Scientific Research and Essays

Volume 9 Number 23 15 December 2014
ISSN 1992-2248

Academic Journals

ABOUT SRE

The **Scientific Research and Essays (SRE)** is published twice monthly (one volume per year) by Academic Journals.

Scientific Research and Essays (SRE) is an open access journal with the objective of publishing quality research articles in science, medicine, agriculture and engineering such as Nanotechnology, Climate Change and Global Warming, Air Pollution Management and Electronics etc. All papers published by SRE are blind peer reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: sre@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Scientific Research and Essays will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editorial Board

n detail.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Scientific Research and Essays is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Scientific Research and Essays

Table of Contents: Volume 9 Number 23 15 December, 2014

ARTICLES

Research Articles

academicJournals

Vol. 9(23), pp. 976-983, 15 December, 2014
DOI: 10.5897/SRE2014.6064
Article Number: DE83AA349120
ISSN 1992-2248 © 2014
Copyright©2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Effect of welding parameters in flux core arc welding (FCAW) with conventional and pulsed current in the efficiency and fusion rate of melting coating

Celso Alves Correa[1], Niederauer Mastelari[2] and João Roberto Sartori Moreno[1]**

[1]Mechanical Engineering Department, Universidade Tecnológica Federal do Paraná – UTFPR, Av. Alberto Carazzai, 1640 - Cornélio Procópio/PR, - Zip Code 86300-000, Brazil.
[2]Mechanical Engineering Department, Universidade Estadual de Campinas - UNICAMP, Rua Mendeleyev, 200; Cidade Universitária "Zeferino Vaz" Barão Geraldo - Campinas, SP - Zip Code: 13083860 Brazil.

Received 2 August, 2014; Accepted 24 November, 2014

This study was conducted over the welding parameters associated with three levels of pulse to lead to important data for the deposition of metal on martensitic stainless steel, through tubular electrode EC410NiMo MC – ESAB by the conventional and pulsed flux core arc welding (FCAW) process in SAE 1020 steel. Were applied three current levels, 170A, 200A e 230A with welding speed of 300 mm/s, 350 mm/s and 400 mm/s, respectively and distance torch tip-piece of 30, 33 and 36 mm to obtain the necessary cords for evaluation. The results showed that the main effect of welding parameters are: the increase in the welding speed and the amperage of the arc tend to increase the fusion rate when we pass from level +1 to -1, for the conventional current and reduced for the pulsed current when evaluating the level -A to +A. However, the efficiency decreases with the increase of welding speed for both processes: conventional and pulsed current, but increases with increasing amperage of the arc when evaluating the level -1 to +1 for conventional and - A to +A only for pulsed process. Also, we observe that the efficiency in both cases with conventional and pulsed current decreases as we increase the distance nozzle/piece. The spectra emitted by the accelerometer showed greater stability of the voltage and current process, but an important instability in the acceleration applied to the three current levels.

Key words: Fusion rate, accelerometer, tubular electrode, welding speed, pulsed flux core arc welding (FCAW).

INTRODUCTION

Fluxed cored arc welding (FCAW) is one of the current processes that have fundamental differences with the metal inert gas (MIG) process, for example, it offers more flexibility in the composition of the alloy, while also allowing higher deposition rates of wire and a greater arc stability, although the efficiency of the MIG process is normally higher as reported Shoeb et al. (2013). Welding with flux cored wire (FCAW) is a process where fusion is

*Corresponding author. E-mail: joaosartori@utfpr.edu.br.

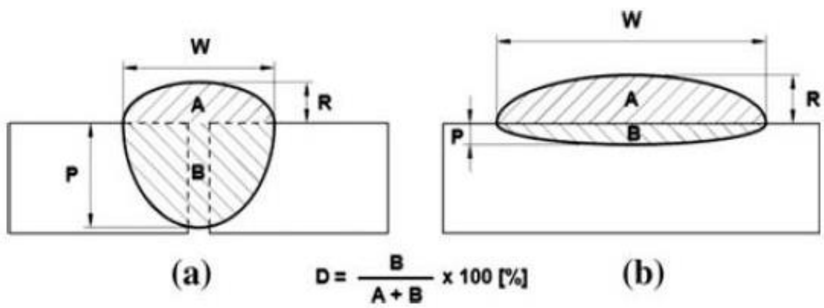

Figure 1. Desired weld bead geometry: (a) union joint (typical applications) and (b) cladding as shown in Gomes (2012).

produced by the action of an electric arc established between the work piece and the consumable wire continuously fed. The protection of the deposited metal and the weld pool are given by the decomposition of the internal flow of consumable wire, and can be further aided by a shielding gas, usually CO_2. For stainless steels, the use of argon mixture with 2% oxygen was recommended which has a slightly oxidizing behavior.

The internal flow of the wire is composed of a mixture of several minerals and organic substances with defined functions as reported by Bracarense et al. (2012). Consumables commonly use in this welding process are usually manufactured with a diameter between 1.0 and 3.2 mm and the geometric cross-sectional configuration can simply be a tube or more complex configurations/metal flow in its cross section (Figure 1).

With the advancement of new technologies, especially electronic sources to manufacture the welding arc, made possible the use of pulsed current for welding processes MIG/MAG, TIG and Flux Cored. Recent studies show that pulsed current can easily weld in all positions and to obtain higher deposition rate when compared to conventional current second (Mohamat et al., 2012).

Another important factor is the use of an average low current welding current used compared to the same conditions with a conventional source, providing less distortion in the pieces. It has been observed that the use of pulsed current provides a reduction in the amount of fumes and spatter by controlling the welding parameters during crude refining and solidification structure of the weld, allowing the decrease of solidification cracks.

When using pulsed current, there are many factors responsible for maintenance of the welding operation. In this sense, it becomes quite complex task of selecting appropriate to the needs of the electric arc welding parameters, the economic advantages, the weld quality and mechanical properties (Sathiya et al., 2012).

Generally, the electrical parameters involved in welding with pulsed current are defined by the peak current (I_p), the base current (I_b), the peak time (t_p), time base (t_b), and the feed speed the wire, welding speed and welding voltage.

The welding coating is employed not only in the recovery of pieces of worn equipment, but also in the manufacture of new products and industrial processes. In welding, the coating on the main aim is to get a welding bead at the lowest possible dilution yielding the morphology of the bead low penetration and the largest possible increase in width for better process efficiency.

Among the materials commonly used for coating, the martensitic stainless steel has important application for resisting corrosion and high-temperature service, it has excellent ductility and mechanical properties appropriate for high temperature and they are easy to be welded. Stainless steels are considered high alloy steels usually containing chromium, nickel, molybdenum in its chemical composition. The alloying elements particularly chromium, gives excellent corrosion resistance when compared to carbon steels. These steels are oxidized, that is, the chromium present in the alloy oxidizes in contact with oxygen in the air, forming a very thin and stable film of chromium oxide. For the oxide, film is effective in protecting against corrosion; the minimum content of chromium should be around 12% according to Ananthan et al. (2012).

Therefore in this study, the influence of important variables such as welding speed, the average amperage arc distance and the tip of the torch-piece was studied in order to determine the efficiency of the processes for both conventional current as for pulsed current controlling the rate of fusion as reported by Kumar et al. (2012).

MATERIALS AND METHODS

The base metal was a steel plate AISI 1020 (185 × 63.5 × 12.7 mm) and for the coating, the EC410NiMo MC 1.2 mm in diameter electrode wire with the shield gas, a mixture of argon and oxygen 2% was used. The chemical composition of the base metal and filler are shown in Table 1.

To perform the welding, a test bench consists of a welding source, a turtle welding and modular data acquisition system with ammeter, voltmeter, accelerometer and thermocouples was used. Figure 2 shows a view of the test bench used during the performance of the weld beads.

Welding is normally limited to the flat and horizontal positions with large diameter wires. Smaller diameter wires are used in all positions. A layer of solidified slag is left on the weld bead that must

Table 1. Chemical composition of base metal and filler metal.

Material	C	Mn	P	S	Si	Ni	Cr	Mo
AISI 1020	0.18/0.23	0.30/0.60	0.03	0.035	0.10/0.30	0.15	0.15	…
EC410NiMo	0.027	0.590	0.024	0.006	0.44	4.86	12.5	0.43

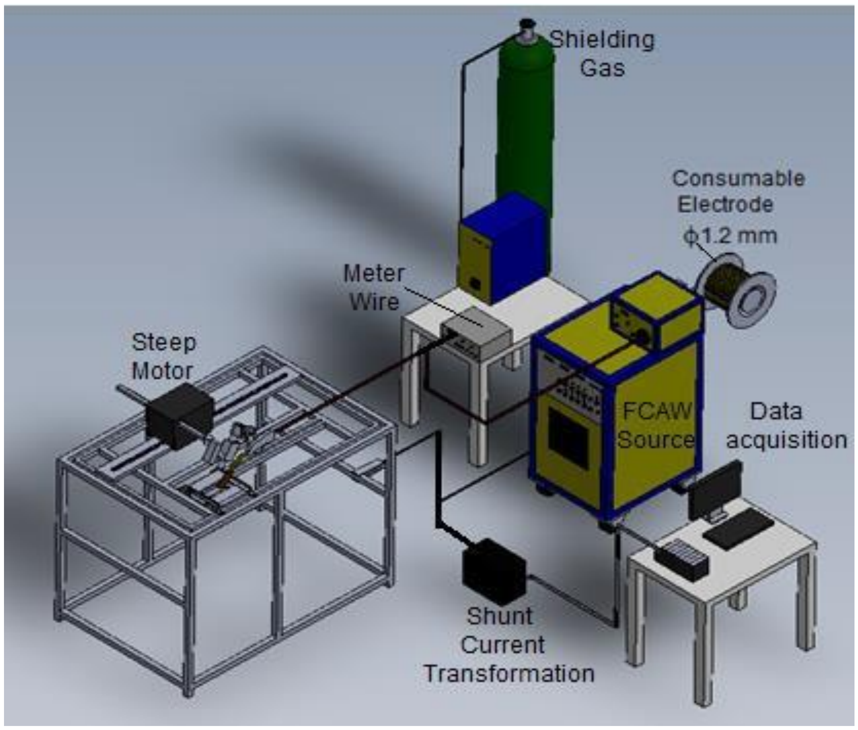

Figure 2. Layout of the test bench.

