ORIGINAL ARTICLE

Encapsulation of Ginseng inside Poly(lactic-*co*-glycolic acid)/ Polyaniline Microcaspsules

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ABSTRACT

Introduction: : Ginseng is a type of traditional medicine that has been used for thousand years to treat various diseases and has been proven effective in treating cardiovascular diseases. Incorporation of polyaniline (PANI) which is a type of conductive polymer together with ginseng into poly(lactic-*co*-glycolic acid) (PLGA) microcapsules is necessary for the treatment of cardiovascular diseases as the polymer will control drug release and the electroconductivity of PANI is beneficial on myocardium cells. **Methods:** Therefore, this project involved the encapsulation of ginseng inside PLGA/PANI microcapsules. The encapsulation of ginseng inside the microcapsules was verified through the identification of chemical composition of ginseng, PLGA and PANI using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). **Results:** The results of scanning electron microscope (SEM) showed the formation of microspheres where the microcapsule size was decreased from 3.14±1.87 μm to 1.98±1.30 μm as the concentration of PANI increased. The distribution of microcapsules size was more homogeneous in the high concentration of PANI as been determined through the histogram analysis. In addition, the fluorescence analysis demonstrated the efficiency of ginseng encapsulation inside PLGA/PANI microcapsules through the appearance of stained ginseng inside the microcapsules. **Conclusion:** As a conclusion, the ginseng was successfully encapsulated within PLGA/PANI microcapsules that will be beneficial in drug delivery application, specifically in the cardiovascular area.

Keywords: Encapsulation, Ginseng, Microcapsule, Polyaniline, Poly(lactic-*co*-glycolic acid)

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INTRODUCTION

Cardiovascular disease is one of the major contributors of death among all other types of non-communicable diseases in Malaysia (1). The increase in number of death due to cardiovascular diseases throughout the years in Malaysia has cause a huge increase in both medical and social costs (2). Studies have shown that unhealthy lifestyle and lack of physical activity are the major causes of cardiovascular diseases (3). Unhealthy lifestyles such as unhealthy diet, tobacco use and excessive consumption of alcoholic beverages have contributed to metabolic diseases such as hypertension, hypercholesterolaemia, overweight and diabetes which may lead to cardiovascular diseases (3, 4).

Ginseng is a type of drug that has been used in Asian countries for thousand years. According to Qi et al. (5), ginseng has been used widely in the United States of America due to its anti-ischemic, anti-arrhythmic and anti-hypertensive properties, especially for patients who suffer from cardiovascular diseases. Ginseng contains a main bioactive component known as ginsenosides or also known as saponins (5). There are different types of ginsenosides, can exist in both hydrophobic and hydrophilic forms of ginseng, depending on their structure (6). The properties of ginsenoside such as antioxidant, antiplatelet, anti-inflammation and etc, make ginseng a suitable drug for human body administration (7, 8).

While poly(lactic-*co*-glycolic acid) (PLGA) is a biocompatible promising polymer being used in biomedical field that has caught numerous researcher's attention. Poly(lactic-co-glycolic acid) can be used for encapsulation of various types of drugs such as hydrophobic and hydrophilic drugs. Since the degradation of PLGA can be controlled by altering the ratio of lactic acid to glycolic acid, it is suitable for the application of controlled drug delivery (9, 10). Due to its properties of biodegradability and biocompatibility, PLGA has been approved by the Food and Drug Administration (FDA) for its administration in the human

body. Poly(lactic-*co*-glycolic acid) has been also studied for hybridization with other materials (11).

Polyaniline (PANI) is a type of polymer which can be prepared through an oxidative polymerisation of aniline. Polyaniline can exists in both conductive and non-conductive states depending on the level of oxidation that it undergo (12). The properties of PANI includes electroconductivity, able to crystallise, has anticorrosion properties, etc (13-15). Since the heart consists of myocytes which responses to electrical impulses, the electroconductivity property of PANI is important to ensure formation of electrical syncytium with the surrounding myocardium (16).

