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DEVELOPMENT OF AN ELECTRONIC AEROSOL SYSTEM FOR GENERATING MICROCAPSULES

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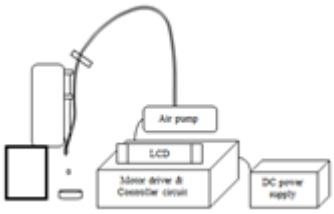
Graphical abstract



Abstract

The encapsulation of living cells in a variety of soft polymers or hydrogels is important, particularly for the generation of microtissues. Various techniques have been developed for the production of microcapsules to encapsulate cells but presented threat to the cells due to the harsh treatment during the encapsulation process. In this paper, we propose a simple, economic and compact design of aerosol electronic system for producing different sizes of microcapsules. The aerosol system was developed with the incorporation of a conventional syringe pump and a customised air pump. The syringe pump purged the droplets of sodium alginate and air pump dispersed the droplets into microdroplets of sodium alginate which was then polymerised in the calcium chloride solution. In this system, the air flow rate from the air pump was controlled by a programmed microcontroller that received input instructions from a potentiometer. The suitable air flow rates that worked synchronously with the speed of the syringe pump were characterised. At 0.2 and 0.3 L/min of air flow and 20 µl/min of alginate solution flow, this device successfully generated round microcapsules with various sizes ranging from 100 to 350 µm.

Keywords: Electronic aerosol system; Arduino-Uno; airflow rate; microcapsules; size distribution

1.0 INTRODUCTION

An aerosol is a suspension of particles in liquid droplets that are dispersed in fine sprays. The dispersion of liquid substance is achieved under pressurised air or gas. The concept of aerosol is widely used in making pressurised cans for packaging perfume or cleaning agent. Similar principles can also be applied to biomedical field in the dispersion of drugs or pharmacological agents under high flow rate of air. One of the applications of microcapsules is in the encapsulation of cells and drug delivery. The cells encapsulated in microbeads are useful as biomaterials or fillers to the artificial tissues [1, 2].

Microencapsulation has wide application in encapsulation of cells, drug delivery, food science and lab experimental testing. A few methods had been developed for the microencapsulation of cells such as the pendant drop, micromolds, microfluidic device [3, 4], vibration, electrostatic droplet generation [5-7], coaxial air-flow [8], and hydrogel techniques [9]. Previous methods applied such as simple dripping technique, under a condition without pre-treatment (or harsh condition), the diameter of the capsules produced is usually ranged between 600-1000 µm [10]. Other techniques such as microfluidic and electrostatic dropping could also produce smaller sized microbeads but these techniques are considered harsh because involvement of the organic solvent, heating, oil phase and ultra-violet treatment. Therefore, these treatments during the cell encapsulation process presented threats to the cell survival in the microbeads [9, 11, 12].

There are various types of biopolymer that can be used to produce microcapsules for encapsulation of cells such as agarose, collagen, alginate, chitosan and gelatin. These materials are different in polymerisation process and hence, influence the design of microcapsules generation system. Encapsulation technology must fulfill the strict requirements that are applicable to therapeutic strategies. Various considerations in terms of performance, biosafety, biocompatibility, retrievability, stability, availability, purity, characterisation and cost [13] are of paramount importance. Amongst previous methods discussed, aerosol technique is a simple, easy and efficient method to generate microcapsules with well controlled size and shape [14-16]. In this study, a new electronic aerosol system has been developed to generate microcapsules of calcium alginate. This system has the advantage of producing calcium alginate microcapsules which can either in simple solid, hollow or core-shell microcapsules without involvement with harsh treatments [17]. The microcapsules produced have high loading efficiency of cells [18] and they were compatible with a wide range of active ingredients [19], micro- or nano-particles other than cells [19-22]. In this paper, we presented a low cost electronic operation aerosol system in combination with the infusion syringe pump dripping technique that can produce microcapsules smaller than the dripping technique alone. The effects of the concentration of solutions, air flow rate and speed of syringe pump were studied in this work.