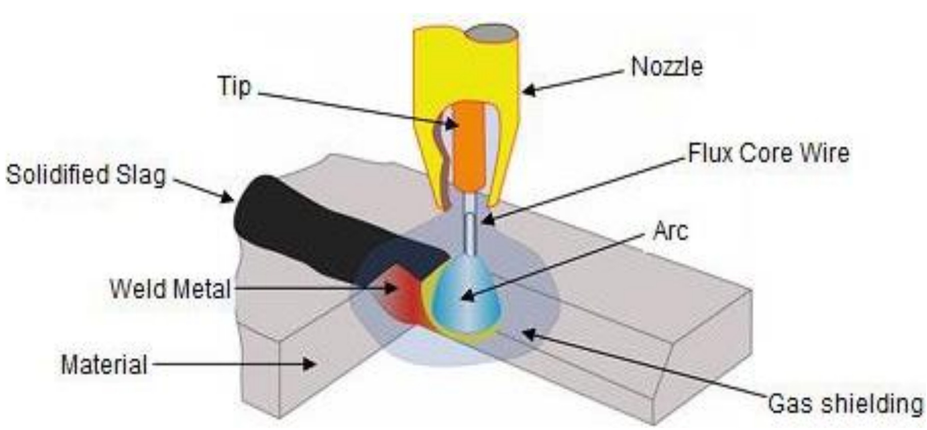

Figure 3. Schematic flux cored arc welding process.

be removed after welding as showed schematically in Figure 3.

The FCAW parameters examined are the welding speed, amperage, distance from torch tip - piece, and the pulse frequency. To define parameters in the FCAW process in previous studies, Om and Pandey (2014) observed that the limit of each variable must be prefixed. Thus, by analyzing previous studies and considering the objectives of this study, the limits of each variable were prefixed. Then, preliminary tests were performed to find the extreme levels for each variable, thus determining whether the process occurred under such conditions. Table 2 shows the parameters and their levels, set at the end of the preliminary tests. Before they were welded, all specimens were subjected to a process of abrasive blasting with steel grit angle G-25 S-280, with hardness D, according to SAE J444 (1993),

Table 2. Parameters applied and maintained constant levels during welding.

Parameter	Level
Polarity of the electrode	CCEP
Protective gas	Argon+2% O_2
Gas flow	18 L/min
Torch angle	90°
Welding position	Plane
Interpass temperature	150°C
Number of cords	11
Peak current (Ip)	350 A
Peak time (tp)	0.01 s

Figure 4. View of the accelerometer model KS80D.

Table 3. Variables and their levels welding.

Variable/level	-1	0	1
Current average (A)	170	200	230
Distance nozzle/piece (mm)	30	35	40
Pulsation frequency (Hz)	18.18	20.00	22.22
Welding speed (cm/min)	30	35	40

for which obtain a surface free of grease, oil and other contaminants. The equipment used in the blast was the case with suction CMV, model GS-9075X.

During welding of the cord sensors (voltmeter, ammeter, thermocouples and accelerometer) to obtain the necessary analyzes and a better interpretation of the results were used. In the acquisition of the temperature control part and interpass temperature two commercial type K thermocouple with a diameter of 3.00 mm, which has a measuring range from 0 to 1350°C were used.

To enable it to monitor the interpass temperature, which is 150°C prior to welding of the samples, were heated in a muffle furnace NT-380 at a temperature of 200°C. When the specimens reached this temperature, they were taken to the welding device for positioning and temperature control via thermocouples.

When this temperature decreases to 150°C welding is performed by monitoring, and this procedure was used in all tests as suggested by Kovacevic and Huang (2011). The welding device was constructed to maintain good contact between thermocouple/specimen; it features an adjustable spring in order to maintain this contact.

To weld properly, a piezoelectric accelerometer industrial KS-80D of MMF Industries as shown in Figure 4, which has a frequency range from 0 to 22000Hz, temperatures of between -20 to 120°C and with magnet stainless steel was used. It is observed that the temperature of the welding part is greater than the operating temperature of the accelerometer which was 90°C and which has been placed laterally on the welding.

Development of L9 matrix

The welding tests were performed aiming to relate the levels applied to the respective variables as shown in Table 3. From the choice of these 4 variables mentioned and 3 levels for each test, we determine parameters that were entered into the MINITAB software with an L9 TAGUCHI matrix according to Palanni et al. (2006) and Aghakhani et al. (2011), which resulted in the parameters in conventional and pulsed current to be applied during the welding trials as shown in Tables 4 and 5, respectively.

RESULTS AND DISCUSSION

In this research, the effect of the main welding parameters on the fusion rate and efficiency of the

Table 4. Data generated by the MINITAB software L9 Taguchi matrix for tests with conventional welding current.

Tests on conventional current			
Experiment	**Welding speed (mm/min)**	**Distance nozzle/piece (mm)**	**Current average (A)**
1	300	30	170
2	300	35	200
3	300	40	230
4	350	30	230
5	350	35	170
6	350	40	200
7	400	30	200
8	400	35	230
9	400	40	170

Table 5. Data generated by the MINITAB software L9 Taguchi matrix for tests with pulsed welding current.

Tests on conventional current				
Experiment	**Welding speed (mm/min)**	**Distance nozzle/piece (mm)**	**Pulsation frequency (Hz)**	**Current average (A)**
1	300	30	18.18	170
2	300	35	20	200
3	300	40	22.22	230
4	350	30	20	230
5	350	35	22.22	170
6	350	40	18.18	200
7	400	30	22.22	200
8	400	35	18.18	230
9	400	40	20	170

welding FCAW process of level -1 to level +1 was investigated (Gomes et. al., 2012). The results show that the main effect of welding parameters are: the increase in the welding speed and the amperage of the arc tend to increase the fusion rate as noted by Chotěborský et al. (2011) when we pass from level -1 to +1, for the conventional current and reduced for the pulsed current when evaluating level -A to +A, as shown in Figure 5. However, the efficiency decreases with the increase of welding speed for both processes, both conventional and pulsed current but increases with increasing amperage of the arc when evaluating level -1 to +1 or –A to +A, second (Figure 6). Also, we can observe that the efficiency in both cases with conventional and pulsed current decreases as we increase the distance of nozzle/piece.

The spectra of Figures 7 and 8 show a behavior definition of important signals in the acceleration, arc voltage and welding current, respectively (Li et al., 2010). All spectra were observed initially, that there was an unstable initial pulse due to the gas flow (acceleration of initial the gas exit) for all parameters (acceleration, arc voltage and welding current).

In the spectra emitted by the accelerometer, we can see the current and open circuit voltage of both processes, conventional pulsed current, open acquisition system, displacement of torch, and thus deposition of weld metal.

Conclusions

This work has presented an application of the parameter of the Taguchi method in optimization of the FCAW parameters. A three-factor of the three level Taguchi experimental design was used to study the relationships between the fusion rate and the five controllable input welding parameters such as, welding current, nozzle/piece distance and welding speed. The following conclusions can be drawn based on the experimental results of this research work:

1. It appears that the Taguchi method is important for the optimization of the welding parameters of FCAW, favoring the control of welding data more easily;
2. The wire feed rate, welding voltage and distance torch

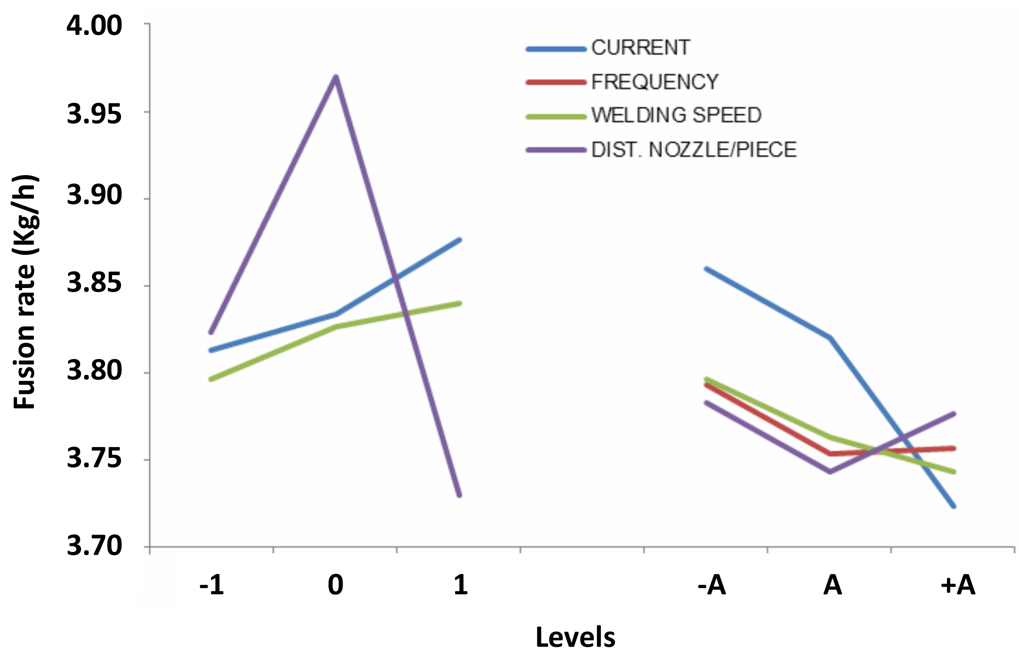

Figure 5. Influence of parameters on the fusion rate (Kg/h) in conventional and pulsed current.

