PREPARATION AND CHARACTERIZATION OF POLYLACTIC ACID/PHYCOCYANIN-ALGINATE COMPOSITES FOR COSMETIC PATCH

BY

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ABSTRACT

Patches has recently emerged and attracting more attention for its versatility in many areas such as cosmetic, pharmaceutical and medical. Patches can either be used to administer selected drug to skin or deliver some beneficial ingredients for cosmetic purposes. With that, as polymer is used as the matrix for patches, the polymer selected must be non-toxic, have adhesive property and non-irritative to the skin. Currently, synthetic polymer had been used as the matrix. However, as time passes, people are more concern with the environment, therefore biopolymer is chosen over synthetic polymer as they are degradable and also safe to use. Among all biopolymers, polylactic acid (PLA) was chosen for its many advantages such as having similar properties with conventional plastic and non-toxic. Our invention combines the use of Polylactic Acid (PLA), spirulina extract (phycocyanin) as active ingredient due to its anti-oxidant, anti-inflammatory and bioactive compounds. Alginate was also incorporated into the patch to help improve the properties of the active layer for rapid release of active ingredient. For the first part of this research, a preliminary study was first carried out in order to identify the cytotoxic effect of the raw material, phycocyanin on skin cell. From the result obtained, it was observed that phycocyanin gives no cytotoxic effect to the skin and is deemed safe to be used in the research. The second part of the research was divided into two section, where for the first section fabrication method was selected between two method which were dip cast and roll over method. From the three tests conducted which was tensile test, releasing test and scanning electron microscopy, sample prepared by roll over method gives the best mechanical and releasing properties and was selected for further studies. For the second part, concentration of PLA was varied to study the effect of PLA film prepared with different concentration on the flexibility and releasing properties of sample formed. The concentration of PLA was varied from 7, 10, 13, 15 to 17% (w/v) and PLA film prepared at 13% (w/v) gave the highest elongation at break and moderate releasing properties which were 9.8 % and 0.6 respectively. For the third part of the study, optimization of preparation conditions (stirring time, temperature and concentration of phycocyanin/alginate) were caried by using one factor at a time (OFAT). Two responses were recorded for each parameter which were elongation at break and absorbance (optical density (OD)). FT-IR analysis was also done to further analyse the composites and results obtained showed that phycocyanin and alginate were fully released after releasing test. Other than that, shiftment in band was observed due to formation of phycocyanin and alginate complex when mixed together. From the tests conducted, PLA/phycocyanin-alginate composite prepared at 20 ^{o}C for 20 hours with a phycocyanin/alginate ratio of 4:6 at 2.5% (w/v) concentration gave the best properties in terms of flexibility and releasing properties.