2.0 MATERIALS AND METHODS

2.1 Development of An Aerosol System

The aerosol system developed consists of three main mechatronic parts: the syringe pump, a customized air pump and motor controller circuits as shown in Figure 1. The solutions used for the preparation of the microcapsules of calcium alginate were 1.5% alginate and 1% calcium chloride solutions (Sigma Aldrich, UK). The alginate solution was filled in a 500 µl and 29 gauge insulin syringe (BD Biosciences, US) while the calcium chloride solution was prepared in a 50 ml beaker. For the dispensing of alginate solution in droplets, a commercial unit of syringe pump (New Era Pump Systems, USA) was used to eject the sodium alginate solutions at a flow rate of 20 µl/min. For fine spraying the dispensed microdroplets of the sodium alginate solution, the sharp needle of the insulin syringe was inserted into a polystyrene hose (inner diameter = 1mm) connected to an air pump (SF4X4-1, Markwort Volcano, China). The motor driver circuit was designed to control the speed of the dc motor in the air pump. A desktop power supply (Figure 2) was used to provide currents to both the air pump and a 2 × 16 liquid crystal display (LCD), respectively. Within the desktop power supply, there were regulated outputs of +12 V and +5V direct current (dc). A power source of 12 Vdc and 14 ampere (A) were supplied to the motor driver and air pump. Another regulated output 5 Vdc was supplied to the Arduino-Uno microcontroller. The same 5 Vdc was also supplied to the LCD. A potentiometer connected to the analog inputs of the microcontroller was used to control the airflow rate. When the device was powered, the potentiometer would be used to set the airflow rate. The selected air flow rate and user inputs would be displayed on a LCD screen. After the selection of speed was performed, the on and off push button were activated to run the motor driver and air pump.

The speed control was achieved by programming an Arduino-Uno-controller that provides Pulse Width Modulation (PWM) signals to control the rotation speed of the DC motor mounted in the air pump. The motor speed was continuously being monitored via a LCD. In addition, the airflow rate of the air pump can be controlled by the user using a potentiometer that either decrease or increase the airflow rate of the air pump. In the overall aerosol system, the syringe pump flow rate must be optimized first with the air flow rate to produce continuous formation of microcapsules. Subsequently, the alginate microdroplets were dispersed by air into the calcium chloride solution leading to the formation of calcium alginate microcapsules.

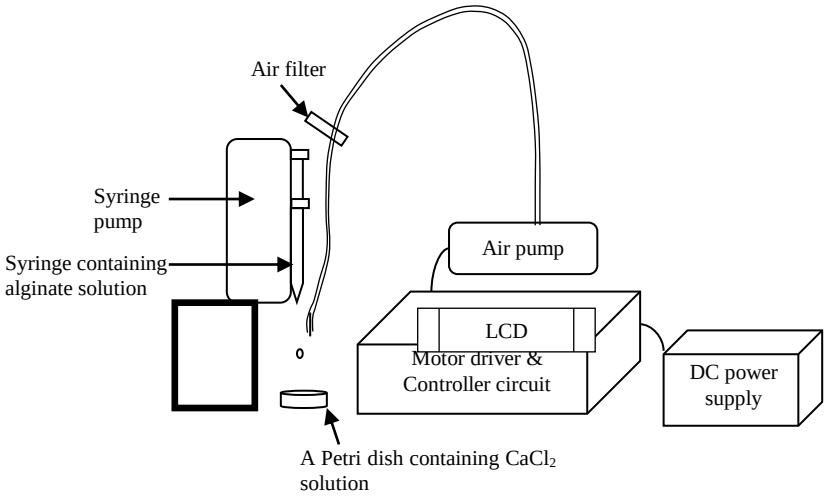


Figure 1 A schematic illustration of an electronic aerosol system for generating microcapsules

