ท่อพอลิไซลอกเซนดัดแปรผิวที่มีสมบัติปล่อยยาแบบควบคุม

นางสาวจิรัชญา โมกขพันธ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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SURFACE-MODIFIED POLYSILOXANE TUBE WITH CONTROLLED DRUG RELEASE PROPERTY

Miss Jiratchaya Mokkaphan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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ในการรักษาโรคทางโสตศอนาสิก จะใช้ท่อพอลิไดเมทิลไซลอกเซน ซึ่งเป็นวัสดุที่ใช้ทางการแพทย์ เพื่อขยายหลอดลมคอของผู้ป่วย โรคปอดอุดกั้นเรื้อรังหรือหลอดลมตีบ และช่วยลดสารคัดหลั่งจากปอดทำให้หายใจได้สะดวกขึ้น อย่างไรก็ตาม มักมีการติดเชื้อได้จากการใส่ท่อช่วยหายใจ ดังนั้นการใช้ท่อพอลิไดเมทิลไซลอกเซนซึ่งมีฤทธิ์ต้านการอักเสบ น่าจะช่วยแก้ปัญหานี้ ในงานวิจัยนี้ บูดีโซไนด์ถูกนำมาห่อหุ้มด้วยเอ ทิลเซลลูโลสโดยวิธีการแทนที่ตัวทำละลาย อนุภาคนาโนพอลิเมอร์ที่ได้ มีบูดีโซไนด์อยู่ 33.26 ± 0.23% (w/w) กระบวนการที่ใช้มีประสิทธิภาพการห่อหุ้ม 49.83 ± 0.11% จากนั้นอนุภาคที่มีบูดีโซไนด์กักเก็บอยู่ภายในจึงถูกกราฟท์ลงบนท่อพอลิไซลอกเซนดัดแปรผิว โดยขั้นแรกจะปรับปรุงพื้นผิวของท่อพอลิไซลอกเซนด้วย กรดคาร์บอกซิลิก จากนั้นกราฟท์อนุภาคที่มีบูดีโซไนด์กักเก็บอยู่กับหมู่ฟังชันก์บนผิวท่อพอลิไซลอกเซน และตรวจสอบการปล่อยบูดีโซไนด์จากท่อพอลิไซลอกเซนดัดแปรผิวในหลอดทดลองที่อุณหภูมิ 37 องซาเซลเซียส , pH 5.8 ท่อพอลิไซลอกเซนแสดงการปล่อยบูดีโซไนด์จากผิวเป็นเวลานานมากกว่า 10 วัน ดังนั้นท่อพอลิไซลอกเซนที่สามารถปลดปล่อยยาต้านการอักเสบได้ จึงถูกสร้างขึ้นในโครงการวิจัยนี้

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

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JIRATCHAYA MOKKAPHAN : SURFACE-MODIFIED POLYSILOXANE TUBE WITH CONTROLLED DRUG RELEASE PROPERTY. ADVISOR : ASSOC. PROF. SUPASON WANICHWECHARUNGRUANG, Ph.D., 68 pp.

In medication of otolaryngology, tracheostomy tube, which is made from polydimethylsiloxane (PDMS), is used in patients who are suffered from chronic obstructive pulmonary disease (COPD), to provide an airway and reduce secretions from the lung. However, intubation usually causes infection. Here we show the fabrication of PDMS tube with anti-inflammatory activity. Budesonide was encapsulated into ethylcellulose (EC) nanoparticles by solvent displacement process. The obtained nanoparticles possessed 33.26 ± 0.23 % (w/w) of budesonide loading and the process gave 49.83 ± 0.11% of encapsulation efficiency. The budesonide-loaded particles were grafted onto PDMS tube by first functionalizing the PDMS with carboxylic acid functionality and then coupling the budesonide-loaded particles with the functionalized PDMS surface. *In vitro* release of budesonide from the modified PDMS tubes at 37°C, pH 5.8 showed prolonged release for more than 10 days. Therefore, the PDMS tube that could slowly release an anti-inflammatory drug, budesonide, could be fabricated successfully.

Field of Study: Petrochemistry and Polymer Science Student's Signature .....