Therefore, in this study, ginseng was encapsulated inside PLGA/PANI microcapsules with the purpose to support drug delivery in cardiovascular area. The ginseng encapsulated PLGA/PANI microcapsules were characterised through chemical composition and morphological analyses of attenuated total reflectance-Fourier-transform infrared spectroscopy (ATR-FTIR) and scanning electron microscopy (SEM). The efficiency of encapsulation was then validated through the fluorescence analysis by viewing the stained ginseng under an inverted fluorescence microscope.

MATERIALS AND METHODS

Materials

Poly(lactic-*co*-glycolic acid) with a lactide/glycolide (85:15) and inherent viscosity (0.63 dL/g) was purchased from LACTEL Absorbable Polymers, UK. Ginseng extract was supplied by Dalian Hongjiu Biotech, China. A fully hydrolyzed polyvinyl alcohol (Mw=30,000) and polyaniline (PANI) (Mw=20,000) was acquired from Sigma Aldrich, USA and dichloromethane (DCM) was obtained from Merck KGaA, Germany.

Sample Preparation

Poly(lactic-*co*-glycolic) acid microcapsules were prepared using a standard double emulsion method. A total of 400 mg of PLGA and a variation composition of PANI (0.5, 1.0 and 1.5 mg) were dissolved in 10 mL of dichloromethane (DCM) at room temperature, separately. An amount of 30 mg of ginseng was dissolved in 1 mL of distilled water at room temperature. Then, 1.25% polyvinyl alcohol (PVA; MW 146,000-186,000) solution was prepared by dissolving 0.75 g of PVA in 60 mL of distilled water at 80.C. The dissolved PLGA and ginseng solution were mixed together and emulsified with a homogeniser for 6 minutes at 20,000 rpm in an ice water bath to obtain a water-oil (w/o) emulsion. The resulting w/o emulsion was injected drop by drop into the dissolved 60 mL of 1.25% w/v PVA solution under stirring condition. The solution was further homogenised for 10 minutes at 20,000 rpm in an ice water bath to obtain a water-oil-water (w/o/w) emulsion. The DCM was then evaporated by stirring the homogenised solution at room temperature overnight (18 hours) at 400 rpm. The microcapsules were isolated by centrifugation at 10,000 rpm for 30 minutes at 4°C. The sediment was finally washed once with 0.9% sodium chloride (NaCl) and twice with distilled water. The resulting encapsulated microcapsules were lyophilised and stored at 4°C prior to use. The PLGA microcapsules encapsulated with stained ginseng was prepared by replacing the ginseng with stained ginseng. The stained ginseng was prepared by dissolving two different amounts of crystal violet (50 µL and 500 µL) in 1 mL of distilled water and stirred for 2 hours.

Sample Characterization

The composition of microcapsules was determined by ATR-FTIR (Nicoler iD5, Thermo Scientific, USA) using ZnSe Crystal at 4 cm^{-1} resolution. The spectra were obtained within 500 - 4000 cm^{-1} frequency range and 32 average scans.

The surface structure and morphology of the microcapsules were then viewed and captured using tabletop SEM (Hitachi, TM300, Japan), viewing at 15 kV accelerating voltage under magnifications of 1000x and 3000x. A thin layer of gold was sputtered on the microcapsules to avoid charging under electron beam. The size of microcapsules was then mapped using imageJ software (NIH, MD, USA).

Encapsulation Efficiency Test

The encapsulation of ginseng inside the microcapsules was clarified and validated through a fluorescence analysis. In this analysis, the ginseng was stained using a crystal violet dye by stirring the mixture of ginseng and crystal violet dye for 2 hours. Two staining observations were performed using different volumes of crystal violet dye: 50 and 500 µL. For the first observation, 500 µL of crystal violet was added to 500 µL of distilled water while for the second observation, 950 µL of crystal violet was added to 50 µL of distilled water. Both stained ginseng solution were used for the preparation of ginseng encapsulated inside PLGA/PANI microcapsules. Finally, the stained ginseng encapsulated inside PLGA/PANI microcapsules were observed under an inverted fluorescence microscope (Carl Zeiss Axio Vert A1, USA) with an excitation at 590 nm wavelength.