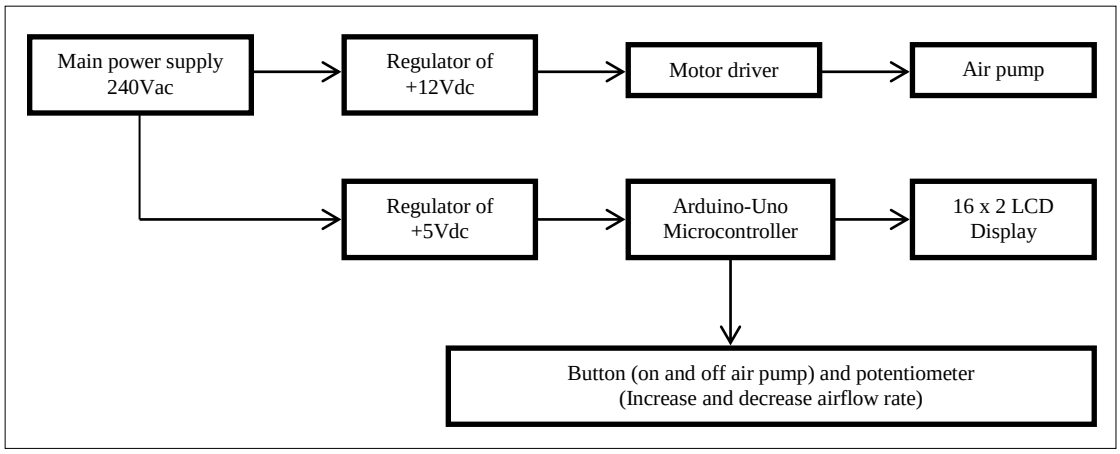


Figure 2 The block diagram of an electronic aerosol system

2.2 Programming The Microcontroller for The Air Pump

The program of the Arduino-uno microcontroller was written in C language to provide user input and information of the current status of the air pump in a LCD. One analog input pin was assigned for the potentiometer. With the Arduino's PWM frequency at about 500 Hz, it would measure 2 milliseconds per signal cycle. An input value was read from the potentiometer and the microcontroller converted to a related digital value ranging from 0-1023. This range of value is then mapped to 0-255 which is the range set for PWM in the Arduino-Uno. In the program, the voltage of the potentiometer would be mapped to the analogWrite() with a scale of 0 - 100, such that analogWrite(100) refers to a 100% duty cycle , and analogWrite(50) relates to 50% duty cycle (on half the time) of square waves to the dc motor of the air pump. The 'stop' instruction was written in order to stop the loop for continuing the function of air pump by a push button.

The initial setup in Arduino-Uno microcontroller is critical. This is to ensure the microcontroller provide a correct flow rate air pump instructions of 0.2, 0.3, 0.4 or 0.5 L/min based on the speed of the dc motor as regulated by the PWM signals. After the initial setup, the programming flow chart of the aerosol system carried on with the operation of air pump and displays of message on a LCD as shown in Figure 3.