Academic Year 2012 Advisor's Signature .....

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LIST OF ABBREVIATIONS

cm	centimeter
CLF M	confocal laser scanning fluorescence microscopy
°C	degree celsius
DMF	dimethyl formamide
DLS	dynamic light scattering
EE	encapsulation efficiency
EtOAc	ethyl acetate
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
g	gram
Hz	hertz
HPLC	high performance liquid chromatography
h	hour
HOBt	hydroxy benzotriazole
kV	kilovolt
LC	loading capacity
µL	microliter
µm	micrometer
mA	milliampere
mg	milligram
ml	milliliter
mm	millimeter
mM	millimolar
mmol	millimole
mtorr	millitorr
mV	millivolt
min	minute
ε	molar absorptivity
Mw	molecular weight
nm	nanometer

ppm	parts per million
%	percent
PDI	polydispersity index
SEM	scanning electron microscope
sec	second
cm^2	square centimeter
TEM	transmission electron microscope
UV	ultraviolet
cm^{-1}	unit of wavenumber
v	volume
λ	wavelength
w	weight

CHAPTER I

INTRODUCTION

1.1 Overview of tracheal tubes and applications for COPD

Tracheal tubes can be used to assist breathing in patients receiving surgery or in intensive care, there are used widely in patients with chronic obstructive pulmonary disease (COPD). Tracheal tubes also play an important role in aerosol delivery to ventilate patients. Nylon, Teflon, silicon, polyvinyl chloride (PVC), polyurethane (PU) and metal are used to produce tracheal tube. These materials can be devided into two categories; metal and plastic. Plastic tubes such as silicone tubes, can conform to the patients' anatomy during movement very well. On the contrary, metal tubes such as silver tubes are rigid and easily cause an irritation to the neck [1-4].

Metal tubes can be fabricated from stainless steel or silver. Silver tube is used on some COPD patients [1]. Silver-Negus and Jackson tracheal tubes are the two most commonly used silver tubes. One of the advantages of metal tubes is that they can be manufactured into very thin tubes. These thin tubes consist of the outer tube and inner tube, which can be removed and cleaned without taking out the whole tube. Silver tubes are appropriate for those requiring long-term tracheostomy because they can remain in situ for up to one month. However, silver tubes have certain disadvantages. For example, it is rigid and cannot bend along the trachea, causing uncomfortable feeling. Additionally, the unit cost is high and silver tubes are not compatible with MRI scanning; they may distort CT scan pictures of the neck, head and chest.

Figure 1.1 Inner tube and outer tube are components in metal tubes [1].

Silicone tubes made from polydimethylsiloxane (PDMS), the most commonly used polymer to manufacture tracheal tubes, are medical devices used widely in patients with COPD and tracheal stenosis to provide airway and remove secretions from the lung. Its specific mechanical properties softness with little or no tissue irritation, relatively good compatibility blood, low toxicity, non-reactive and non-allergic materials [5-9], have attracted our interest.

Figure 1.2 Tracheal tube made of silicone.

For medical practices, the tubes are usually in range 2 to 10.5 mm of internal diameter, and the patient's body size is used for determining which tubes will be selected for the patient. Because of a variety of diameters and materials, it is important for medical staff to understand and know how to choose a tube which will be compatible or appropriately fit with the patient.

In 2007, Björling et al. demonstrated, after 30 days of exposure in the trachea, the surface of tracheal tube changes because of degradation of the polymeric chains. The data indicated that the degradation of the polymeric chains was related to the biofilm formation. They also observed the least change in surface of silicone tube when compared with PVC and PU [2].

In 2007, Schrader et al. reported the male patient with symptomatic tracheal stenosis that was not responsive to surgical resection. This treatment, T-tube tracheal stent was chosen because T-tubes are benefit particularly for patients who possess

respiratory abnormality. Silicone is general material, used to make a T-tube stents. This material is smooth, good flexible and forestall binding of dried mucosa and less probable to cause damage to the tracheal mucosa. The rim are slightly curved, which decreases the amount of injury toward the trachea [7].