خلاصة البحث

ظهرت الرُقع في الآونة الأخيرة وجذب تنوعها المزيد من الاهتمام في العديد من المجالات مثل مستحضرات التجميل و مجالات الأدوية والطب. يمكن استخدام البقع في أدوية البشرة، أو في تقديم بعض المكونات المفيدة لأغراض التجميل. مع ذلك ، فإنه عند استخدام البوليمر كمصفوفة للبقع ، يجب أن يكون البوليمر المحدد غير سام وله خاصية لاصقة وغير مُهيج للجلد. حاليًا ، يتم استخدام البوليمر الاصطناعي كمصفوفة. ومع ذلك ، مع مرور الوقت ، يصبح الناس أكثر اهتمامًا بالبيئة ، وبالتالي يتم اختيار البوليمرات الحيوية على البوليمرات الصناعية لقابليتها للتحلل ولكونها أيضًا آمنه من حيث الاستخدام. من بين جميع البوليمرات الحيوية ، تم اختيار متعدد حمض اللاكتيك (PLA)لمزاياه العديدة كوجود خصائص مماثلة مع البلاستيك التقليدي وغير السام .أما بالنسبة للعنصر النشط ، فقد تم اختيار طحالب سبيرولينا بشكل خاص لفوائدها للجلد: وذلك لكونها مضادة للأكسدة ، مضادة للالتهابات، وتحتوي على العديد من المركبات النشطة بيولوجيا. في هذه الدراسة ، تم استخدام متعدد حمض اللاكتيك كمصفوفة وطحالب السبيرولينا كنعصر نشط للرقعة. تم دمج الآجينيات مع السبيرولينا للمساعدة في تصنيع مصفوفة متعدد حمض اللاكتيك. بالنسبة للجزء الأول من هذا البحث ، تم إجراء دراسة أولية لتحديد التأثير السام للخلايا الخاص بالمواد الخام و مستخلص السبيرولينا (Phycocyanin) على خلايا الجلد. وبناء على النتائج ، لوحظ أن مستخلص السبيرولينا لا يعطي أي تأثير سام للخلايا على الجلد ويعتبر آمنًا ليتم استخدامه في البحث. تم تقسيم الجزء الثاني من البحث إلى قسمين ، حيث تم اختيار طريقة تصنيع القسم الأول من ثلاثة طرق، وهي الغمس ، الدحرجة (RA) والتجفيف لمدة يوم ومن ثم الدحرجة (1DAY). من بين اختبارات الثلاثة التي تم إجراؤها- اختبار الشد ، واختبار الإفراز والمسح الإلكتروني المجهري- أعطت العينة المجهزة من خلال الدحرجة *(RA)* أفضل الخصائص الميكانيكية وخصائص الإفراز وتم اختيارها لإجراء المزيد من الدراسات. بالنسبة للجزء الثاني ، تم أخذ تركيزات متنوعة من فيلم متعدد حمض اللاكتيك لدراسة تأثير فيلم متعدد حمض اللاكتيك على المرونة وخصائص الإفراز لدى العينة المشَكلة. كان تركيز متعدد حمض اللاكتيك يتراوح بين 7 ، 10 ، 13 ، 15 لنسبة 17%، أما فيلم متعدد حمض اللاكتيك لنسبة 13٪ أعطى أعلى نتيجة استطالة عند الاستراحة وخصائص إفراز معتدلة وهي 9.8٪ و 0.6 على التوالي. وأخيرًا بالنسبة للجزء الأخير من الدراسة ، تم تحسين ظروف التحضير (وقت التحريك ودرجة الحرارة وتركيز مستخلص السبيرولينا / الآجينات) باستخدام عامل واحد في وقت واحد (*OFAT*) .تم تسجيل استجابتين لكل عامل -الاستطالة عند الاستراحة والامتصاص (الكثافة البصرية -OD). تم إجراء تحليل FT-IR لتحليلات أوسع للتركيبات. بناءً على الاختبارات التي أجريت ، أعطى مركب PLA /آلجينات-السبيرولينا والذي تم تحضيره في 20 درجة حرارة مئوية لمدة 20 ساعة مع تركيز سبيرولينا /آلجينات في 60/40 أفضل الخصائص من حيث المرونة وخصائص الإفراز.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology Engineering)

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

ASTM	American Society for Testing Material
AA	Ascorbic acid
CO_2	Carbon dioxide
DOE	Design of experiment
DMEM	Dulbecco's modified eagle medium
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
HPMC	Hydroxypropylmethylcellulose
kN	kilo Newton
Mw	molecular weight
mL	mililiter
µL	Microliter
µm	Micro meter
MTT	3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NF	Nanofiber
nm	nanometer
OD	Optical density
OFAT	One factor at a time
OLAs	Oligomers of lactic acid
PBS	Phosphate-buffered saline
PET	Polyethylene Terepthalate

PVC	Polyvinyl chloride
PS	Polystyrene
PLA	Polylactic Acid
PMMA	Poly methyl methacrylate
PLGA	Poly(D,L-lactide-co-glycolide) acid
PCL	Polycaprolactone
PGA	Polyglycolide
PHA	Poly(hydroxyalkanoates)
PP	Polypropylene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
RA	Right after
RPM	Revolution per minute
SEM	Scanning Electron Microscopy
T_g	Glass transition temperature
T_m	Melting temperature
TiO_2	Titanium dioxide
T_{cc}	Cold crystallization temperature
USFDA	United State Food and Drug Administration
^oC	Celcius
VCO	Virgin coconut oil

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

In the past, patches are usually made up of cotton wool or bandages in order to protect the targeted area from surrounding. However in the usage of cotton, as the outer part become moistened to activate the patch then the protection is lost as the patch is vulnerable to bacterial contamination (Sofokleous et al., 2013). The usage of fabrics such as cotton wool and gauze then no longer serve the purpose to be use as a patch. Another thing to be taken into consideration is that when using these fabrics as a patch, moisture will loss with time and this will cause discomfort when removing it (Elsner & Zilberman, 2009; Cui et al., 2010).

Patches have high in demand of usage for its versatility of applications such as cosmetic (Mahdavi et al., 2006; Nechushtai, et al., 2001; Mohammadi et al., 2014), pharmaceutical (Venkateswaran et al., 1996; Schroeder et al., 2006; Jung et al., 2018) and medical (Ahmad et al., 2009; Sofokleous et al., 2013; Nayak & Gupta, 2017). As patches works by delivering active compounds or medication, it must possess adhesive material and properties for the patches to work within a desired expanse of time (Byeon et al., 2017). Other than that, the patches need to be fabricated with active ingredients that will give beneficial effect to the skin such as anti-oxidant property (Romay et al., 1998) and anti-inflammatory effect (Ammala et al., 2013).

