VOL. 11, NO. 14, JULY 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

DEVELOPMENT OF A MICRO-EXTRUDER WITH VIBRATIONAL MODE FOR MICROENCAPSULATION OF CELLS

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ABSTRACT

Microencapsulation techniques have been developed for various applications such as bioengineering, pharmaceutical, drug delivery, cosmetics and food technology. Some of these techniques involve with complex process and harsh chemicals and may not be suitable for the microencapsulation of cells. Micro-extrusion is a simple yet efficient technique to produce large number of microcapsules. However the simple extrusion only enables the production of microcapsules in a few millimetres that limits the transportation of oxygen and nutrients. In this paper, a micro-extruder with an inclusion of vibration mode was developed to reduce the size of the microcapsules. Smaller size of microcapsule provides better transportation of nutrients and oxygen. The effects of adding a vibrator motor to the customised micro-extruder were studied and characterized. The experimental results showed that the size of the microcapsules can be controlled and reduced to approximately 800-1000 µm using vibrational frequency ranging from 10 to 60 Hz at flow rate of 0.6 ml/min. A lower flow rate of the micro-extruder and a high frequency of vibration can produce smaller size of microcapsules. The technique developed is potentially easy and safe for microencapsulation of cells.

Keywords: micro-extruder, vibration, microencapsulation, microcapsules, calcium alginate.

INTRODUCTION

Microencapsulation was originated for pharmaceutical industrial process in 1930's. This pharmaceutical process involved the solid, liquid and even gases to be enclosed in polymeric material around the substances that produced the tiny droplets of solid or liquid material (Agnihotri, Mishra, Goda, and Arora, 2012). The material was used for shielding or protecting the substances from the surrounding environment (Dubey, 2009). In 1931, gelatin was used to encapsulate drugs based on coacervation technique (Gupta and Dey, 2013; Sachan, Singh, and Rao, 2006). Therefore after, various techniques have been developed to produce capsules of different size, composition and functionality (Fukui *et al.*, 2010; Kang, Park, Ju, Jeong, and Lee, 2014; Tomaro-Duchesneau, Saha, Malhotra, Kahouli, and Prakash, 2012).

Electro-spray in dripping mode is one of the popular techniques used to form microcapsules in large quantity(Fukui *et al.*, 2010). The dripping technique via a glass capillary could produce beads easily but the beads produced are rather large (100-1000 µm). High voltage and control of flow rate were applied to the conductive nozzle of the syringe in order to reduce the droplet size. Some of other techniques were based on phase separation in microfluidic and elevated temperature control to produce microcapsules. However these techniques required harsh condition to succeed in the production of microcapsules. In addition to the control parameters such as the temperature and phase separation, microencapsulation can also be improved by the selection of materials (Wan, 2012).

Micro-extrusion is a simple technique used for the encapsulation of cells, drug, deoxyribonucleic acids and food substance (Fukui *et al.*, 2010). For the micro-extrusion technique, micro-beads or microcapsules can be produced via the extrusion of droplets out of a needle or aperture in a micro-extruder. The solution contained in the syringe could be a monomer or a polymer that are polymerized through a series of subsequent processes (Draget and Taylor, 2011; Freitas, Merkle, & Gander, 2005).

The physically encapsulated cell can be protected from the outside environment and restoring their cell-cell integrity in three dimensional (3D) environment such as calcium alginate (Wan, 2012). Alginate is a biopolymer with cross-linked hydrophilic polymers network that can absorb water or other biochemical component. The gelling properties of alginate is subjected to the binding of divalent cations such as calcium and the linear binary copolymers consisting of guluronic acid (G) block and mannuronic acid (M) (Capretto, Mazzitelli, Luca, & Nastruzzi, 2010; Choi *et al.*, 2007; Draget & Taylor, 2011).

In supporting the growth of cells into microtissues, most of the techniques reported produced microcapsules in the range of 100-300 µm (Martín-Banderas, Gañán-Calvo, and Fernández-Arévalo, 2010). Smaller size of microcapsules offers some advantages such as providing a better transportation of nutrients and oxygen to the cells, better mechanical strength, easier for implantation, have sufficient and predictable diffusive mass transport and have a potential access to new implantation sites (Sugiura *et al.*, 2005; Wan, 2012).

However, the micro-extruder method produced large sized microcapsules due to the gravity effect (Bressel *et al.*, 2008). In this work, the development of micro-extruder with vibrational mode was proposed to improve the current system. As part of the system improvement, vibration mode was added to the complement the micro-extrusion process. The vibration would disperse the extruded droplets of alginate in to smaller size. Although

the techniques presented earlier could be used to encapsulate cells (Bressel *et al*., 2008) but this technique is suitable to be used to encapsulate the cell because of the simplicity and stability. A huge and consistent amount of microcapsules may be produced using this technique.