RESULTS

ATR-FTIR Analysis

The ATR-FTIR spectra of all samples are shown in Figure 1. The presence of PLGA, ginseng and PANI were determined through the identification of the functional groups from the spectra. The presence of PLGA can be seen on the microcapsules through the appearance of sharp carbonyl (C=O) peaks and C–O peaks. The C–H peaks also shows the presence of PLGA on microcapsules. The incorporation of ginseng was determined through the emergence of broad peaks of hydroxyl group (O–H)

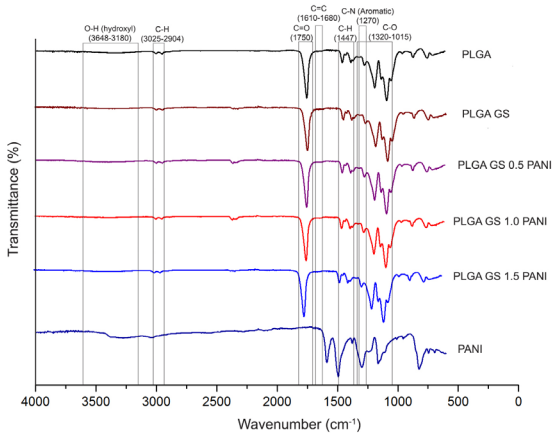

Figure 1: ATR-FTIR spectra of PLGA microcapsules, ginseng encapsulated inside PLGA/PANI microcapsules and PANI. All the microcapsules samples were analysed using ATR-FTIR spectroscopy method. The chemical composition of the samples were determined by the emergence of the peaks from the graph.

and also small peaks of C=C. Detection of PANI can be observed through the appearance of C–N peaks.

Scanning Electron Microscope Analysis

The SEM images visualised successful formation of microcapsules with a spherical shape, non-porous and smooth wall surface as shown in Figure 2. From the SEM images, the diameter of the microcapsules was determined using ImageJ software. The PLGA microcapsules has an average diameter of 3.14±1.87 µm whereas the ginseng encapsulated inside PLGA microcapsules has an average diameter of 2.76±2.03 µm. Further reduction of microcapsule size was recorded on the ginseng encapsulated inside PLGA with 0.5 mg, 1.0 mg and 1.5 mg of PANI with an average diameter of 2.21±1.52 µm, 2.02±1.51 µm and 1.98±1.30 µm, respectively.

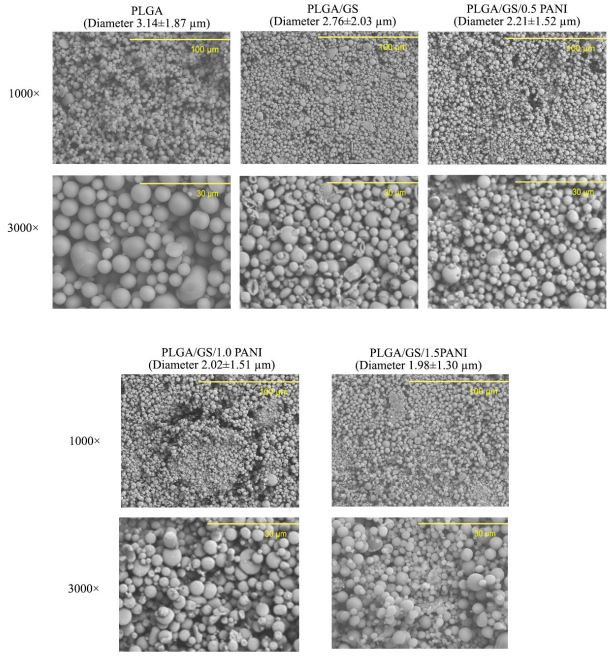

Figure 2: SEM images of PLGA microcapsules, ginseng encapsulated inside PLGA and PLGA/PANI microcapsules. All of the microcapsules samples were observed under scanning electron microscope to identify its structure and shape. From the scanning electron microscope, microcapsules with smooth and round surface were observed.

Histogram Analysis

Through histogram analysis, the distribution of the size of microcapsules can be determined. Figure 3 shows the histograms of the microcapsules distribution. From Figure 3, it can be seen that the diameter of the microcapsules was distributed in the range of 1 µm to 9 µm where most of the PLGA microcapsules have the diameter of 4 µm.