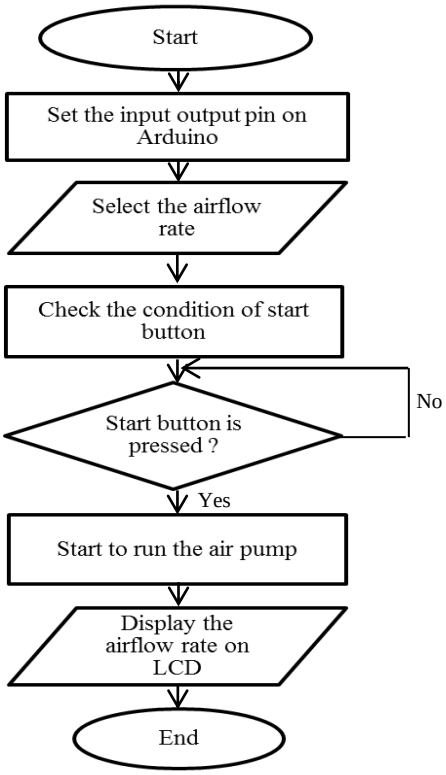


Figure 3 The programming flow of the aerosol system

2.3 Calibration The Flow Rate of The Air Pump

By manipulating different duty cycles of the PWM, different frequencies of dc motor rotations or air pump can be measured. The PWM was manipulated at 20, 25, 30, 35, and 40 % of duty cycle. The measured frequencies were used to quantify the flow rate in 60 second. The average frequencies were then calculated in order to obtain the result of airflow rate in the unit of L/min based on Equation 1:

$$Q = (f / 98) \ L/min \quad (1)$$

where, Q is the airflow rate, in the unit of litre per minute (L/min) and F is the frequency measured, in the unit of Hertz (Hz).

2.4 Measuring The Size of The Microcapsules

In this study, the microcapsules produced at different flow rates were determined. The images of the microcapsules were acquired using an optical microscope (Olympus BX60M microscope, JAPAN) linked to a CCD camera (Olympus U-PMTVC, JAPAN). The diameter of the microcapsules was measured using the ImageJ version 1.8 software (NIH, USA). For determining the size distribution, 300 microcapsules from the three repeats of experiment sample were captured and the diameter of each microcapsule was measured.

3.0 RESULTS AND DISCUSSION

3.1 Duty Cycle of Pulse Width Modulation (PWM)

Figure 4 shows the generation of PWM oscillation of an aerosol system measured by oscilloscope. The PWM pulse oscillation measured (Figure 4) at the digital output pin of the microcontroller was correlated with the controlled voltage of the potentiometer (Figure 5). The duty cycle of the PWM pulse in turn regulated the flow rate of the air pump; the higher the duty cycle of PWM, the higher is the air flow rate. The PWM of 20, 40, 60, 80 and 100 % duty cycle produced signals with pulse on time of 400, 800, 1200, 1600 and 2000 µs, respectively.

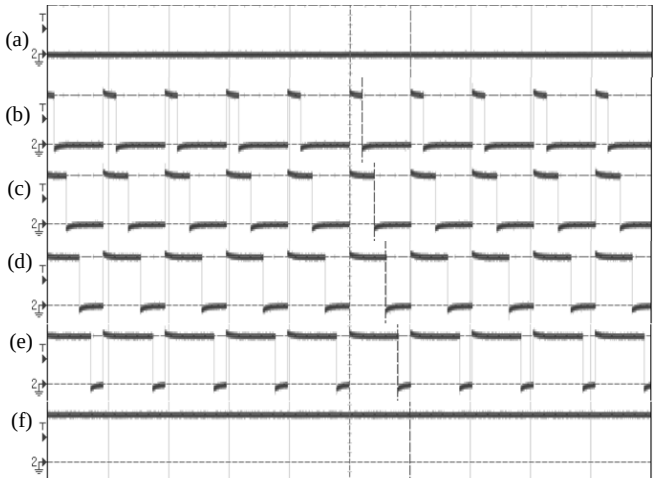


Figure 4 The signals of PWM oscillation at duty cycle of (a) 0%, (b) 20%, (c) 40%, (d) 60%, (e) 80% and (f) 100%, respectively