MATERIALS AND METHOD

Preparation of sodium alginate and calcium chloride solutions

A solution of sodium alginate at 1.5 w/v % (Sigma-Aldrich, United Kingdom) and calcium chloride at 1w/v% were prepared in distilled water. Subsequently, the 1.5 w/v% sodium alginate solution filled in a 5 ml syringe (brand, country) while 1 w/v % of calcium chloride was prepared in a 50 ml beaker. Calcium alginate will be formed when these two solutions are mixed together. The gelation of alginate would happen when the calcium chloride is mixed with the sodium alginate.

Cell culture and preparation of cell suspensions

Human Keratinocyte cell lines (HaCaTs) were purchased from Cell Line Services (CLS, Germany). In a $25cm^2$ culture flask, the cell were cultured with Dulbecco's Modified Eagle's medium (DMEM, Sigma-Aldrich, UK) which was supplemented with L Glutamine (2Mm, Sigma-Aldrich, UK), Penicillin (100 units/ml, Sigma-Aldrich, UK), Streptomycin (100mg/ml, Sigma-Aldrich, UK), Fungizone (2.5 mg/l, Sigma-Aldrich, UK) and 10%Fetal Calf Serum (Promocell, UK). The culture flask was maintained in incubator at 37℃ with 5% CO_2. As soon as the existing cells in culture flask reached confluences, the culture media was discarded from the culture flask and washed three times in Hank's Balanced Salt Solution (HBSS, Sigma-Aldrich, UK). After that, 1ml of crude 0.25% EDTA-trypsin was deposited into the culture flask and incubated at 37℃ for 5 minutes to detach the cell from culture flask. After the incubation, 5 ml of DMEM was deposited into culture flask and the cells were transferred to a centrifuge tube to be centrifuged at 1200 rpm for 5 minutes. The cells pellet at bottom of the tube was re-suspended in 1 ml of media.

Development of a micro-extruder with vibration mode

The conceptual model of the micro-extruder with vibration mode was designed using SketchUp software 2014 (Trimble Navigation Limited version 14.0.4900) as shown in Figure-1. The prototype developed consists of three main mechatronic parts including a syringe pump, a vibrator circuit and a 0.5 ml syringe containing the alginate solution. In this design, the vibrator is placed in contact with the syringe. The syringe pump is made of a motorised linear slider, syringe holder and plungerclamping plate. A 2.5 minibea stepper motor (1.8^o, 200 steps per rotation) was used to drive the linear screw to exert force that push the plunger through the syringe in extruding the fluid containing in the syringe. The position, velocity and rotation angle of the linear slide was controlled via an Arduino-uno microcontroller. A potentiometer was used to provide control voltage to the microcontroller which determined the rotation velocity of the motor. The higher the input voltage of the potentiometer, the higher is the rotation velocity of the motor and vice versa. While the customized syringe pump pumping fluid in a control velocity, the vibrator simultaneously vibrates the syringe at variable frequency ranging from 10 Hz to 60 Hz. The vibration caused the extruded alginate droplets from the needle to be dispersed and dropped into the calcium chloride solution leading to the polymerization of the calcium alginate microcapsules. At different intensity of the vibration and syringe extrusion speed, the effects of both the extrusion speed and vibration to the microcapsules production were studied. The challenge was to determine the suitable synchronization speed between the vibrator and velocity of the syringe pump in achieving different sizes of microcapsules.

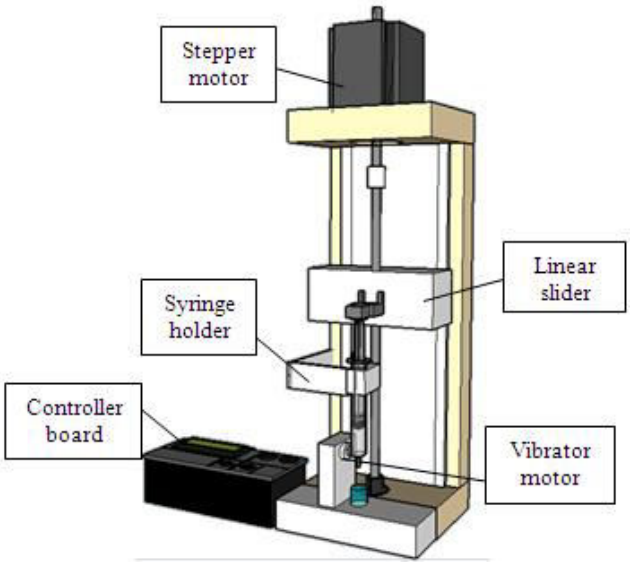

Figure-1. Micro-extruder design.

