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Abstract. Type II diabetes mellitus caused adverse impact on almost 3.5 million patients locally. Conventional treatment for such disorder includes oral administration of anti-diabetic drugs or herbs supplement. However, the performance of this drugs in regulating blood glucose level is not too reliable due to the occurrence of weight gain, hypoglycaemia and low tolerability as well as structural breakdown of herbal supplements during consumption. In this project, an alternative approach was attempted by immobilising saponin, a known insulin stimulant extracted from plants (e.g. bitter gourd, eggplant or soapbark) onto chitosan beads via microfluidic technology. This technology offers advantages of producing micro-sized droplet with high surface area to volume ratio and minimal coefficient of variation for more accurate dosage. The droplets undergo solidification via cross-linking with NaOH as solvent. These beads are approximately 550 microns with 5.12 % polydispersity in size. The solidified beads were used as carrier for the immobilisation of saponin. This was achieved through altering the surface charge of the microbeads with Phosphate Buffer Solution (PBS), a commonly used electrolyte for the surface modification of chitosan before coating saponin onto its surface. Successful adsorption is rectified through FTIR, FESEM and EDX analysis.

1. Introduction

Diabetes mellitus (DM) has affected approximately 3.6 million Malaysians of age 18 and above [1]. DM are segregated into 2 types which relates to their respective severity. Type I DM (T1DM) is caused by defective pancreas that are unable to produce insulin. This type is often hereditary, and there is by far no preventive actions to be done to avoid such disorder. As a contrary, Type II DM (T2DM) patients have high blood sugar level either due to insulin resistance or lack of insulin production by the pancreas. 99% of diabetic cases in Malaysia are the second type [2]. The focus of this research is a to establish a preventive measure for T2DM. Insulin plays an important role to regulate blood glucose level by allowing glucose to be taken up by cells and be used for energy. One of the new trend of the development in the field includes an introduction of herbs supplements in the form of tablets, capsules or liquid can be used to replace these drugs. They are the preferred alternatives for diabetic patients at mild stage [3]. However, the antidiabetic compounds from herbs experiences structural breakdown and low absorption in digestion system during consumption [4]. This drawback causes fluctuation in serum glucose levels. An improvement on the compound release has been proposed by immobilization of

herbal extract onto solid carriers as a structural support. One of the most effective antidiabetic herbal extracts discovered is from bitter gourd (*Momordica Charantia sp.*) [5]. Through some vivo tests, saponin, the extract helps in the release of pancreatic insulin via increase in plasma insulin level [6] and hinders glucose formation in the bloodstream [7]. Other health benefits of the saponin and clinical studies are well documented in *Alam et al.* [8]. Chitosan, a derivative from chitin, is a natural bio-membrane extracted from exoskeleton of crabs, shrimps, or other crustaceans. It has been broadly applied as therapeutics material as it is biodegradable, non-toxic, antacid, and low antigenic characteristic [9]. Usage on chitosan in oral sustained release preparation was also established [10] using microfluidic approach. Microfluidics is the study of the behavior of fluids interactions at microscale with application including imaging, drug delivery and diagnostics [11].

However, the immobilization of saponin onto chitosan-based carrier has yet to be reported. This project therefore attempts to produce a controllable size carrier for the immobilization of saponin. To do so, chitosan droplets were first produced with a co-axial flow geometry device in dripping regime using microfluidic technologies. The microfluidic device was configured via off-the-shelf technology with dispensing needles and a mini crosslink. In the second stage, droplets generated were solidified with NaOH solution to produce chitosan microbeads. In the third stage, chitosan microbeads were used as a carrier for the immobilization of saponin via PBS soaking.

2. Experiment Setup

2.1. Droplet Generation

The needle-based microfluidic devices fabricated based on co-flow geometry. The plastic head of a 19G needle was removed from the stainless-steel needle as shown in figure 1 (a) below. The dissembled needle and a 23G dispensing needle were inserted into two opposite ends of a cross-link and affixed with ultraviolet-curing adhesive. Needles are aligned to the center of the junction before exposed to an UV torch light for 3 minutes for cross-link purpose, as seen in figure 1 (b). From Figure 1 (c), 3 device size combinations were fabricated (i.e. 23G-19G, 25G-20G and 27G-21G.