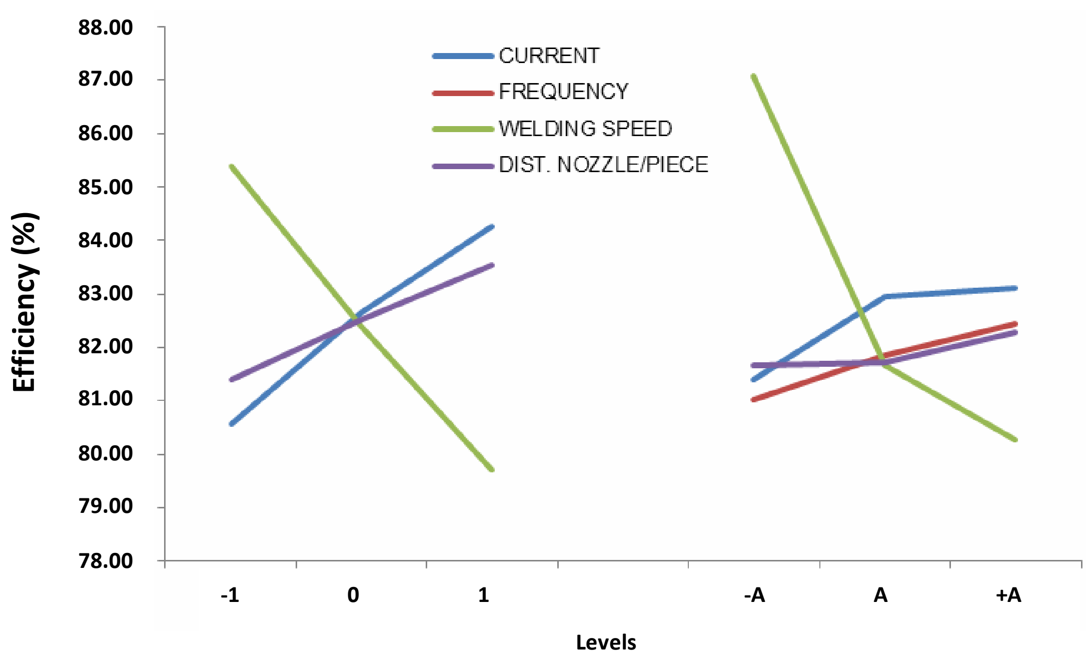

Figure 6. Influence of parameters on the efficiency (%) in conventional and pulsed current.

tip-piece have a significant effect on the fusion rate and efficiency, e.g, the welding speed and the arc amperage increase the fusion rate for the process of conventional arc and reduces the process to a pulsed arc;
3. The spectra emitted by the accelerometer showed a very stable voltage and current process, however showed some instability in the acceleration applied in the three stages amperage (170 A, 200 A and 230 A);
4. Increasing welding current value influenced the depth of penetration increased. Other than that, the factors that can influence the value of depth of penetration are arc voltage and welding speed.

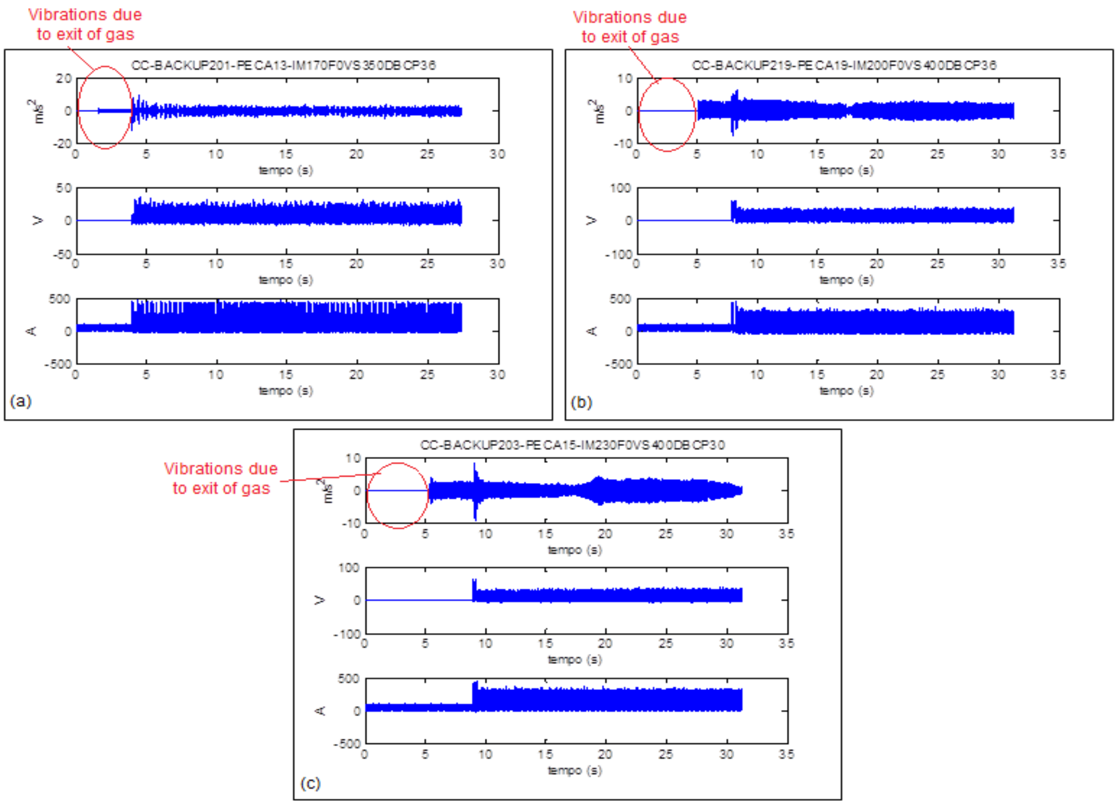

Figure 7. Pulse spectra emitted by accelerometer at (a) 170 A, (b) 200 A and (c) 230 A in conventional current.

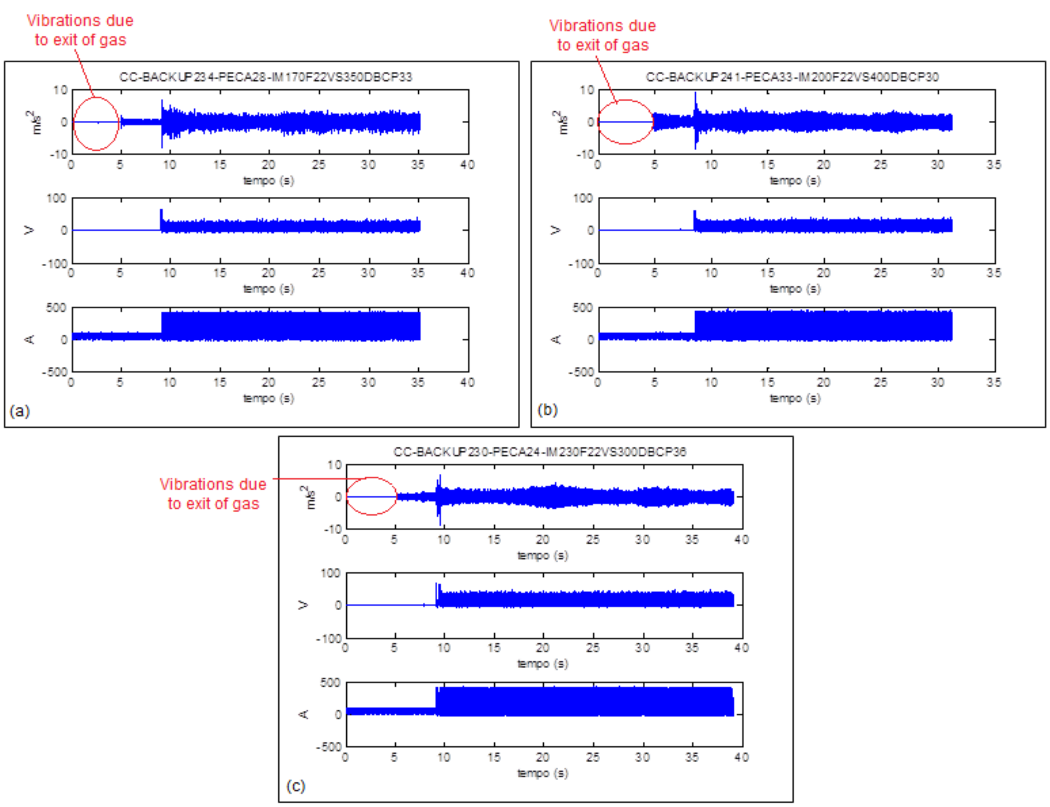

Figure 8. Pulse spectra emitted by accelerometer at (a) 170 A, (b) 200 A and (c) 230 A in pulsed current.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This research paper is made possible through the help from Mechanics Engineering Department of the UTFPR – Cornélio Procópio - PR - Brazil and UNICAMP– University Estadual de Campinas for their unconditional support - SP - Brazil.