Nowadays, consumers are demanding for more effective product that is not only good for the appearance of their skin but also the health of their skin. Therefore, various versatile cosmetic patches with different active ingredients have been introduced (Park et al., 2015; Herman, 2012). As invention evolve with time, researchers have now found an alternative for the fabrics used in the past. These days, patches are usually made up of polymer to support their matrix where the active ingredients are imbedded. As the final product of a cosmetic patches need to be transparent and not harmful to the skin, the polymer used as a matrix needs to be ensured safe for contact with human skin and approved by United State Food and Drug Administration (USFDA) (Kwak, Jeong, & Suh, 2011).

Polylactic acid (PLA), a biodegradable polymer in particular stands out for this purpose compared to other types of biodegradable polymers. As PLA is produced from agriculture crop, PLA is deemed safe by the FDA (Jamshidian et al., 2010). PLA is also distinctive from other polymers as it degrades naturally into the environment and possesses similar properties to the conventional plastic such as polystyrene and polyethylene (Silva, 2011). PLA has been used extensively in variety of applications such as food packaging, automotive and medical (Jamshidian et al., 2010; Bulota & Budtova 2015; Wu et al., 2015).

PLA was first discovered by Wallace Carothers in 1932. They were mainly produced from basic materials like sugarcane, corn, whey or cellulose biomass. The basic materials are first processed into lactic acid via bacterial fermentation. Lactic acid is the basic monomer of polylactic acid. According to Hassan and Balakrishnan (2013), preparation of PLA can be done in three ways. The first is by ring-opening polymerization (ROP) of the dehydrated ring-formed dimer, second by polycondensation and manipulation of the equilibrium between lactic acid and polylactide by removal of reaction water using drying agents, or third by polycondensation and linking of lactic acid monomers.

As mentioned previously, the polymer used for patches need to be impregnated with active ingredients for it to be beneficial to the skin (Ravanan et al., 2016). The active ingredients can be from many type of sources such as fruits, plants, aquatic and many more, as long as they possess ingredients that are good for skin (Viyoch et al., 2003; Yhirayha et al., 2014). Many researchers have used spirulina extract as their active ingredients for application in both cosmetic patches and medical patches as spirulina contains many kinds of bioactive compound that works as an anti-oxidant and has anti-inflammatory effect (Byeon et al., 2017; Jung et al., 2013). Hence in this study, a patch comprising of PLA as its matrix and spirulina extract (phycocyanin) as the active ingredient was developed based on its benefits.

1.2 PROBLEM STATEMENT

A patch usually comprises of a backing layer (polymer) and active layer (active ingredient) (Baker & Heller, 1989). Though there had been many studies done in the past on using drugs and medications as active ingredient, natural compounds actually offers more biological effects (Jensen et al., 2016). For example, phycocyanin bioactive ability had been proven from its many applications such as in skin cream (Gunes et al., 2017), scaffold (Jung et al., 2013) and dressing film (Kim et al., 2018). Although phycocyanin is an FDA approved compound, the maximum amount known to be cytotoxic to skin cell is unknown. Thus, cell cytotoxicity test is the most suitable test to identify the cytotoxic effect of phycocyanin on skin cell.

Next, to produce a patch, a suitable and efficient fabrication method is needed. Electrospinning is a conventional method that can produce a nanofiber with uniform diameter for imbedding active compound, however the method consumes a high amount of voltage (Heydarkhan et al., 2008). Solvent casting on the other hand is a more energy and cost saving method compared to electrospinning. Furthermore, patch produced by solvent casting method exhibit colour uniformity and smooth transparent surface (Nisa et al., 2016). Though, a patch with uniform thickness is hard to produce with only solvent casting method. Thus, in this research, new methods had been developed with the basis of solvent casting method in order to form a patch with desired thickness. Therefore, two methods were proposed which were dip cast and roll over method.

Thickness of patch is one of the factors affecting the properties of a patch. Other than adjusting the thickness when fabricating the patch, concentration of PLA also plays an important role on the thickness of patch produced. This is because the thickness of the patch differs according to the ratio of PLA to solvent, where a higher ratio of PLA to solvent will produce a thicker patch. Nevertheless, not many studies had been done on varying the concentration of PLA. In this research, the concentration of PLA had been varied according to the ranges selected to study the effect of different PLA layer thickness on the properties of patch produced.

1.3 IMPORTANCE OF STUDY

The development of transdermal cosmetic patches that are biocompatible with skin is important in order to avoid skin irritation and to control release of active ingredients. The usage of biopolymer in this study is imperative as the polymer used for the patches not only need to have adhesive properties, but also must be biocompatible with skin and can release active ingredients to the skin effectively. Moreover, as a biopolymer PLA is used, the worries of the product endangering the environment can be reduced as biopolymer is known to degrade naturally into the environment.