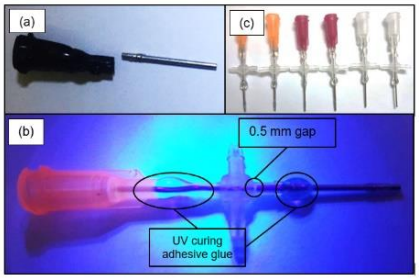


Figure 1. (a) Dissembled 19G dispensing needle; (b) Illustration of fabricated and assembled 23G-19G device; (c) 3 pairs of devices configuration assembled (23G-19G, 25G-20G and 27G-21G).

2.2. Chitosan Microdroplet and Solidification.

0.2M acetic acid was first diluted to prepare 2 wt% chitosan solution. For chitosan solution, 2 g of chitosan powder was added into 98 g of 0.2 M acetic acid. The mixture was stirred for 3 hours using magnetic stirrer (IKA C-MAG HS 7) until fully dissolved to form chitosan solution. Meanwhile, in a separate beaker, 20 mL of 10 wt% NaOH solution was topped with 2.5 mL of sunflower oil. The experimental set up was done with chitosan solution as disperse phase. Flowrate of chitosan solution and sunflower oil was set as 1 mL/hr and 10 mL/hr respectively. After 30 minutes, the beaker was swirled gently to allow settling of the chitosan microbeads. The beads were set aside for another 2 hours to allow complete crosslinking. Next, the beads will undergo washing step with 5 mL distilled water repeatedly to remove residual alkali, oil and soap. They were stored in 5 mL of distilled water in the centrifuge tube for later use.

2.3. Immobilization of Saponin onto Chitosan Microbeads

10x Phosphate Buffer Solution (PBS) was prepared by adding 20 g KCl, 800 g NaCl, 24 g of KH_2PO_4 and 144 g $Na_2HPO_4 \cdot 2H_2O$ into 8 liters of distilled water. The solution was topped up to 10 liters as the final PBS solution after complete mixing. Microbeads from stage 2 were removed from distilled water medium and soaked in 15 mL of PBS solution for 24 hours. The beads were rinsed 3 times and dried on a petri dish in a sealed box with silica gel (figure 2 (a)) for 3 days. Saponin solution was prepared by mixing 0.5 g of saponin powder with 100 mL of liquid ethanol. The solution was stirred with magnetic stirrer for 3 hours. A colloidal saponin solution is then formed. Undissolved saponin powder were left for a day to settle (figure 2 (b) & (c)). The top layer of the settled saponin solution were used to carry out saponin adsorption. Dried microbeads were transferred to a beaker together with 40 mL of top layer saponin solution. To prevent spillage, the beaker was covered with parafilm and inserted into a larger sized plastic beaker with Styrofoam and Blu-Tack as shown in figure 2 (d). The beaker was secured on a shaker (IKA KS 4000) for 6 hours at 200 RPM.

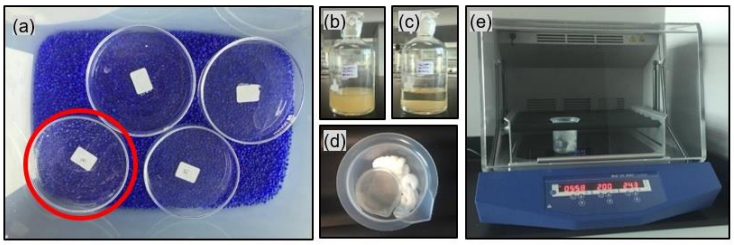


Figure 2. (a) Beads dried on petri dish in a sealed box with silica gel; (b) Colloidal saponin solution formed from saponin powder and ethanol solution; (c) Settled saponin solution forming 2 layers; (d) Top view of beaker secured on the shaker top view; (e) Side view of the shaker.

3. Result and Discussion

3.1. Droplet Generation

Several co-flow geometry devices were fabricated to generate droplets through dripping regime. Study was carried out to determine the relationship between Q_d/Q_c (above 0.1) and the frequency of droplet generated by different size configuration of devices. The introduced continuous phase was oil whereas the disperse phase was distilled water with 0.5 wt% of surfactant sodium dodecyl sulphate (SDS) to avoid coalescence of the droplets generated.