REFERENCES

Aghakhani M, Mehrdad E, Hayati E (2011). Parametric Optimization of Gas Metal Arc Welding Process by Taguchi Method on Weld Dilution. Int. J. Model. Optimiz. 1(3):216-220. http://dx.doi.org/10.7763/IJMO.2011.V1.38

Ananthan SS, Senthil Kumar G, Natarajan U (2012). Vision inspection system for the identification and classification of defects in MIG welding joints. Int. J. Adv. Manuf. Technol. 61(9):923-933.

Bracarense AQ, Lacerda de Souza RL, Souza Costa MCM, Faria PE, Liu S (2002). Welding current effect on diffusible hydrogen content in flux cored arc weld metal. J. Braz. Soc. Mech. Sci. 24(2):278-285. http://dx.doi.org/10.1590/S0100-73862002000400005

Chotěborský R, Navrátilová M, Hrabě P (2011). Effects of MIG process parameters on the geometry and dilution of the bead in the automatic surfacing. Res. Agric. Eng. 57(2):56-62.

Gomes JHF, Costa SC, Paiva AP, Balestrassi PP (2012). Mathematical Modeling of Weld Bead Geometry, Quality, and Productivity for Stainless Steel Claddings Deposited by FCAW. J. Mater. Eng. Kovacevic R, Huang W (2011). A Laser-Based Vision System for Weld Quality Inspection. Sensors 11(1):506-521.

Kumar V, Singh G, Yusufzai MZK (2012). Effects of Process Parameters of Gas Metal Arc Welding on Dilution in Cladding of Stainless Steel on Mild Steel. MIT Int. J. Mech. Eng. 2(2):127-131.

Li Y, Li FL, Wang Q, Xu D, Tan M (2010). Measurement and Defect Detection of the Weld Bead Based on Online Vision Inspection. IEEE Trans. Instrum. Meas. 59(7):1841-1849. http://dx.doi.org/10.1109/TIM.2009.2028222

Mohamat SA, Ibrahim IA, Amir A, Ghalib A (2012). The Effect of Flux Core Arc Welding (FCAW) Processes On Different Parameters. Proc Eng. 41:1497-1501. http://dx.doi.org/10.1016/j.proeng.2012.07.341

Om H, Pandey S (2014). Effect of Process Parameters on Weld Penetration Shape Factor in ASAW Based Surfacing. Int. J. Innovat. Res. Sci. Eng. Technol. 3(6):13590-13596.

Palanni PK, Murungan N, Karthikeyan B (2006). Process parameter selection for optimising weld bead geometry in stainless steel cladding using Taguchi's approach. Mater. Sci. Technol. 22(10):1193-1200. http://dx.doi.org/10.1179/174328406X118294

SAE J444/827/1993 (ISO 11124-3) (1993). Cast Shot and Grit Size Specifications for Peening and Cleaning.

Sathiya P, Mishra MK, Shanmugarajan B (2012). Effect of shielding gases on microstructure and mechanical properties of super austenitic stainless steel by hybrid welding. Mater. Des. 33:203-212. http://dx.doi.org/10.1016/j.matdes.2011.06.065

Shoeb M, Parvez M, Kumari P (2013). Effect of MIG Welding Input Process Parameters on Weld Bead Geometry on HSLA Steel. Int. J. Eng. Sci. Technol. 5(1):200-212.

academicJournals

Vol. 9(23), pp. 984-987, 15 December, 2014
DOI: 10.5897/SRE2014.6133
Article Number: 4B18A3F49121
ISSN 1992-2248 © 2014

http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Queue size comparison for standard transmission control protocol variants over high-speed traffics in long term evolution advanced (LTE-A) network

Ghassan A. Abed

Department of Computer Techniques Engineering, Dijlah University College, Baghdad, Iraq.

Received 8 November, 2014; Accepted 25 November, 2014

The employment of the existing protocols such as Transmission Control Protocol (TCP) cannot achieve the requirements of high-speed networks such as long term evolution advanced (LTE-A) due to the large-bandwidth and low-latency links used in this network. LTE-A network is the continuation of 3GPP-LTE (3GPP: 3rd Generation Partnership Project) and it is targeted to advanced development of the requirements of LTE in terms of throughput and coverage. Then, LTE-A is not new as a radio access technology, but it is an evolution of LTE to enhance the performance. Therefore, it is necessary to enhance the TCP variants such as improving the congestion control performance over LTE-A to make these variants fulfills the needs of the huge data which transferred over the secured links. This article offered comparative evaluation and estimation of the queue size performed by different TCP source variants over traffic model of LTE-A system using the Network Simulator 2 (NS-2).The queue size measurement proved that TCP Vegas performed better than TCP Tahoe and TCP Reno that are used in the simulation scenario.

Keywords: Long Term Evolution Advanced (LTE-A), queue size, Transmission Control Protocol (TCP), Network Simulator 2 (NS-2).

INTRODUCTION

3GPP have prepared the official suggestion to the proposed new International Telecommunication Union (ITU) systems, represented by LTE with Release 10 and beyond to be the appraised and the candidate toward IMT-Advanced (IMT: International Mobile Telecommunications). After attaining the requirements, the main object to bring LTE to the line call of IMT-Advanced is that IMT systems must be candidates for coming spectrum bands that are still to be acknowledged (Kottkamp, 2010) (Kiiski, 2010). LTE-A is applying various bands of the spectrum which are already valid in LTE along with the future of bands of IMT-Advanced.

More developments of the spectral efficacy in downlink and uplink are embattled, specifically if users serve as an edge of the cell. In addition, LTE-A aims quicker exchanging between the resource of radio states and between additional enhancements of the latency. All at once, the bit cost must be decreased (Stencel et al., 2010). IMT-Advanced represents the next generation in systems of wireless communications, which aim to accomplish other main advances of the current third-generation systems, by reaching to uplink (UL) rate of

E-mail: dr.ghassan@duc.edu.iq. Tel: +964 771 124 8438.

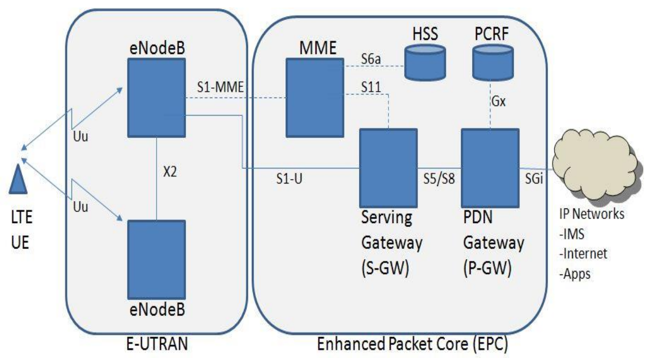

Figure 1. General architecture of LTE-A.

500 Mbps and to 1 Gbps in the downlink (DL) (Nam et al., 2010). The contributions approved by this research are providing a new comparison for the performance evaluation in queue size for some standard TCP source variants. This will help the network developers to modify the current TCPs to run efficiently over large bandwidth and low latency network such as the new generation networks LTE and LTE-A.

The methodology of this research is based on using three TCP variants, Vegas, Reno, and Tahoe over a traffic model of LTE-A network and then measuring the queue size in packets when a loss packet injected in a simulation scenario using NS-2 simulator. The next section includes the architecture of LTE and LTE-A network while the other sections are including the queue estimation formula and then the results and discussion and finally the conclusion section with the recommendation to the future work.

MATERIALS AND METHODS

Architecture of LTE-A network

3GPP identified in Release 8 the requirements, features, and requirements of the architecture of Evolved Packet Core (EPC) which that serving as a base for the next-generation systems. This identification specified two main work objects, called LTE and System Architecture Evolution (SAE) that leading to the description of the Evolved Packet Core (EPC), Evolved Universal Terrestrial Radio Access Network (E-UTRAN), and Evolved Universal Terrestrial Radio Access (E-UTRA). Figure 1 illustrates the architecture of LTE-A networks based on EPC and E-UTRAN. Each of these parts corresponded respectively to the network core, system air interface, and the radio access network.

EPC is responsible to provide an IP connection between an external packet data network by using E-UTRAN and the User Equipment (UE). In the environment of 4G systems, the radio access network and the air interface are actually improving, while the architecture of the core network (that is, EPC) is not suffering large modifications from the previous systematized architecture of SAE.