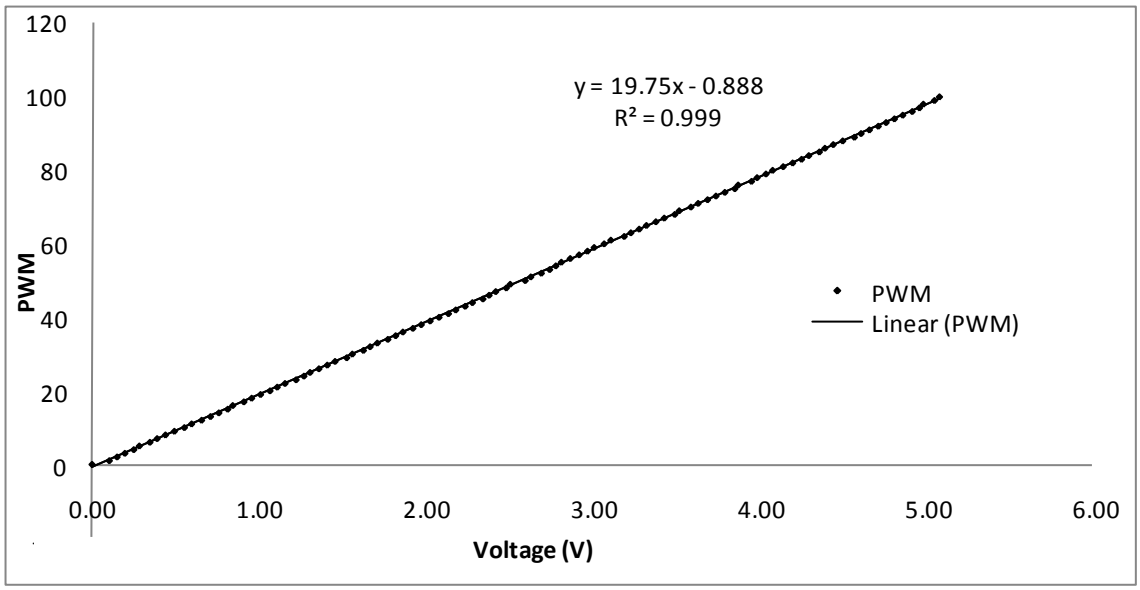


Figure 5 The corresponding duty cycle of PWM to the voltage of the potentiometer for controlling the air pump

3.2 Airflow Rate Measurement

The output of the air pump was connected directly with an airflow sensor (YF-S401, Sea, China) to measure the frequencies. The airflow sensor measured was in the working range of 0.1-6 L/min [23]. The airflow rates were calculated (Equation 1) and average frequencies were tabulated in Table 1. With the specified duty cycle of PWM, the average frequencies recorded were increased accordingly to the airflow rate calculated.

Figure 6 shows the quantification of airflow rate by manipulating the speed of the motor (PWM). From the plotted graph, the PWM showed a linear relationship with the airflow rate. From the characterised airflow rate (Figure 6), the desired airflow at 0.2, 0.3, 0.4 and 0.5 L/min were selected to determine the corresponding duty cycle of the air pump. The corresponding duty cycle of the air pump was determined at 21, 27, 32 and 38 % duty cycle, respectively. Figure 6 shows the flow rate selected from the graph.

Table 1 The air flow rate measurement for different PWM recorded

No.	Duty cycle of the PWM (%)				
	20	25	30	35	40
Average frequency (Hz)	18.07	27.08	34.57	43.69	52.80
Airflow rate, Q = (f/98) L/min	0.18	0.28	0.35	0.45	0.54