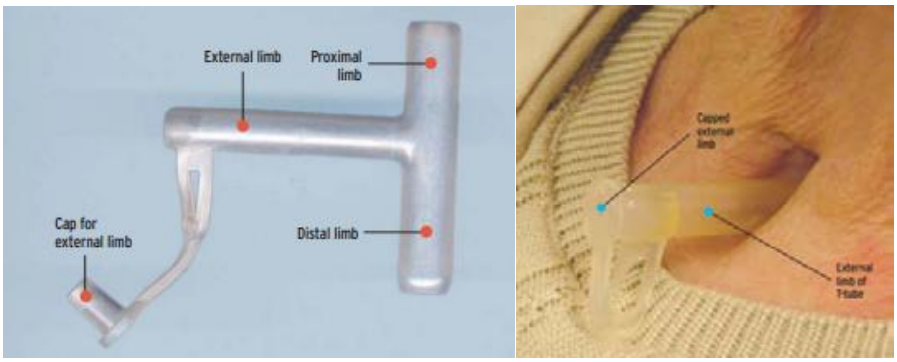


Figure 1.3 The T-Tube stent in place [7]

The most common indication for tracheotomy is patients need long-term mechanical ventilation. Patients with COPD, with poor control of airway secretions may require tracheotomy not only to prevent aspiration of particulates but also to allow their removal by airway suctioning. A tracheotomy is a surgical procedure to make an opening through the neck into the trachea (windpipe). A tube is usually placed through this opening to provide an airway and to remove secretions from the lungs [Appendix D]. This tubes are called as tracheal tube or a tracheostomy tube. Tracheal tubes are frequently used for airway management, critical care, mechanical ventilation and emergency medicine. They may also be used as a route for administration of certain medications such as bronchodilators, inhaled corticosteroids such as budesonide, and drugs used in treating cardiac arrest such as atropine, epinephrine, lidocaine and vasopressin [10-15].

However, the common problems found in using tracheal tubes are tracheal injury and infection of the device caused by contamination of the patients' airway and the infection of gram-negative bacilli so the tubes incorporate with anti-inflammatory drugs are needed [16].

In humans, normal pH values in tracheal mucosa range is 6.9–9.0. Moreover, infection can be attended by pH values as low as pH 5.8 in mucosa [17-21].

In 2002, Hunt et al demonstrated that human airway epithelial cells in vitro have biochemical evidence for glutaminas activity and express mRNA for two glutaminase isoforms (KGA and GAC). Glutaminase activity increased in response to acidic stress (media pH 5.8) and was associated with both increased culture medium pH and improved cell survival. In contrast, activity was inhibited by interferon and tumor necrosis factoron. Glutaminase protein was expressed in the human airway in vivo. This demonstrate that glutaminase is expressed and active in the human airway epithelium and may be relevant both to the regulation of airway pH and to the pathophysiology of acute asthmatic airway inflammation. The conclusion shown that glutaminase is expressed and active in the human airway epithelium and production of ammonia by airway epithelial cells serves a pH homeostatic role increasing protectively in response to acidic stress and is inhibited by inflammatory cytokines. Direct relevance of this pathway to human disease is suggested by the permissive effect of ammonia depletion on exhaled airway fluid acidification during asthma exacerbations [21].

1.2 Overview of budesonide

Trade Names: Pulmicort Turbuhaler, Pulmicort Respules, Pulmicort Flexhaler

Mechanism of Action & Side Effects:

- ♦ Budesonide can bind to intracellular glucocorticoid receptors and modulates gene expression. Approximately 20% of expressing genes in a cell are regulated by glucocorticoids.
- ♦ Glucocorticoids have multiple anti-inflammatory effects, inhibiting both inflammatory cells and release of inflammatory mediators.
- ♦ Improvement in asthma control following inhalation can occur within 24 hours of beginning treatment in some patients, although maximum benefit may not be achieved for 1 to 2 weeks**,** or longer**.**

Pharmacokinetics:

- ♦ Aerosol inhalation
- ♦ Most of budesonide delivered to the lungs is systemically absorbed. In healthy patients, 34% of the metered dose is deposited in the lungs with an absolute systemic availability of 39% of the metered dose
- ♦ If it is taken orally, budesonide has a very low (6-13%) oral bioavailability