EPC is responsible to provide an IP connection between an external packet data network by using E-UTRAN and the User Equipment (UE). In the environment of 4G systems, the radio access network and the air interface are actually improving, while the architecture of the core network (that is, EPC) is not suffering large modifications from the previous systematized architecture of SAE. The main part in the architecture of E-UTRAN is the improved Node B (eNB or eNodeB), that providing the air interface between the termination of control plane protocol and the user plane towards the user equipment (UE). Both eNodeBs are a logical element that serving one or more E-UTRAN cells and the interface between the eNodeBs is termed the X2 interface. The interfaces of network are built on IP protocols. The eNodeBs are connected by an X2 interface and to the MME/GW (Mobility Management Entity/Gateway) object with an S1 interface. The interface S1 supported many relationships between eNodeBs and MME/GW (Khan, 2009). The two entities of the logical gateway are termed Serving Gateway (S-GW), and the other is Packet Data Network Gateway (P-GW).

X2 is the interface between the eNodeBs and also involving two interfaces; the first is X2-C, which is the control plane interface between eNodeBs, and X2-U are user plane interface between eNodeBs. It is always supposed that there is an X2 interface between eNodeBs which is to provide communicating between each other (Ghosh et al., 2010). S1-MME represents the S1 control plane interface between MME and eNodeB. Similarly, the transport network layer and user plane are based on IP transport and in case of a reliable transport to the signaling messages; the Stream Control Transmission Protocol (SCTP) is applied over IP. These protocol functions analogously to TCP confirming a reliable, in sequence transmission of all messages with congestion control. SCTP drives analogous to TCP certifying reliable and offer in-sequence transport of messages with congestion control (Abed et al., 2011). The application layer signaling protocols is mentioned to S1 application protocol (S1-AP) and X2 application protocol (X2-AP) for S1 and X2 interface control planes respectively. LTE, 3GPP is also defining IP-based, flat network architecture. This architecture is defined as part of the (SAE) effort. The LTE/SAE architecture and concepts have been designed for efficient support of mass-market usage of any IP based service.

Queue size estimation

The queue size at a bottleneck would affect the performance of TCP protocols, especially with the single TCP flow and large bandwidth path. There are many studies on TCP/RTT determined by evaluating packet traces, but it is not efficient if the maximum RTT is used to estimate the bottleneck queue size. In this study, the maximum values of one-way delays are immediately measured when a packet loss occurs and leads to queue overflow. To estimate the number of queued packets for each RTT interval in the slow-start phase, the following formula in Equation 1 is used (Hirabaru, 2006).

$$N = \frac{W * RTT(R_i - R_o)}{BW} \quad (1)$$

Where N is the number of queued packets, W is the window size in packets, BW is the link bandwidth capacity, R_o is the bottleneck rate, W_i is the initial window size, and R_i is the burst rate at which the TCP source generates the traffic.

To estimate the number of queued packets for each RTT interval in the slow-start phase, the following formula in Equation (2) is used. In Equation (2), the standard TCP increases the window size by two when an ACK is received so that R_i is limited to $2R_o$.

$$W = \frac{W_i * 2 * Time}{RTT} \quad (2)$$

The queue size at a network bottleneck would affect the performance of TCP protocols, particularly when executed in a single TCP flow in networks (Abed et al., 2012a).

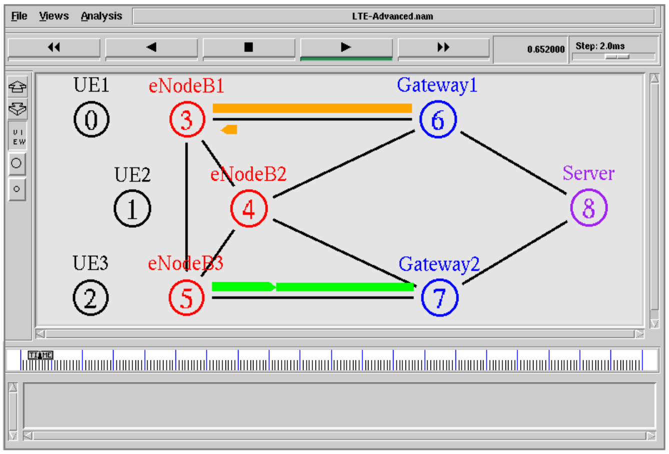

Figure 2. The proposed model of LTE-A.

One of the important metrics to validate the performance is to know the queue sizes of network pipeline, because the dynamic behavior of TCP protocol implementations varies according to bottleneck queue size. The next section focuses on evaluating and investigates the performance of TCP source variants according to the queue length by taking all the packet traces at the sender (Abed et al., 2012b).

Simulation parameters

The proposed model is shown in Figure 2. A new scheme is proposed based on modifying the forwarding packet to add some packet loss to the simulation scenario (Sookhak et al., 2012). It involves one main server for serving data as FTP and HTTP, also to providing source connection for the TCP. The routers Gateway1 and Gateway2 are connected directly to the Server with a duplex link with bandwidth reach to 1Gbps, and propagation delay of three msec. In fact, the propagation delay for all links over the proposed model kept the same value of 3msec, where this value represents the practical latency of the links interfacing and connections on LTE-A networks. The function of Gateway routers is to control the flow rate of the streaming data from the server to the base stations eNodeB1, eNodeB2, and eNodeB3. Gateway within the wired simplex link with Bandwidth reaches to 10 Mbps. The interface between base stations (X2) is very important in a model setting due to the relation between eNodeBs will detect the handover scenario when the UEs move from one eNodeB to another. The base station nodes are responsible for buffering the data packets to the User Equipment's (UEs).

Each base station (eNodeB) is connected to the corresponding the average bandwidth size of X2 proposed to be 20 Mbps and this represent the estimated and practical range. In the proposed model, three UEs used with wireless features and each UE coupled to eNodeB, these UE nodes don't have full mobility features because avoiding the handover scenario in this model where that represents the next step. All linksare kept for one propagation delay of 3msec, and the maximum packet size used sets to 1500 Byte, with minimum window size of 128 packets.

RESULTS AND DISCUSSION

Here the performance of TCP variants that are affected by the queue length by taking all the packet traces at the sender were investigated, where it is possible to calculate the number of queued packets at the bottleneck by simulating drop-tail token buckets. The estimation of queue size over TCP running is subject to several factors and limitations; one of these limitations is at what average rate TCP expects to transmit, but there is no control of the rate within the slow-start period or discovery of the available bandwidth capacity rapidly. Therefore, TCP transmits packets as a burst per RTT intervals at a rate up to the transmitter capability. Because standard TCP's increasing window size almost jumps for each RTT interval, the final growth of window size may save overflowing a bottleneck queue till a packet loss indicates because congestion reaches the sender at most afterward RTT.

The default queue length proposed in LTE-A model is set to 12 packets and the queue monitoring focused on the link between eNodeB1-Gateway and eNodeB2-Gateway as shown in Figure 1. The motive of using 2 bottlenecks in the queue monitor instead of many bottlenecks relates to the testing of the queue management of standard TCP (Tahoe) and comparing it with the management by other TCP's due to the main goal here is not to test the model, but to evaluate the TCP versions. That is why we should expect the queue size not to exceed 12 packets, but simultaneously it should be in large level to avoid congestion in bottlenecks as far as possible. The queue size of Tahoe, Reno, and Vegas TCP are shown in the Figure 3.

Large difference in the queue management performed by the candidate TCP versions in both of steady run and during initialization phase was noted. In Figure 3, Tahoe showed a reasonable queuing control in spite of the poor size in the first 4 seconds but it keeps decent queue level until the simulation end.

Despite Reno and Tahoe are based on similar congestion control mechanism (the difference only happens with multiple packet loss) but they behaved differently. The reason behind the performance difference between Tahoe and Reno, that when Reno is used with multiple packet scenarios will not perform well while Tahoe can perform better.

All TCP variants used in this study used same slow-start mechanism and share many routines in congestion control except TCP-Vegas, where Vegas uses a different approach to avoid congestion in the pipeline network. Vegas congestion control senses the available bandwidth and compares it with the target bandwidth before increase the congestion window. Even though the throughput is steady but it will not be in high level. Figure 3 shows the excessive queue size performed by Vegas over LTE-A model where the size oscillated near the maximum size and the size of queue opened earlier than the other TCP variants.