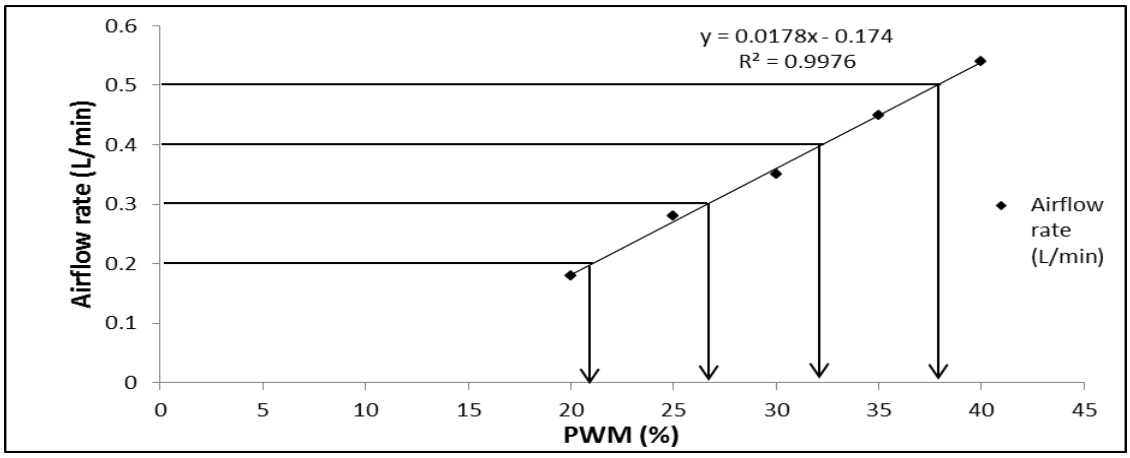


Figure 6 The results of correspond PWM duty cycle to the airflow rate of the air pump

3.3 LCD Display Output

The LCD display in Figure 7 shows an example output of the aerosol system. The airflow rate was displayed according to the voltage adjustment of the potentiometer.

Figure 7 The LCD display of the electronic aerosol system

3.4 Size Distribution of The Alginate Microcapsules

Size control of microcapsules is an important parameter for practical applications. Calcium alginate microcapsules were formed by the reaction between the alginate droplets and the $CaCl_2$ solution. In order to control the microcapsules diameter, we investigated the effect of the airflow rate to the microcapsules-formation process (Figure 8). Most of the microcapsules produced were in spherical shapes. The microencapsulation system proposed in this study can be applied for encapsulation of cells, since the system work under sterile conditions and without chemical irritants, such as a surfactant [24]. According to Figure 8, microcapsules showed regular round shape and smooth surface with different size of diameter ranging from 100 to 350 µm. Lower flow rate of the aerosol system at 0.2 (Figure 8(a)) and 0.3 L/min (Figure 8(b)) produced more rounded microcapsules. The microcapsules produced by aerosol system were not demonstrated with the problem of fragmented or deformed of shape [11], tail shaped microbeads [25], and an undesirable oil layer that are to be applied for the culture of cells [12].

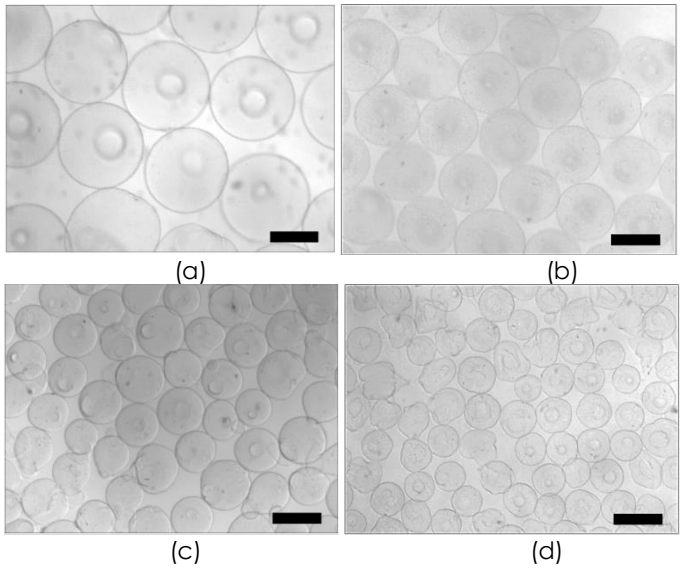


Figure 8 Morphological and size distribution microscope photographs of prepared microcapsules with the airflow rate of (a) 0.2 L/min, (b) 0.3 L/min, (c) 0.4 L/min and (d) 0.5 L/min. (Scale bar = 200 µm)