From figure 3, the device with larger diameter (23G-19G) produces the greatest number of droplets at constant flow ratio. This is because device with larger needles restricts the flow of the outer continuous phase. Thus, at constant Q_d/Q_c, smaller continuous phase flow area induces a higher continuous phase flow velocity, U_c. Since U_c is directly proportional to Ca_c, the droplet size generated will decrease non-linearly with the increase of Ca_c. Smaller droplet size represents a higher breakup frequency and ultimately higher number of droplets generated, which is in line with the reported literature [12]. On the other hand, by using the same device size configuration, an increase in Q_d/Q_c causes U_c to be relatively lower. By using the relationship as explained above, the frequency of droplets generated should reduce. Hence the results shown in Figure 4 is further clarified.

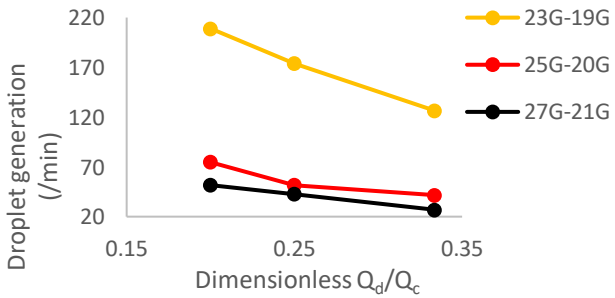


Figure 3. Graph of frequency of droplet generation against Q_d/Q_c.

3.2. Chitosan Solidification

The solidified microbeads were characterized with an optical microscope (Nikon, H600L). The mean ± standard deviation was calculated to be 552 ± 28 microns. The size range distribution of bead diameters is 499 to 613 microns with a polydispersity index of 5.12%. In the solidification step, uneven shrinking of chitosan beads between 11 to 13% were reported due to crosslinking of microbeads [13]. The differences of the mean diameter as compared to the literatures was due to the type of disperse and continuous phase used and their respective flowrates. From figure 4 (a), majority of beads produced are either droplet like shaped or spherical with a small tail whereas some are oval shaped. This is caused by the suspension of beads between oil and NaOH layer. The interfacial tension as mentioned and low-density difference between chitosan droplet and NaOH solution. Thus, cross-linking took place at the bottom of the drops to form a perfect spherical bottom whereas the top surfaces of the beads were immersed in oil layer are yet to exposed to NaOH solution as illustrated in figure 4 (b). Solidification of the top surface only initiated once the bead penetrated the oil – NaOH interface. For droplet shaped spherical beads, the tail was ignored while taking 2 diameter readings (d_1 and d_2). Both diameter readings are taken perpendicularly and pass through the centre of the circle. An average diameter (d_{avg}) between d_1 and d_2 was taken for a single bead. For oval or ellipsoidal beads, Feret diameter approached was used by measuring the length of both major and minor axis of the bead, average values were taken as the d_{avg} reading as seen in figure 4 (c).

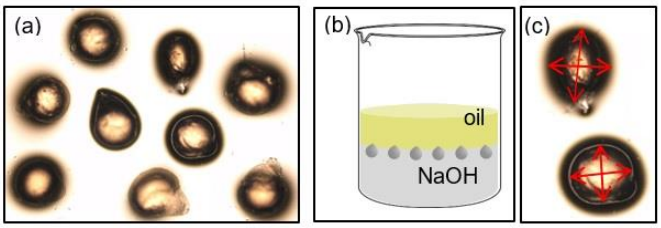
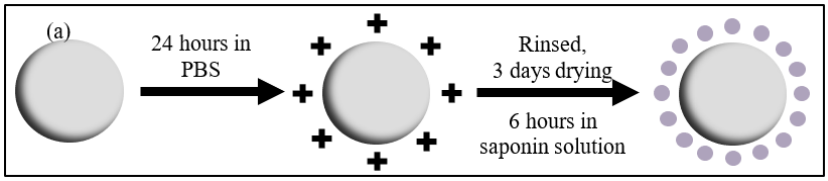


Figure 4. (a) Microscope image of the microbeads; (b) Illustration of droplet shaped microbeads stuck at interface; (c) Method of reading d_1 and d_2.