Conclusions

This article provides experimented results to estimate the

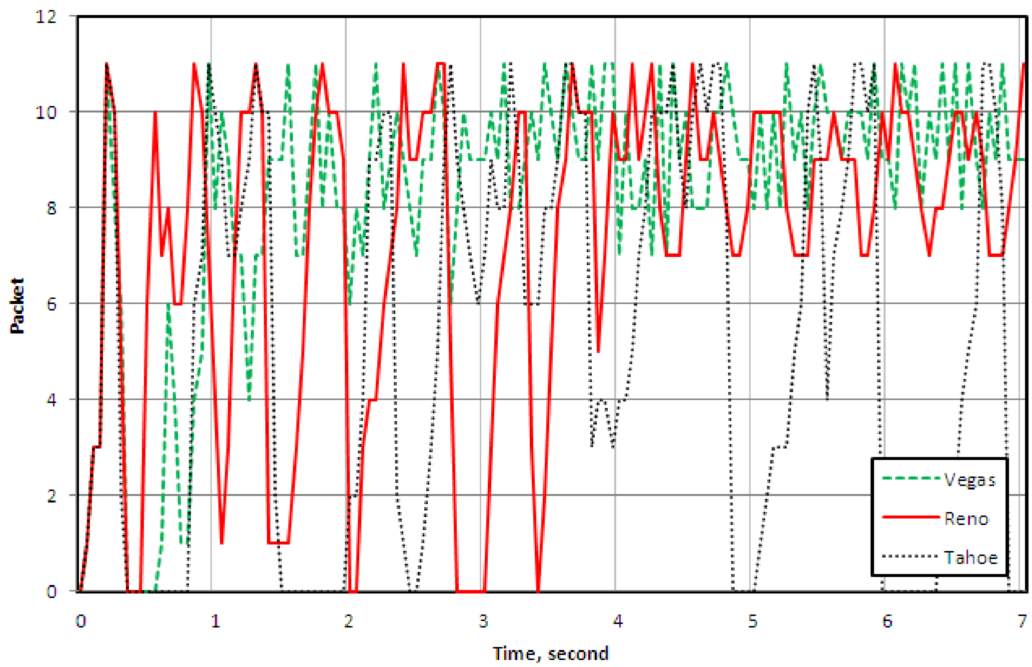

Figure 3. Queue size behavior comparison of Tahoe, Reno and Vegas.

queue size given by three TCP variants, Tahoe, Reno, and Vegas over a proposed traffic model of LTE-A system. Furthermore, this article provides the basic procedures to implement the link interface for LTE-A networks by using network simulator NS-2. In addition, it offers the main features of the user plane protocol, control plane protocol, and the link interface of the protocol stack and illustrate the parameter values which make the limitation to the behavior of these protocols over LTE-A. The comparative queue size estimation proved that TCP-Vegas performed better than other TCP versions while Reno performed better than Tahoe.

FUTURE WORK

The future work of the current research will focus to estimate the packet loss of the source TCP variants over the same traffic model of LTE-A.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Abed GA, Ismail M, Jumari K (2011). Traffic modeling of LTE mobile broadband network based on NS-2 simulator. International Conference on Computational Intelligence, Communication Systems and Networks (CICSyN), pp. 120-125.

Abed GA, Ismail M, Jumari K (2012a). Exploration and evaluation of traditional TCP congestion control techniques. J. King Saud Univ. Comput. Inf. Sci. 24(2):145-155.

Abed GA, Ismail M, Jumari K (2012b). Links Interfacing Demonstration of LTE-Advanced Networks Using NS-2 Modeller. Third International Conference on Intelligent Systems, Modelling and Simulation (ISMS), pp. 669-673. http://dx.doi.org/10.1109/ISMS.2012.11

Ghosh A, Zhang J, Muhamed R, Andrews JG (2010). Fundamentals of LTE. Prentice Hall.

Hirabaru M (2006). Impact of bottleneck queue size on TCP protocols and its measurement. IEICE transactions on information and systems 89(1):132-138. http://dx.doi.org/10.1093/ietisy/e89-d.1.132

Khan F (2009). LTE for 4G mobile broadband: air interface technologies and performance. Cambridge University Press. http://dx.doi.org/10.1017/CBO9780511810336

Kiiski M (2010). LTE-Advanced: The mainstream in mobile broadband evolution. European Wireless Conference, pp. 983-988.

Kottkamp M (2010). LTE-Advanced technology introduction. Rohde and Schwarz, pp. 1-23.

Nam YH, Liu L, Wang Y, Zhang C, Cho J, Han JK (2010). Cooperative communication technologies for LTE-advanced. IEEE International Conference on Acoustics Speech and Signal Processing (ICASSP), pp. 5610-5613.

Sookhak M, Haghparast M, Gani A (2012). Anomaly Detection in Geographic Routing Protocols. International Conference on Education and e-Learning Innovations (ICEELI), pp. 1-6. http://dx.doi.org/10.1109/ICEELI.2012.6360604

Stencel V, Muller A, Frank P (2010). LTE Advanced—A further evolutionary step for Next Generation Mobile Networks 20th International Conference In Radioelektronika, pp. 1-5.

academicJournals

Vol. 9(23), pp. 988-992, 15 December, 2014
DOI: 10.5897/SRE2014.6003
Article Number: AE6C3DD49123
ISSN 1992-2248 © 2014

http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Lipoxygenase assay and cutaneous erythema test have discovered a potent anti-inflammatory activity shown by some genus *Prunus* plants

Gulfishan[1], Naveed Akhtar[1], Tariq Mahmood[2], Qazi Adnan Jamil[1], Shafia Arshad[3], Hira Khan[1] and Muhammad Sohail[1]

[1]Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Bahawalpur, Pakistan.
[2]Department of Pharmacy, University of Central Punjab, Lahore, Pakistan.
[3]University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Bahawalpur, Pakistan.

Received 10 June, 2014; Accepted 2 December, 2014

Medicinal plants have gained considerable interest in treatment of various medical conditions including inflammation. *Rosaceae* (the rose family) family of flowering plants having largest genus *Prunus* (plums, peaches, cherries and almonds) is one of the most economically important families. In current study, anti-inflammatory activities of three fruit species *Prunus persica, Prunus avium* and *Prunus domestica* of family *Rosaceae* and genus *Prunus* were evaluated by both *in-vitro* and *in-vivo* assays. Non-invasive bioengineering probe Mexameter MPA-5 was used to measure cutaneous erythema level *in-vivo*, while for *in-vitro* evaluation lipoxygenase assay was used. These medicinal plant extracts were entrapped in oil-in-water emulsions and applied on 33 healthy human female volunteers (22 years) to assess cutaneous erythema level. Methanol extracts of *P. persica, P. avium* and *P. domestica* depicted significant (P ≤ 0.05) lipoxygenase activity *in-vitro*, that is, 58.73, 51.29 and 47.87%, respectively. Results of *in-vivo* assay revealed that active formulations loaded with botanical extracts produced statistically significant decrease in skin erythema over respective controls after 48 h *P. persica, P. avium and P. domestica* extracts can be used for isolation of new lead compounds with better anti-inflammatory activity. Results indicate that studied plants present potential for many natural products studies with high safety profile.

Key words: Anti-inflammatory, *Prunus persica*, *Prunus avium*, *Prunus domestica*, Lipoxygenase, *Rosaceae*.

INTRODUCTION

Inflammation is a protective response of the body towards various injuries and infections (Vijayalakshmi et al., 2011). Most anti-inflammatory drugs used to alleviate the cardinal signs of inflammation particularly pain, redness and swelling are synthetic agents having various side effects (Dharmasiri et al., 2003; Bepary et al., 2008). In many applications, it is possible to use crude plant materials or extracts to screen lipoxygenase enzyme

*Corresponding author. E-mail: gulbintezia@gmail.com or gulfishan@iub.edu.pk.

(Taner and Aslıhan, 2007). The screening of medicinal plants for lead anti-inflammatory compounds may guide to the discovery of more safer and effective compounds (Fazli et al., 2014).

Lipoxygenase (LOX) is an enzyme found in many plants which catalyses the oxygenation of polyunsaturated fatty acids (PUFA) to form fatty acid hydroperoxides that plays important roles in wound response and defense mechanism. Lipoxygenases have antioxidant status in plant-based foods. Lipoxygenase in vegetative tissues provide hydroperoxide substrates that can be metabolized to compounds that play important roles during wound response in plants. Linoleic acid is the most common substrate in plant-based foods (Taner and Aslıhan, 2007).

In peaches, LOX activity increases in conjunction with the ripening processes (Mingyu et al., 2011). Peaches (*Prunus persica*) are enriched in water, potassium and Vitamin C. Potassium helps to maintain the water balance, counteracting the negative effect that sodium could have in the retention of liquids; therefore it is appropriate to eliminate liquids of the organism. Peach protects us from dryness in the skin, juvenile acne or a bigger easiness for the infections (Daymi et al., 2010). Cherries boost the immune system. Cherry (*Prunus avium*) is rich in water, Vitamins C, phenolics and anthocyanins (Bourquin et al., 2003). Several studies have demonstrated that cherry intake inhibits inflammatory pathways (Kelley et al., 2006). It is skin cleanser and reduces inflammation (Usenik et al., 2008). Plums (*Prunus domestica L.*) are good sources of natural antioxidants. Masks of these juicy fruits are usually applied on skin to produce soothing and moisturizing effects (Dae et al., 2003). But limited data is available on the Lipoxygenase activity of these botanicals.

Lipoxygenase assay (*in vitro*) is related to the anti inflammatory activity (Fazli et al., 2014) of *P. persica, P. avium* and/or *P. domestica* fruits of family *Rosaceae*. For *in-vivo* analysis of skin irritation, redness or swelling subjective visual assessment methods are commonly applied but better results can only be interpreted by trained researchers (Rogiers et al*.,* 1999). Thereby, non-invasive bioengineering techniques could be used to make the data more objective and to measure subclinical symptoms like skin erythema, redness or inflammation. Primary skin irritation test or patch test is the most valid and widely used test for evaluation of irritancy or erythema potential of skin. 24 to 48 h occlusive patch test is usually applied to observe the type of any allergic/inflammatory reaction to a particular topical pharmacological or dermatological agent (Nigam, 2009).