The size distribution of the microcapsules at 0.2 L/min airflow rate had an average hydrodynamic diameter of 334.38 ± 6.72 µm. Meanwhile, for the size distribution of the microcapsules with 0.3, 0.4 and 0.5 L/min airflow rate, the average hydrodynamic diameter were 247.63 ± 7.75, 169.66 ± 15.77 and 127.42 ± 15.30 µm respectively, as can be seen from Figure 9. The size of microcapsules at 0.3 L/min

airflow rate was suggested for the encapsulation of cells because this flow rate could produce the desired size (200-300 µm) of microcapsules to grow cells *in vivo* [26-29]. Nonetheless, smaller beads size presented with more advantages such as transportation of nutrients and oxygen [30], better dispersion, better mechanical strength, easier implantation and access to new implantation sites [31].

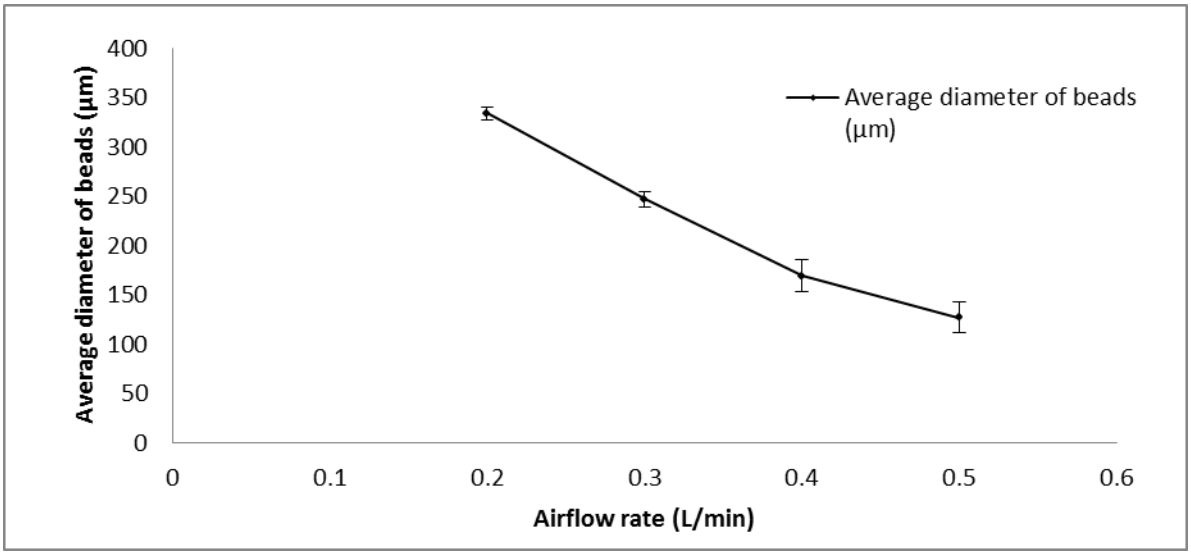


Figure 9 The effect of airflow rate of the aerosol device to the average diameter of the microcapsules. Average microcapsules diameter and coefficient of variation of microcapsules are shown in (|) and (♦), respectively

4.0 CONCLUSIONS

An electronic aerosol system has been developed to generate the various sized calcium alginate microcapsules. The system developed has the potential to be applied for microencapsulation of cells. In a hose, the control of the air flow pressurised the flow of alginate droplets sprayed from the needle inserted. The flow rate of the air pump was achieved by controlling the potentiometer providing input signals to the microcontroller of the air pump. The duty cycle of the air pump is linearly proportional to the voltage input of the potentiometer. Lower flow rate of the aerosol system at 0.2 and 0.3 L/min compared to higher flow rate produced more rounded microcapsules. The microcapsules seemed to be distorted when the flow rate was at set 0.4 and 0.5 L/min.

Acknowledgment

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