Figure-2 shows the circuit block diagram of the controller board for the overall system. A 12V DC supply was used to supply the power to an Arduino-uno microcontroller and a L293N motor driver. The L298N driver module regulates the speed and direction of motor via the instruction from the microcontroller. Upon reading inputs from the forward or reverse switches, the stepper motor rotates accordingly based on the pulsed signals provide by the microcontroller and driver circuit. The Arduino-unoboard can operate on an external supply of 6 to 20 V. If supplied with less than 7V, however, the 5V pin may supply less than five volts and the board may be unstable. If using more than 12V, the voltage regulator may overheat and damage the board. The recommended range is 7 to 12 V.

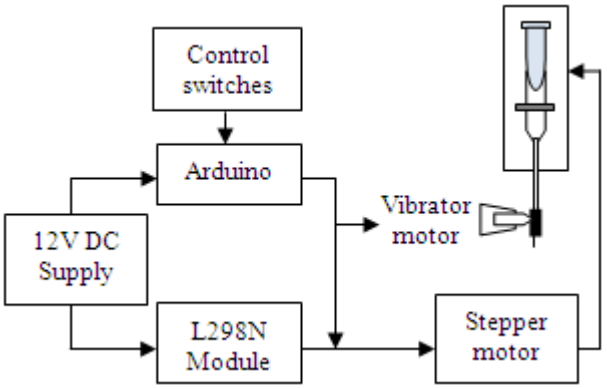

Figure-2. Circuit block diagram for the overall of project.

To get the better performance of vibrator motor, the different configuration of this component were set up. The vibrator motor (DC 1.5- 6 V 1500 RPM) was given a power supply of 9V battery even though it can be powered up to 12 V of supply. The higher the voltage supply, the faster is the speed of vibration of motor because the vibrator is direct current motor with internal displacement.

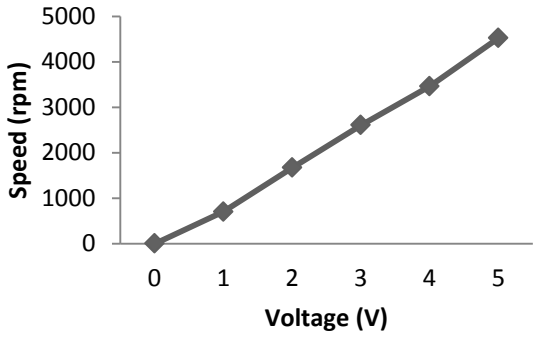

Figure-3. Relationship between speed of vibrator motor and voltage.

Software development

The Arduino Uno used can be reprogrammable in circuit via the USB connection to the computer. The algorithm for the control of the stepper motor was programmed in C language. The flowchart in Figure-4 shows the programming of Arduino for the development of this micro-extruder device. Begin with initialize the program; the LCD will display the welcoming instruction. Then, the user will be prompted to select the infusion or diffusion of the syringe pump based on the activating the forward or reverse switches available on the control panel. Subsequently, speed of rotation ranging from 10 rpm to 100 rpm can also be selected.