This novel work was aimed to screen and correlate the *in-vitro* anti inflammatory activity of natural medicinal extracts prepared from Peach, Cherry and Plum fruits by Lipoxygenase assay, to evaluate *in-vivo* cutaneous erythema of topical soothing and cooling oil-in-water emulsions; loaded with those pleasant fruity medicinal extracts; by the use of non-invasive method; Mexameter MPA-5.

MATERIALS AND METHODS

Chemicals

Linoleic acid and Baicalein (Sigma, USA), Methanol (Merck KGaA Darmstadt, Germany), Hydrochloric Acid and Sodium Phosphate Buffers (Merck, Germany), Stearic-acid and Paraffin oil (Merck, Germany), Propyl and Methyl paraben (Acros, USA) and Potassium Hydroxide (Riedel, USA) were the chemicals used in this study.

Apparatus

Ultraviolet (UV) Spectrophotometer (Shimadzu Japan), Elisa Plate Reader (Biotek Synergy HT), Rotary evaporator (Eyela, Co. Ltd. Japan), Incubator (Shimadzu Japan), Electrical Balance (Precisa BJ-210, Switzerland), Refrigerator (Dawlance, Pakistan), micropipettes, separating funnel and water bath (HH.S214, China) were used.

Collection and identification of fruit materials

P. persica and *P. domestica* were collected from local market of Bahawalpur, Pakistan. The identification of the fruit sample; *P. persica,* voucher number 705-12-2119; was performed at the Cholistan Institute of Desert Studies (CIDS), the Islamia University of Bahawalpur, Pakistan. Identification of the fruit sample (*P. domestica,* voucher number 18247) was performed at Herbarium Department of Pharmacology, Sir Sadiq Muhammad Khan Post Graduate Medical College, The Islamia University of Bahawalpur, Pakistan. *P. avium* (voucher number IUB 7-821) was identified at Herbarium University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Pakistan.

Extraction methods

Maceration and hot percolation methods were used in extraction of fruit samples. *P. persica, P. avium* and *P. domestica* fruits were extracted whole (without peeling); after removing the stones.

Preparation of *P. persica* extract

One hundred gram fresh fruits of *P. persica* (pulp with peel) were crushed and extracted three times by maceration with 80% aqueous methyl alcohol, by acidifying with a 0.25 ml of 1% hydrochloric acid, at room temperature for 24 h. The extract residue was removed by filtration through multiple layers of muslin cloth to get a coarse filtrate. The coarse filtrate was then filtered through a Whattman No.1 filter paper to get particle free extract. The methyl alcohol was removed under vacuum at 40°C. The concentrated extract was collected by rotary evaporator and stored under refrigeration.

Preparation of *P. avium* extract

P. avium fruit sample (100 g) was crushed and successively macerated (individually) in a mixture of 400 ml of 80% methanol and water in a ratio of 80:20 respectively; with 0.25 ml of 1% HCL; for 24 h at room temperature in dark. Then, the mixture was filtered through sixteen layers of muslin cloth and then through Whattman

Table 1. Ingredients of the active formulation (O/W emulsion) loaded with *Prunus persica / Prunus avium / Prunus domestica* extract.

	S\N	Ingredients	Quantity (g)
A	1	Stearic acid	22
	2	Paraffin oil	6
	3	Propyl paraben	0.02
B	4	Potassium hydroxide	1.5
	5	Methyl paraben	0.10
	6	Active ingredient (i.e. Peach/Cherry/Plum Extract)	5
	7	Distilled water	65.38
	8	Perfume	Nil

Where; A indicates the composition of oily phase and B indicates aqueous phase.

No.1 filter paper paper to remove all the coarse particles and to get a clear filtrate. The extract was then concentrated at rotary evaporator under reduced pressure at 40°C and stored in a refrigerator for further use.

Preparation of *P. domestica* extract

Fruit sample (*P. domestica*) was accurately weighed to 100 grams. After carefully removing stones; the whole fruit was crushed with spatula and quantitatively transferred to a measuring flask with about 200 mL of a methanol: water (80:20) solvent mixture and homogenized in a blender. The homogenized fruit mixture was transferred to a large beaker, by repeating the addition of 200 mL methanol: water (80:20) and 0.25 ml of hydrochloric acid (1%); for 24 h at room temperature in dark. The macerated fruit materials were filtered through 16 layers of muslin cloth for coarse filtration. The coarse filtrates were then filtered through a Whattman No.1 filter paper to get particle free extract. To obtain concentrated extract, the filtrates was evaporated under vacuum at 40°C in a rotary evaporator and stored in amber containers; under refrigeration (-15°C) until used for further analysis.

***In-vitro* evaluation of anti-inflammatory activity**

Lipoxygenase (LOX) inhibition assay: Lipoxygenase (LOX) activity was assayed according to the method (Tappel, 1953; Baylac and Racine, 2003) with slight modifications. A total volume of 200 µL lipoxygenase assay mixture contained 150 µL sodium phosphate buffers (100 mM, pH 8.0), 10 µL test compound and 15 µL purified lipoxygenase enzyme (600 units well-1, Sigma Inc.). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 min at 25°C. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm using 96-well plate reader Synergy HT, Biotek, USA. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalin (0.5 mM well-1) was used as a positive control (Khalid et al., 2013).

The decrease in absorbance indicates increased enzyme activity which was determined by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{(Absorbance of control – Absorbance of test solution)}}{\text{Absorbance of control}} \times 100$$

Where; Absorbance of Control = Total enzyme activity without inhibitor, Absorbance of Test = Activity in the presence of test compound.

IC_{50} values were calculated using EZ–Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA). LOX activity (% inhibition) was measured in triplicate.

***In-vivo* evaluation in healthy human volunteers**

Alternative to animal testing in present work; 33 healthy human volunteers (all female of 22 years average age) were included after their written informed consent. Individuals were divided into three groups (11 each) for the application of three different formulations loaded with *P. persica* (peach), *P. avium* (cherry), and/or *P. domestica* (plum) extracts. Subjects were strictly prohibited to use any topical or oral antihistamine or steroidal drug during and one week preceding the study period to void any false perception of the results. Participants could unclose the patches due to any irritant or uncomfortable feeling during the study period but all volunteers completed the study safely and effectively.

Anti-inflammatory activity of all the three medicinal plant extracts was evaluated *in-vivo* by entrapping them in topical oil-in-water emulsion formulations. Three oil-in-water emulsion formulations were tested along with their respective vehicle controls (placebo). All the three formulations were having different composition and named as Peach Extract Cream, Cherry Extract Cream and Plum Extract Cream; with 5% extract of *P. persica, P. avium* and *P. domestica*, respectively. Vehicle control of each emulsion formulation was without any active ingredient (extract), for comparative analysis of actual inflammatory or erythema potential of active ingredients in the formulations. Oil-in-water emulsion formulations were prepared by the addition of oil-phase (A) to aqueous-phase (B) with regular stirring (Table 1). Both phases were individually heated to 70 ± 1°C. Then oil-phase was added drop by drop to aqueous-phase with stirring at 1300 rpm for about 10 min. No fragrance was added to the formulations to avoid any irritancy or allergic reaction on topical application. After complete mixing, the speed of mechanical stirrer was minimized to 800 rpm for 8 to 10 min then final homogenization was carried out at 500 rounds per minute for another 10 min. Vehicle control formula was also prepared by same procedure but without any respective active ingredient.

To decide the final stable formula of active formulation (Table 1), 25 approx. formulas with varying quantity of different ingredients of both oil phase and aqueous phase were prepared. Stability tests such as change in pH, electrical conductivity test and centrifugation test for phase separation were performed for active formulations and respective control formulations at different storage conditions, that is, 4, 25, 40 and 40°C with 75% relative humidity (RH) for a

Table 2. Lipoxygenase activities of *Prunus persica, Prunus avium* and *Prunus domestica* extracts.

Lipoxygenase assay			
Crude extract	***Prunus persica***	***Prunus avium***	***Prunus domestica***
Inhibition (%)	58.73±0.78	51.29±0.23	47.87±0.54
IC50 (µg/ml)	15.31±0.76	18.01±1.21	21.07±1.13

Data are expressed as means ± standard deviations (*n* = 3); values are not significantly different ($P \geq 0.05$).

study period of 90 days.

Vehicle control was prepared by using same composition but without any active ingredient or extract. For single application closed patch test (48 h), area (1 × 1 cm) was marked on the inner right and left forearms of all human female volunteers; for the applications of active formulations on right forearms and their respective vehicle controls on left forearms. A small amount of each active formulation was applied on the right forearms and respective vehicle controls (without active ingredient) on left forearms of all volunteers in the three groups, that is, in Group-I (11 volunteers) Peach Extract Cream was applied on right forearms and its vehicle control (without peach extract) was applied on left forearms, in Group-II (11 volunteers) Cherry Extract Cream was applied on right forearms and its vehicle control (without cherry extract) was applied on left forearms. Similarly, in Group-III (11 volunteers) Plum Extract Cream was applied on right forearms and its control (without plum extract) was applied on left forearm for comparison. Area was covered with semi-occlusive cotton bandage, fixed with adhesive tape (closed patch test). Non-invasive biophysical technique used was Mexameter MPA-5 (Courage + Khazaka, Germany) for measurement of erythema index. Erythema level is expressed in arbitrary units which range from 0 to 1000 erythema index. The higher the erythema level observed the higher the capacitance was and vice versa. The skin erythema level on both forearms (right and left) of each volunteer was noted on zero hour, that is, before application of any product on the marked sites. Then after 48 h, the enclosed patches for primary skin irritation testing were removed and measurements were performed for erythema testing to observe any increase or decrease in erythema index.