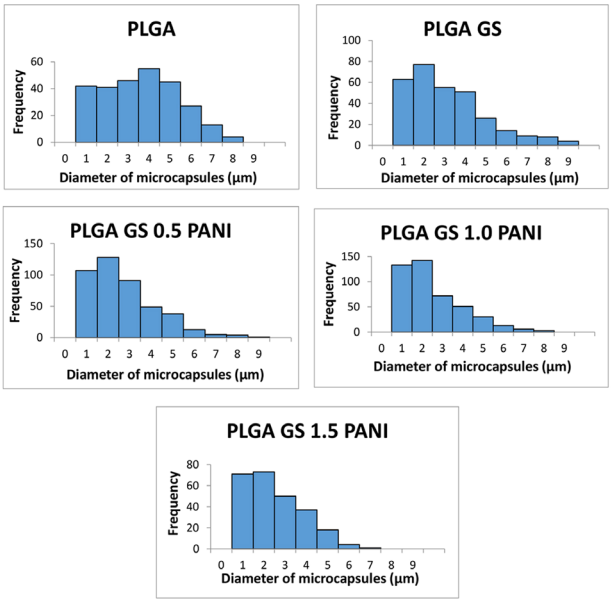

Figure 3: Histogram analysis of microcapsules size. A histogram was plotted to determine the distribution of the size of the microcapsules. From the histogram plotted, the size of the microcapsules followed the normal distribution.

Encapsulation Efficiency Analysis

The encapsulation efficiency was determined by staining the ginseng with crystal violet dye when encapsulating the ginseng in the PLGA microcapsules and observed using a fluorescence microscope. The appearance of tiny blue spots shows the presence of ginseng encapsulated in the PLGA microcapsules. Figure 4 (a) shows that the staining of the crystal violet dye distributed all over the microcapsule surfaces while in Figure 4 (b), the staining of crystal violet dye was seen as tiny blue spots.

DISCUSSION

From the ATR-FTIR spectra, the appearance of sharp carbonyl (C=O) peaks can be observed at 1750 cm-1 and C–O peaks in between, 1015 cm-1 and 1320 cm-1 indicates the presence of PLGA on all the microcapsules. The C–H peaks at 2904 cm-1 to 3025 cm-1 and 1345

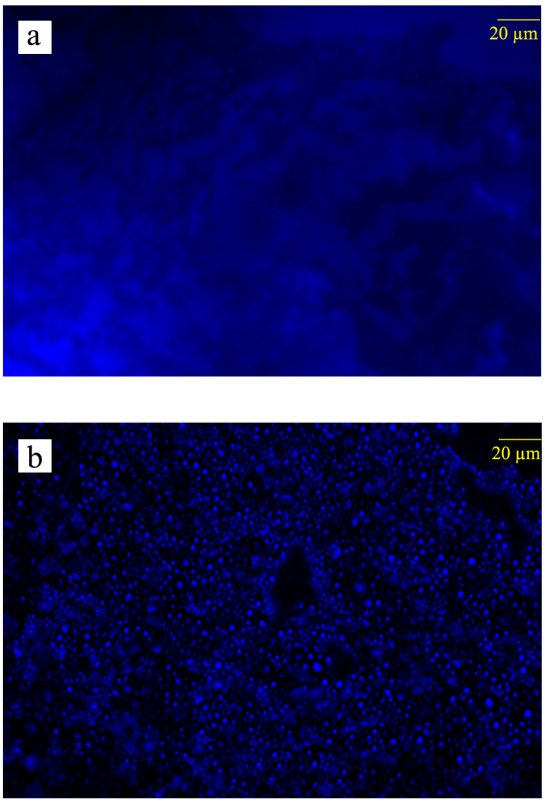

Figure 4: Fluorescence observations with (a) 500 µL crystal violet and (b) 50 µL crystal violet. Crystal violet dye was used to determine the encapsulation of ginseng inside the microcapsules. The encapsulation of the ginseng can be seen through the presence of tiny blue spots.

cm-1 to 1487 cm-1 also annotated the presence of PLGA on microcapsules. The incorporation of ginseng was determined through the emergence of broad peaks of hydroxyl group (O–H) between 3180 cm-1 and 3648 cm-1 and also small peaks of C=C in the range of 1610 cm-1 and 1680 cm-1. Detection of PANI can be observed through the appearance of C–N peaks at 1270 cm-1 (17), making the C–N peaks overlapping with the range of C–O bond.