Budesonide is an anti-inflammatory drug for the treatment of asthma and COPD. It blocks the inflammatory cell influx and inhibits inflammatory mediator release by inhibition of the arachidonic acid pathway. Budesonide is one of the corticosteroids representing the cornerstone of asthma management. Budesonide has been proven very effective for a long time [22]. This agent is available in different formulations, from metered dose (MDI) and dry powder inhalers (DPI) to produce for nebulisation, to meet the needs of the heterogeneous population of asthmatic patients. Nevertheless, one of the major limitations of such inhalation products is the variability in the pulmonary drug deposition, which in turn leads to potential differences in clinical responses [24]. Budesonide could be a powerful tools for treating ventilated patients if delivered effectively.

A budesonide solution is stable at a pH lower than 6.0 [23], but it has limited light stability. These problems could be solved by encapsulation method.

1.3 Overview of polymeric nanoparticles

Polymeric nanoparticles have gained considerable attention as potential drug delivery systems due to its biocompatibility, non-immunogenicity, non-toxicity, simple preparation methods, high physical stability, target ability to particular tissue and ability to deliver protein and peptide via oral route. Nanoparticles for drug delivery are generally made up of biocompatible polymers obtained from either natural or synthetic source [25].

Polymeric nanoparticles offer some specific advantages over another form, for instance, help increasing drug stability and give controlled release property.

1.4 Overview of methods in preparation of nanoparticles

Nanoparticles are receiving considerable attention for the delivery of therapeutic drugs. The desired effect of many medical treatments is usually obtained when the drug concentration is in the therapeutic range, especially true for highly potent drugs with potent side effect [26]. There are several techniques to prepare nanoparticles for controlled release and various forms of nanoparticles have been studied, e.g., ceramic nanoparticles, polymeric nanoparticles, polymeric micelles, liposomes and dendrimer [27].

In 2006, Neufeld et al. demonstrated the most important methods for the preparation of nanoparticulate drug carriers, using the natural polymer, its advantages and disadvantages are summarized in Table 1.1. The chosen method should minimize loss of the drug, according to a particular application [28].

Table 1.1 Preparation methods, general advantages and drawbacks of the polymeric nanoparticles.

Method	Simplicity of procedure	Need for purification	Facility scaling-up	EE (%)	Safety of compounds
Polymerization of monomers					
Emulsion polymerization					
Organic	Low	High	NR	Low	Low
Aqueous	High	High	High	High	Medium
Interfacial polymerization	Low	High	Medium	High	Low
Preformed polymers					
Synthetic					
Emulsification/					
Solvent evaporation	High	Low	Low	Medium	Medium
Solvent displacement and					
interfacial deposition	High	NR	NR	High	Medium
Salting out	High	High	High	High	Low
Emulsion/solvent diffusion	Medium	Medium	High	High	Medium
Natural					
Albumin	NR	High	NR	Medium	Low
Gelatin	NR	High	NR	Medium	Low
Polysaccharides					
Alginate	High	Medium	High	High	High
Chitosan	High	Medium	High	High	High
Agarose	Medium	High	NR	NR	High
Desolvation	NR	High	NR	Low	Low

EE, encapsulation efficiency; NR, no reference available.

The advantages of using the polymeric nanoparticles is that they could be an effective drug delivery carrier, their small size can help them penetrating through small blood capillaries, thus can be taken up by tissue cell.

According to Neufeld and coworkers [28], solvent displacement method gave high encapsulation efficiency and simple preparation methods. Therefore, we chose this method to prepare our polymeric nanoparticles.

1.5 Overview of ethyl cellulose (EC) nanoparticles for drug encapsulation

Ethyl cellulose (EC), one of many polymers used as shell materials for encapsulation technology which offers a new level of physical stability, is a derivative of cellulose in which some of the hydroxyl groups of the repeating glucose units are converted into ethyl ether groups. The number of ethyl groups can vary depending on the manufacturer. EC is slightly soluble in water and well soluble in ethanol. It is widely used in various applications such as pharmaceuticals, food and thin-film coating material because of its biocompatibility and non-toxicity, leading to advanced development i.e. chemical modifications [30, 31].