Ethical considerations

This work was approved by the Board of Advance Studies and Research (BASR) and Ethical Review Committee (ERC), The Islamia University of Bahawalpur. Study was conducted according to ethical guidelines of GCP (Good Clinical Practice). Human volunteers were included in this study after taking their written informed consent. They were informed about any possible adverse reaction, protocols and objectives of this work.

Statistical analysis

All the measurements were done in triplicate. At 0.05 level of significance; data was compared to assess the significance of difference using Graph Pad Prism (GPP) version 5.01. The results obtained were expressed as Mean ± S.D.

RESULTS AND DISCUSSION

Lipoxygenase (LOX) assay

The LOX activities (% inhibition and & IC_{50} µg/ml) of various medicinal plant extracts were determined and shown in Table 2. More the percentage inhibition more the Lipoxygenase activity whereas, less the IC_{50} µg/ml (minimum inhibitory concentration required) more good is the activity. Various crude extracts of same family (*Rosaceaae*) and same genus (*Prunus*) were found to have good and comparable activities; maximum in *P. persica L.* extract and then in *P. avium L.* and *P. domestica L.* extracts when Baicalein was used as control.

Cutaneous erythema testing

Patch tests for erythema were performed for *P. persica*, *P. avium* and *P. domestica* formualtions with their respective vehicle controls and percentage of changes in erythema index for all the three formualtions with their vehicle controls has been shown in Figure 1. Where; A_1 indicates *P. persica* active formulation with its respective control (A_2), B_1 indicates *P. avium* active formulation with its respective control (B_2) and C_1 indicates *P. domestica* active formulation with its respective control (C_2).

All the active formulations (A_1, B_1 and C_1) significantly decreased cutaneous erythema level after performing patch test of 48 h; whereas their respective vehicle controls (A_2, B_2 and C_2) statistically produced insignificant change ($P \geq 0.05$) in skin erythema index, so the active formulations loaded with medicinal plant extracts of *P. persica, P. avium* and *P. domestica* can be safely used on human skin for *in-vivo* evaluation. All the three medicinal plant extracts produced significant anti-inflammatory effects ($P \leq 0.05$). No skin irritation was observed rather all the volunteers felt very soothing, cooling and moisturizing feeling during the application of closed patch of medical plant extract formulations. It may be attributed to the presence of various natural antioxidants and/or lipoxygenase in these fruit extracts which has the ability to reduce skin erythema or redness.

Conclusion

P. persica, P. avium and *P. domestica* extracts have positive significant correlation of Lipoxygenase activity (*in-vitro*) and produced no skin erythema after *in-vivo* testing. All those fruit extracts produced cooling and soothing effects on human (female) skin; thereby could

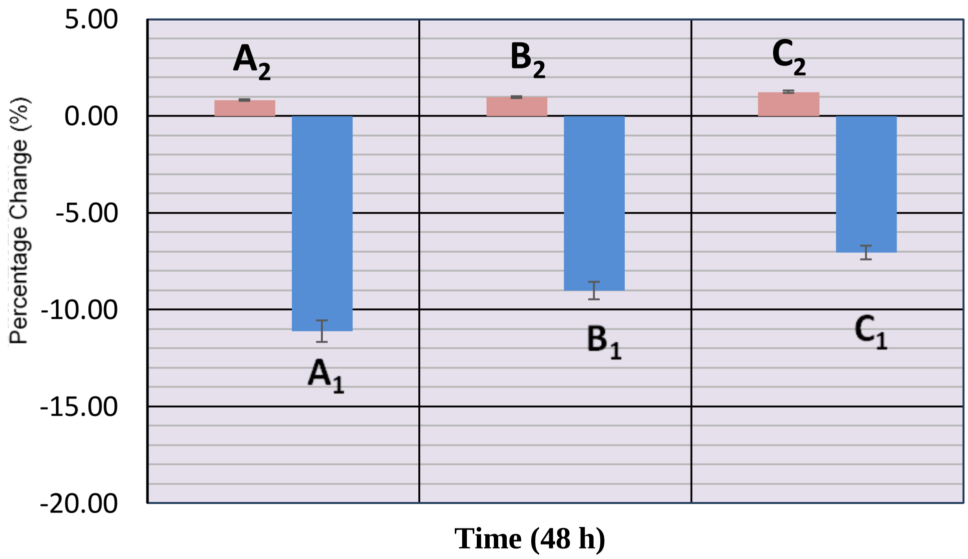

Figure 1. Percentage of change in erythema in case of active formulations (A_1, B_1, C_1) and respective controls (A_2, B_2, C_2) after 48 h.

be considered for use in skin care safely. The fruit sample extracts were proved to be pleasant, economical, non-irritant and anti-erythemic for topical use which makes them acceptable by the consumer. However, this study can be further elaborated to study the effects of these botanical extracts on other aspects of inflammation in future.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank Chairman Department of Pharmacy and Dean, Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan for providing necessary laboratory facilities and moral support for carrying out this study.

REFRENCES

Baylac S, Racine P (2003). Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int. J. Aromatherap. 13:138-142. http://dx.doi.org/10.1016/S0962-4562(03)00083-3

Bepary S, Biplab KD, Sitesh CB, Joydev KK, Abu SSR, Bidyut KD (2008). Anti-inflammatory activity of indanyltetrazole derivatives. Pak. J. Pharm. Sci. 21:295-298.

Bourquin LD, Nair MG, Seeram NP (2003). Tart cherry anthocyanins inhibit tumor development in Apc (Min) mice and reduce proliferation of human colon cancer cells. Cancer Lett. 194:13-19. http://dx.doi.org/10.1016/S0304-3940(02)00583-9

Dae OK, Young JK, Hae YM, Chang YL (2003). Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums. J. Agric. Food Chem. 51:6509-6515. http://dx.doi.org/10.1021/jf0343074

Daymi C, Paloma R, Ana O, Ana J (2010). Antioxidant System and Protein Pattern in Peach Fruits at Two Maturation Stages. J. Agric. Food Chem. 58:11140-11147. http://dx.doi.org/10.1021/jf102807t

Dharmasiri MG, Jayakody J, Galhena G, Liyanage SP, Ratnasooriya WD (2003). Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. J. Ethnopharmacol. 87:199-206. http://dx.doi.org/10.1016/S0378-8741(03)00159-4

Fazli K, Zafar I, Ayub K, Zakiullah, Yasar S, Lateef A, Fazli N, Muhammad H, Ismail, Waheed AS (2014). Evaluation of anti-inflammatory activity of selected medicinal plants of Khyber Pakhtunkhwa, Pakistan. Pak. J. Pharm. Sci. 27:365-368.

Khalid H, Rehman A, Abbasi MA, Khan MK, Ashraf M, Ahmad I, Ejaz SA (2013). Synthesis of biologically active O-substituted derivatives of 1-[(3, 5-dichloro-2-hydroxyphenyl) sulfonyl] piperidine. Pak. J. Pharm. Sci. 26 (3): 479-485.

Kelley DS, Rasooly R, Jacob RA, Kader AA, Mackey BE (2006). Consumption of Bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women. J. Nutr. 136:981-986.

Mingyu H, Tao Z, Caiping Z, Junhai Z (2011). Regulation of the expression of lipoxygenase genes in P. persica fruit ripening. Acta Physiol. Planta 33:1345-1352. http://dx.doi.org/10.1007/s11738-010-0668-6

Nigam PK (2009). Adverse reactions to cosmetics and methods of testing. Indian J. Dermatol. 75:10-18.

Rogiers V, Balls M, Basketter D, Berardesca E, Edwards C, Elsner P (1999). The potential use of non-invasive methods in the safety assessment of cosmetic products. 36th ECVAM Workshop ATLA. 27:515-537.

Taner B, Aslıhan D (2007). Lipoxygenase in fruits and vegetables: A review. Enzy. Micro. Technol. 40:491-496. http://dx.doi.org/10.1016/j.enzmictec.2006.11.025

Tappel AL (1953). The mechanism of the oxidation of unsaturated fatty acid catalyzed by hematin compounds. Arch. Biochem. Biophys. 44:378-395. http://dx.doi.org/10.1016/0003-9861(53)90056-3

Usenik V, Fabcic J, Stampar F (2008). Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry. Food Chem. 107:185-192. http://dx.doi.org/10.1016/j.foodchem.2007.08.004

Vijayalakshmi A, Ravichandiran V, Velraj M, Hemalatha S, Sudharani G, Jayakumari S (2011). Antianaphylactic and anti-inflammatory activities of a bioactive alkaloid from the root bark of Plumeria acutifolia Poir. Asian Pac. J. Trop. Biomed. 10:401-405. http://dx.doi.org/10.1016/S2221-1691(11)60088-9