For the active ingredient, spirulina extract (phycocyanin) is selected for its many advantages such as possessing anti-oxidant and anti-inflammatory properties. Spirulina is a photosynthetic microorganism and is from the diversified microalgae group, and it stood out because of its high protein content and vitamins as well as presence of essential fatty acids (Costa et al., 2017). Spirulina had been extensively studied and consumed as health supplement. In this study we focused more on its benefits and contributions in cosmetic patch in order to expand the application of phycocyanin and utilize its advantages.

1.4 OBJECTIVES

The objectives of the research are as follow:

1. To characterize the phycocyanin content and the biosafety of phycocyanin cell cytotoxicity study
2. To determine the most suitable method for fabrication (between dip cast and roll over methods) and then select the best concentration of PLA to be use as a matrix foundation for the patch
3. To optimize the preparation conditions (ie: stirring time, temperature and phycocyanin/alginate ratio) of PLA and phycocyanin/alginate composite for maximum extract release

1.5 SCOPE OF STUDY

In this study, a preliminary test was first conducted on the raw material, spirulina extract (Phycocyanin) for cell cytotoxicity test. After it was confirmed that phycocyanin gave no cytotoxicity to the cell, preparation method selection was then conducted. The preparation method of the patch was selected from two methods, dip cast or roll over method. Analysis for the patch obtained from these methods were done by using tensile, releasing and scanning electron microscopy (SEM) tests and then based on the analysis, roll over method was selected. Then, selection of suitable PLA concentration was also conducted in order to study the effect of PLA concentration on releasing properties and flexibility of patch produced (Range: 1 to 23 wt%). PLA/Phycocyanin-Alginate composite was then fabricated using roll over method and suitable PLA concentration and the stirring time (Range: 4 to 24 hours), temperature (Range: 20 to 45 ^{o}C) and phycocyanin/alginate ratio (Range: Range: 9:1 to 1:9). Lastly, the properties of PLA/ Phycocyanin -Alginate composite were evaluated by releasing test to study the release properties, scanning electron microscopy (SEM) to study the morphological properties, FT-IR to observe the changes in the chemical structure and chain functional group and tensile testing to evaluate the elongation at break, tensile strength and Young's Modulus.

1.6 THESIS ORGANIZATION

This thesis consists of five chapters, Chapter One started with a brief background about the research including the issue of using non-degradable polymer as the main matrix for a cosmetic patch and biopolymer as an option to substitute non-degradable polymer. Other than that, this chapter also include a brief introduction to PLA and phycocyanin as the main material for this research. In addition, problem statement, objectives, scope and importance of study were described in this chapter.

Chapter Two begin with an introduction to cosmetic patch and the materials currently used as the main matrix, with PLA introduced as the biopolymer to substitute conventional plastic used nowadays. This chapter also includes literature review on biodegradable polymer, fabrication method and preparation conditions for the cosmetic patch and active ingredients currently used in cosmetic industry.

Chapter Three described in details the materials and equipment used in this research. The experimental procedures were followed and described in details starting from the preliminary study on the raw material, fabrication method, optimization study on preparation conditions and characterization test involved.

Chapter Four comprises of results and discussion of this research, starting with the preliminary test on the cytotoxicity study for phycocyanin. Fabrication method was selected from dip cast or roll over method and the concentration of PLA and preparation conditions (phycocyanin/alginate ratio, stirring time, temperature) were optimized. Characterizations were done on the composites to study the mechanical, releasing and morphological properties of the composites. FT-IR was also conducted to observed any changes in chemical properties of the composites.

Finally, in Chapter Five work done in the research was conclude and recommendations to improve this study in the future was outlined.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

A cosmetic patch is defined as an adhesive patch or film which is place above the skin to deliver a certain amount of active ingredients (Byeon et al., 2017). Delivering of active ingredients through dermal layer is better, safe and pain free, convenient, cheaper, and the delivery of active ingredients to the skin can be stopped simply just by removing the patches (Duppala et al., 2016). The material used to make the patches can either be from synthetic polymer (Zaman et al., 2017) or biopolymer (Suksaeree et al., 2018).

Polymer is the main foundation (matrix) for a transdermal patch. The polymer used need to be stable and non-reactive, easily fabricated, exhibit excellent properties and should be able to release active ingredients consistently (Duppala et al., 2016). As mentioned above, the polymer can be either synthetic or natural based. Synthetic polymers are polyvinylchloride, polyethylene, polyvinyl alcohol and polypropylene while some example of biopolymer polymer use are cellulose derivatives, chitosan, poly caprolactone (PCL), polylactic acid (PLA) and polyglycolide (PGA) (Duppala et al., 2016).

As people are becoming more concern with environmental issues, the use of synthetic polymer raised an issue as they are non-degradable and can cause major pollution. Therefore, bio-derived polymer was introduced as substitute to conventional petroleum based polymer. To add, these polymers are also fully biodegradable, which discard the need for recycling as they will decompose in the soil. The degradation is