed with control diet and other experimental diets were evident. However, rats fed with *D* (2.07 mmol/l) and *E* (1.86 mmol/l) showed much lower concentrations in cholesterol when compared with the *A* (2.36 mmol/l), *B* (2.25 mmol/l) and *C* (2.27 mmol/l) after the final day



	A	B	C	D	E
🟦 0 day	213.78	246.62	245.81	223.73	256.48
🟥 28 day	310.01	342.48	357.37	304.01	368.73
🟩 % Weight gain	31	28	31	26	30

*Percentage of body weight gain for A^a, B^a, C^a, D^a, E^a (p>0.05), showed no significant differences.

Figure 1. Body weight gain before and after treatments of rats fed with control and experimental diets.

Table 2. Effects of lipid absorption of rats after treatment with control diet and experimental sample.

Group	Cholesterol (mmol/l)	TAG (mmol/l)	LDL-cholesterol (mmol/l)	HDL-cholesterol (mmol/l)
A	2.36^a	0.65^a	1.531^a	0.54^a
B	2.25^a	0.805^a	1.407^a	0.47^a
C	2.27^a	0.985^a	1.314^a	0.51^a
D	2.07^a	1.51^a	0.859^a	0.53^a
E	1.86^a	1.02^a	0.878^a	0.52^a

^{a} Means in the same column followed by same lowercase alphabet is not significantly different at p>0.05.

of treatment. Same things occurred for LDL-cholesterols of *D* and *E* group showed lower concentrations, 859 mmol/l and 878 mmol/l, respectively. These results as an indicator to explained the effective reaction of blood cholesterol in human body. These findings suggest that the MCFA was metabolized quickly for energy, and that different fatty acids (FAs) in the diet may eventually lead to change in fatty acid composition of the liver (Osborn & Akoh 2002). SCBS2 containing MCFAs and PUFAs could be a therapeutic or medical lipid source, and may be useful in enteral and parenteral nutrition. This SCBS2 could decrease serum cholesterols and TAGs. It may also reduce the rate of body weight gain, because the MCFAs were metabolized more rapidly in the body.

Pancreatic Lipase Study

R. miehei preferentially hydrolyzes the fatty acids at the *sn*-1 and 3 positions of TAG. This characteristic is especially desirable when the nutritional benefits of TAG are considered, largely because specialty oils with desired fatty acids at specific positions can be prepared with *sn*-1,3-specific lipases. Several studies have shown that the nature of the fatty acids and their positions in a glycerol molecule can affect their metabolism, availability as an energy source, and as an essential fatty acid source. Jandacek *et al*. reported that a TAG with octanoic acid at the *sn*-1 and 3 positions and long-chain fatty acids (LCFA) at the *sn*-2 position were hydrolyzed and absorbed more efficiently than TAG composed of only LCFA. Christensen *et al*. reported that a defined TAG molecule with MCFA at the *sn*-1,3 positions could be a better source of fat. They observed increased absorption of essential fatty acids at the *sn*-2 position of glycerol. In this study, a pancreatic lipase hydrolysis was performed to determine the fatty acid composition of the *sn*-2 position of the SL. Pancreatic lipase hydrolysis was performed to determine the fatty acid composition at the *sn*-2 position (*Table 3*). Values for SCBS showed that lipase incorporated 17.8% of MCFA and 75.7% of LCFA at the *sn*-2 position. While at *sn*-1,3 position showed slightly high content of MCFA (23%) and only 72.7% of LCFA. Lipase RM IM was able to produce SL that contained specific fatty acids at the *sn*-1,3 positions. The SCBS2 prepared in this study are potentially useful as reduced-calorie oils similar to Salatrim®. It contains desirable functional fatty acids in the same molecule and may provide rapid delivery of energy.

Table 3. Fatty acids content at position *sn*-1, 2 and 3.

Sample	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:0
CBS5										
sn-2			52.1	25.5	9.9		1.4	9.5	1.5	
sn-1& 3	2	2.8	55	21.9	8.7		2	6.6	1	
CBS1										
sn-2				0.7	14.7	0.6	1.6	75.3	7	
sn-1& 3				0.8	57		6	32.1	3.3	0.4
CBS2										
sn-2	3.5	3.7	14.4	6.9	12.5		1.4	52.5	5.5	
sn-1 & 3	4.5	3.9	11.2	4.6	56.6		6.7	12.2	1.2	
SCBS2										
sn-2	0.7	3.1	14	6.5	12.1		1.2	56.5	5.9	
sn-1 & 3	7.3	4.5	11.2	4.9	57.0		6.5	8.4	0.8	

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