3.3. Saponin Immobilization

Chitosan microbeads acts as a bio-membrane for the adsorption of saponin. The blank chitosan beads are soaked into 10x PBS solution for 24 hours. Surface modification causes the chitosan microbeads to have positively charged surface. This allows electrostatic interaction to occur between PBS and microbeads surface. Adsorption of anionic saponin molecules onto the cationic microbead surface is then possible as illustrated in figure 5 (a).

The blank beads were modified with PBS and saponin solution. The saponin coated chitosan beads were analyzed with FTIR (PerkinElmer) spectroscopy with pure saponin powder as a control. From figure 5 (b), saponin coated chitosan beads has similar FTIR trend as the pure saponin powder (control sample). The peak observed close to 1000 cm^{-1} indicated the presence of -C-O-C- bond from saponin molecules [14].



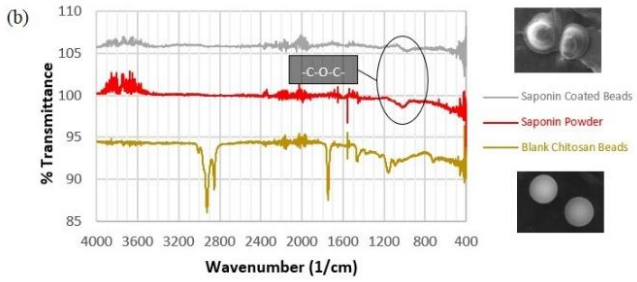
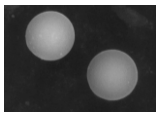
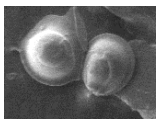


Figure 5. (a) Immobilization of saponin onto chitosan microbeads mechanism; (b) FTIR tests on blank chitosan beads (without saponin adsorption), saponin powder and saponin coated beads with FESEM images of blank chitosan beads and saponin coated beads.

3.4. FESEM and EDX Analysis

The surface morphology of the blank and modified microbeads was observed under FESEM (Quanta 400F, FEI) and analyzed with EDX (Quanta 400F, FEI) to determine the sphericity and elements present on the surface of the beads as shown in Table 1. For blank chitosan beads, the amount of C atoms and O atoms are approximately 85% and 15%. This is because chitosan molecules are mainly made up of those 2 atoms as seen in literature [15]. Oxygen atom present in both modified beads was comparatively more than those in the blank microbeads. This can be explained with the molecular structure of saponin itself as seen in literature [16]. The O element present in a single saponin molecule are more than that in chitosan molecule. This further validate the successful surface adsorption done on chitosan microbeads.

Table 1. EDX analysis of microbeads.

FESEM image	Beads type	No.	Normalised atomic %		Remark
			C	O	
	Blank Microbeads	1	86.95	13.05	-
		2	86.84	13.16	-
	Saponin Modified Microbeads	1	65.69	34.31	With traces of Cl element
		2	74.22	25.79	With traces of Si element

4. Conclusion

Microfluidics technology has been well established but the report of using dispensing needles as microfluidic devices is very limited. This work has proven the reliability of this device to generated highly monodisperse droplet. Chitosan droplet has been generated by using a 23G-19G microfluidic device, cross-linked with NaOH solution and immobilized with saponin extracted from Momordica Charantia species. Inevitable saponification during the solidification process has hindered the crosslinking of microbeads, which was resolved by gently swirling of the NaOH solution. Microbeads produced are in an acceptable size range with polydispersity index of 5.12% despite the uneven shrinking from chitosan cross-linkage. Successful immobilization has been proven through FTIR and FESEM analysis. The ongoing research in process optimization can be done on several aspects to produce drugs with the best effect. Of the current studied parameters of the drug production process, the type of buffer electrolyte used, its concentration and soaking time can be varied to allow uniform and maximum saponin adsorption. Other parameters such as saponin concentration, its adsorption time and chitosan carrier's concentration can also be manipulated for better saponin adsorption. In addition,

there is much analytical work to be carried out such as the effectiveness of saponin from the synthesis route to ensure the function of saponin is not compromised from this synthesis route.

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