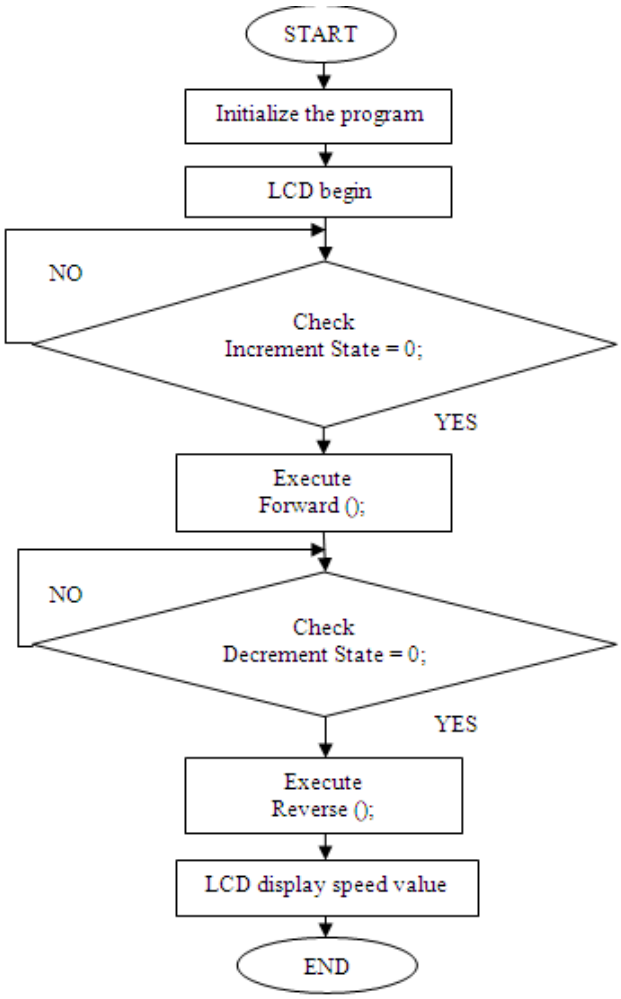

Figure-4. Flowchart of Arduino programming.

RESULT AND DISCUSSIONS

Encapsulation of calcium alginate

Using this method, the encapsulation of cells with calcium alginate can be produced. The size of spherical beads was produce consistently but not in micron in size. The shape of beads also will change due to the changing of speed of motor.

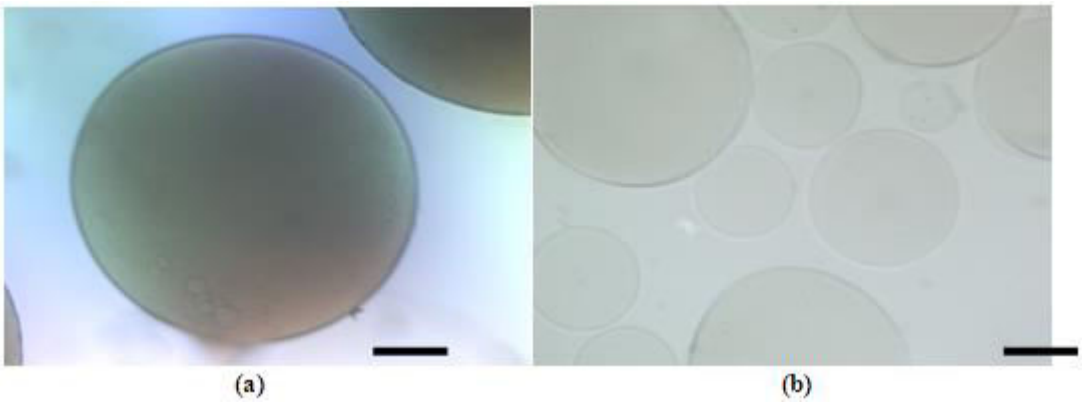

Figure-5. Microcapsule alginate produced (a) without and (b) with vibration mode. (Scale bar: 100µm).

Figure-5 show two different images were captured using different microscopic technique. The size of beads produce is in the range of 800-1000µm. The Production of beads may become a thread-like structure with increasing speed value. Even the size is bigger compared to the others study; this method can be modified more on the technical aspects. The vibration mode will help the extruder to produce smaller size of beads.

From Figure-6 below shows the relationship between the size of beads produce and speed of stepper motor. For the increment of speed at 10, 20, 30, 40, 50 and 60 rotation per minute (rpm), the beads size obtained getting bigger which are 831.59, 858.72, 976.56, 1082.73 and 1096.06 µm respectively. The unit of rotation per minute (rpm) were used because of the faster rotation of stepper motor. The size of beads depends on the speed of stepper motor. The result shows higher the speed, the bigger the size of beads produce.

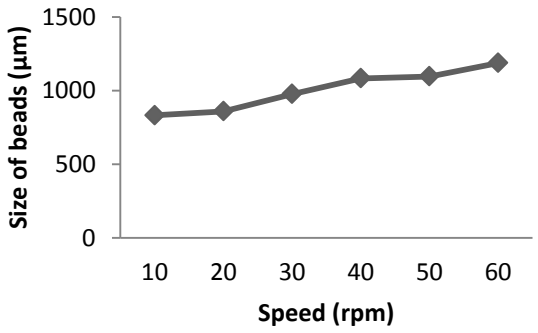

Figure-6. Size of beads against the speed of stepper motor.

Figure-7, the bar chart shows how the frequency effects the size of beads produce. For increment of frequency at 20, 40 and 60 Hz, the beads were obtained in size which is 1096.06, 967.69 and 830.63 µm respectively. From the result obtained, the increment of frequency of vibration motor was decreasing the standard deviation of the beads size (±29.478,±35.817and ±34.560) which means the size was nearly consistent with each frequency range from 20 to 60 Hz. The result show of frequency will decrease the size of beads.