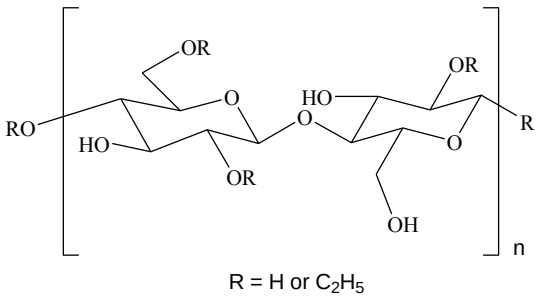


Figure 1.4 Chemical structure of ethyl cellulose (R = H or C_2H_5)

In 1991, Sheorey et al. developed a technique for encapsulation of water insoluble drugs. Sulphadiazine was dissolved in organic solvent, containing EC and the solution was emulsified into an aqueous bentonite. Solvent evaporation method induced phase separation. The data clearly showed that encapsulation process is able to produce microcapsules with varying drug to shell ratios [29].

In 2008, Kamel et al. describes the chemically modification of cellulose to produce cellulose derivatives which are in general strong, low cost, reproducible, recyclable and biocompatible, so they can be tailored for pharmaceutical applications. Cellulose derivatives are often used to modify the release of drugs in tablet and capsule formulations and also as tablet binding, thickening and rheology control agents for film formation, water retention and improving adhesive strength for suspending and emulsifying. EC can be used in hydrophobic matrix system. Also, liquid and semi-solid pharmaceutical dosage forms are important physicochemical systems for medical treatment which require rheological control and stabilizing as essential additives [30].

In 2003, Morales et al. designed a morphine oral suspension, as sustained release pharmaceutical formulations. Dividing into two methods, different EC suspensions were prepared. In the first group, the drug incorporated during synthesis (suspension A) so that the drug was inside the polymeric microparticles. In the second group of suspensions, the drug was incorporated after synthesis (suspension B), thus resulting in the drug being adsorbed on the surface. The transfer of drug from the particles is comparatively rapid and the polymeric particles can adsorb morphine hydrocloride on their surface, the amount of adsorbed drug is approximately 15%. On the other hand, EC is able to spontaneously encapsulate approximately 95% of the drug molecules when they are in the aqueous phase during synthesis. This fact offers the possibility of controlling the release rate of morphine without complex technological processes. The result indicated the EC suspension is suitable for the sustained release of morphine hydrochloride [32].

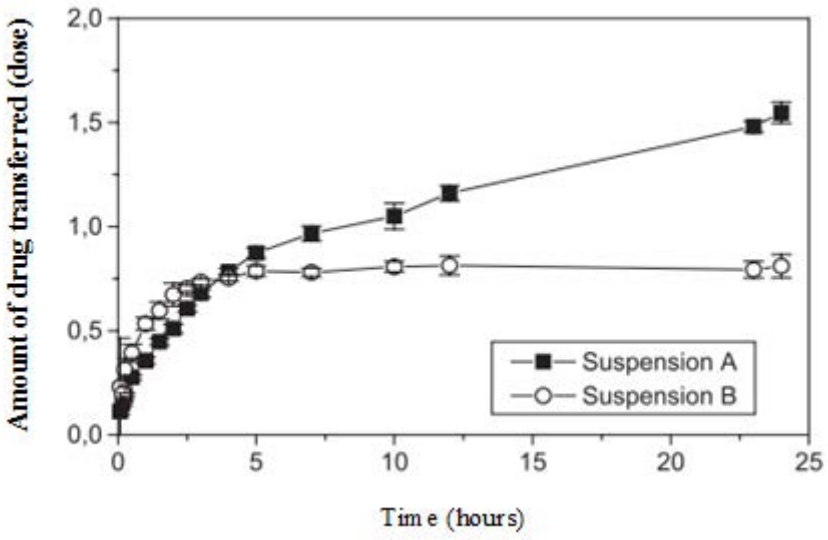
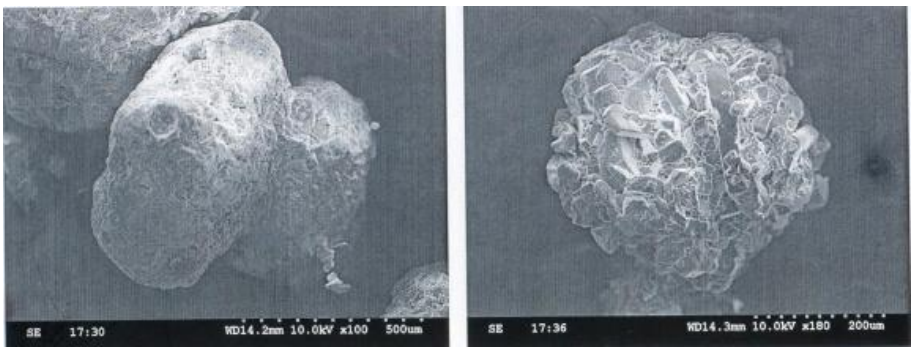