From the SEM result, the average diameter of the PLGA microcapsules was 3.14±1.87 µm while the ginseng encapsulated inside PLGA microcapsules was 2.76±2.03 µm. When different amount of PANI (0.5 mg, 1.0 mg and 1.5 mg) was added, the average diameter of the ginseng encapsulated inside PLGA microcapsules becomes 2.21±1.52 µm, 2.02±1.51 µm and 1.98±1.30 µm respectively. It can be seen that, as the amount of PANI increased, the average diameter of the microcapsules decreased. The shrinkage of the microcapsules might be due to the increase in electroconductivity property as the PANI possessed electroconductive characteristic that cause repulsion of the surface charge (18). However, there were still no facts stating how the change in electroconductivity will affect the size of microcapsule. More research and findings need to be conducted to determine how electroconductivity affects the size of microcapsules.

From the histogram analysis, the diameter of the microcapsules was distributed in the range of 1 µm to 9 µm. The histogram of the PLGA microcapsules appears to follow the shape of a normal distribution curve where most of the PLGA microcapsules have the diameter of 4 µm. When ginseng was introduced into the PLGA microcapsules, the normal distribution curve was shifted to the left where the most tabulated diameter of ginseng encapsulated inside PLGA microcapsules was 2 µm showing the reduction in the size of microcapsules. Since ginseng is classified as a type of high water-soluble drugs, it causes the reduction in microcapsules size during the encapsulation (19).

Addition of different concentrations of PANI (0.5 mg, 1.0 mg and 1.5 mg) caused the histograms to shift further to the left, decreasing the size of microcapsules even more due to the increase in electrical conductivity of the microcapsules (18). However, the highest frequency of the diameter of ginseng encapsulated inside PLGA/PANI microcapsules still remains at 2 µm but with decreasing in the average diameter of the microcapsules as the concentration of PANI increased. Hence, from the histogram analysis, it can be further justified that the size of the microcapsules was decreased when PANI was added.

From the plotted histogram, information regarding homogeneity of the microcapsule size can also be extracted. The diameter of the PLGA/PANI microcapsules fell in the range of 1 µm to 7 µm compared to the diameter of PLGA microcapsules and ginseng encapsulated inside PLGA microcapsules with the range of 1 µm to 9 µm. Hence, this depicts the sign of increase in homogeneity of the PLGA size as more PANI was added.
In Figure 4 (a), the staining of the crystal violet dye was distributed all over the microcapsule surfaces while in Figure 4 (b), the staining of crystal violet dye was distributed as tiny blue spots, featuring the encapsulated drug. The distribution of crystal violet dye all over the microcapsule surfaces was due to the utilisation of excess crystal violet dye that also stained the PLGA particles when the stained ginseng solution was mixed with the dissolved PLGA solution. The distinctive layer between the stained ginseng solution and dissolved PLGA solutions was absent as shown in Figure 4(a), showing that the PLGA particles were stained with crystal violet dye.

In Figure 4 (b), the ginseng stained with crystal violet dye was appeared in tiny blue spots, indicated that the PLGA was not stained with crystal violet dye during the mixing of stained ginseng solution and the dissolved PLGA solution. There was formation of two distinctive layers of stained ginseng solution and dissolved PLGA solution as shown in Figure 4 (b). The presence of tiny

blue spots on the fluorescence image demonstrated the encapsulation efficiency of the stained ginseng within the PLGA microcapsules.

CONCLUSION

As a whole, ginseng had been successfully encapsulated inside PLGA/PANI which was identified through FTIR, SEM and fluorescence analysis. The degradable microcapsules would be beneficial in delivering therapeutic effects of ginseng and PANI for cardiovascular application.

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