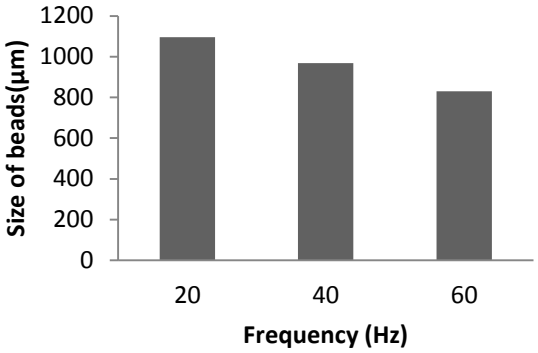

Figure-7. Relationship betweem size of beads with frequency.

Figure-8 shows how the voltage affects the speed of the stepper motor. The variable value of voltage from 10 kΩ potentiometer will make the speed of stepper motor changing. From the graph, there were rapidly increase in speed of stepper motor rotation when the given voltage is around 10-100mV. Means that, slow speed of stepper motor can produce the small size of microcapsule. So, the higher the voltage reading, the faster the speed of stepper motor rotation.

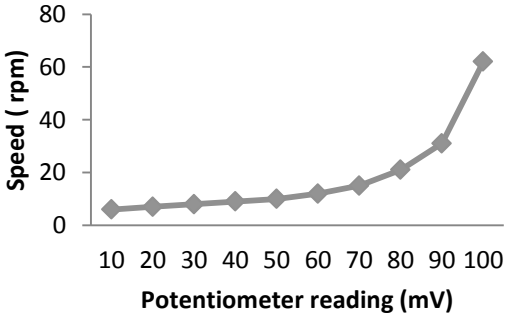

Figure-8. Speed of stepper motor against voltage reading.

This is different with the study by (Xie and Wang, 2007) which used the high voltage generator with ring electrode to make a better droplet formation. Their study focus more on the effect of diameter of nozzle, viscosity of alginate solution and flow rate of polymer solution used on the microcapsules size. The size of droplet could decrease with the increasing voltage supply.

CONCLUSIONS

The collected data was presented in Figure-9. From the graph, there are three parameter being observed which are the speed, volume of alginate and flow rate. There was increasing trending of flow rate with the increasing of speed value as shown in Figure-9. The smallest amount of alginate which are 0.1ml give the linear reading of flow rate with increasing of speed. With the different volume of alginate used, it was clearly shown that the flow rate is depend on the speed of stepper motor.For 0.1 ml of calcium alginate, the flow rate increase slightly around 0.1ml/min to 0.2 ml/min when the speed of stepper motor increase. Compared to 0.4ml of calcium alginate, the flow rate drastically increased due to the speed of stepper motor.

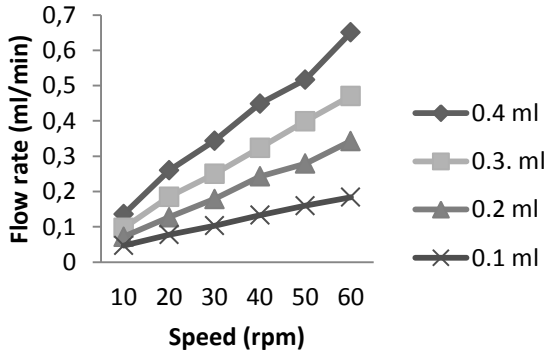

Figure-9. Relationship between flow rate and speed of stepper motor.

With the help of stepper motor controller, step motors convert electrical energy into precise mechanical motion. The stepper motor rotates a specific incremental distance per each step. The number of steps that are executed controls the degree of rotation of the motor's shaft. This characteristic makes stepper motors excellent for positioning applications. For example, a 1.8° stepper motor executing 100 steps will rotate exactly 180° with some small amount of non-cumulative error. The speed of step execution controls the rate of motor rotation. A 1.8° step motor executing steps at a speed of 200 steps per second will rotate exactly 1 revolution per second. In summary, the development of micro-extruder for microencapsulation is the simplest method to encapsulate cells. The small size of microcapsules can be produced with the addition of vibrational mode. High speed of stepper motor with high voltage of vibrator motor can produce smaller size of microcapsules. The analysis related to speed control will show how the linearity of their relationship when different speed we applied to stepper motor. The controlled speed value was able to produce the encapsulated cell in well distribution and size.

ACKNOWLEDGEMENT

The authors would like dedicate special thanks to Malaysia Ministry of Higher Education for providing research funding under FRGS Grant (Vote 1482) and GIPS (Vote U327).

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