Figure 1.5 Amount of drug transferred, normalized according to dose [32].

In 2010, Midhun et al. demonstrated that electrospraying is a viable single-step method to generate drug-entrapped nanoscale particles with biopolymer matrices. Corticosteroids such as budesonide is the drug of choice for the treatment of inflammatory disorders, with an inherent limitation, budesonide was encapsulated in a biodegradable polymer, polycaprolactone (PCL), by electrospraying with high drug-entrapment efficiency and sustained drug release [33].

In 2011, Chowdary et al. evaluated EC as a shell for controlled release microcapsules of diclofenac. EC shell microcapsules were prepared by an emulsion-solvent evaporation method. The prepared microcapsules were found to be spherical shape. Drug content was uniform in each batch of microcapsules and the microencapsulation efficiency was in the range of 98.85-101.81%. EC was found to be an efficient microencapsulating agent and the EC microcapsules also exhibited good controlled release characteristics and were found suitable for oral controlled release of diclofenac over 12-16 h [34].



(a) (b)

Figure 1.6 SEM of EC coated microcapsules of diclofenac. Prepared employing chloroform (a) and microcapsules prepared employing dichloromethane (b) as solvents for the polymer [34].

As presented above, EC is an appropriate polymer for encapsulation and controlled release of insoluble drug such as budesonide. Thus, this research, budesonide, an anti-inflammatory drug was loaded into nanoparticles in which EC polymers was used as shell materials. Then the budesonide loaded particles were covalently linked to the surface of the PDMS tube. Finally, the release of the budesonide from the tube was evaluated.

1.6 Overview of used techniques in surface modified PDMS tube

1.6.1 Chemical modification of PDMS surface

In 2008, Grundke et al. designed a stable surface functionalization of PDMS layers screen printed onto silicon wafers. In the first step, the PDMS surfaces were activated by oxygen-plasma or ammonia-plasma. In the case of oxygen plasma, it is rather intense treatment conditions and long treatment times are required to functionalize the PDMS surface. Reactive silanol groups were generated. Then the surface functionalization was established with the formation of a weak boundary layer that can be easily washed off by polar liquids. A strong tendency to recover their hydrophobicity was observed when the plasma-treated PDMS samples were stored under ambient conditions. The amino functional groups created by the attachment of 3-aminopropyl trimethoxysilane (γ-APS) onto oxygen plasma-treated PDMS surfaces

or created directly by the ammonia-plasma, respectively, were used in the second step to graft a thin layer of poly(ethylene-alt-maleicanhydride) (PEMA) copolymer onto the PDMS surface [36].

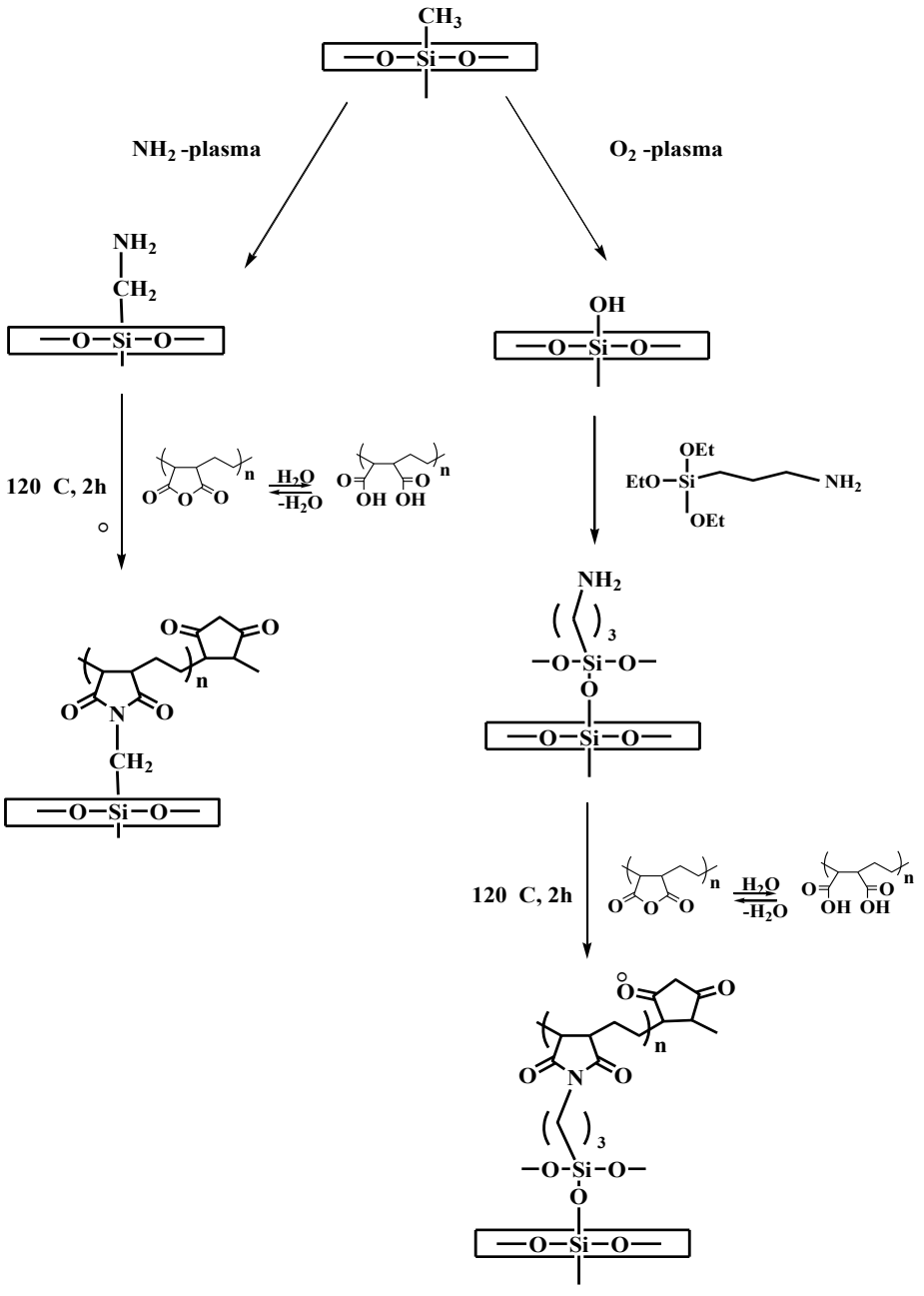


Figure 1.7 Schematic of the grafting procedure of PEMA on plasma-treated PDMS surfaces.

In 2009, Guittard et al. reported the preparation of superhydrophobic PDMS surface. First, surface roughness was generated by means of acid corrosion with sulfuric or hydrofluoric acids. Second, the chemical grafting of the semifluorinated agent was achieved by means of silane derivatization in order to create a highly fluorinated monomolecular layer on the surface. Two pathways were investigated: the direct grafting of a highly fluorinated silane and a two-step process where the PDMS supports were first functionalized with γ-APS and then allowed to react with a semifluorinated acid chloride, providing to the surface monolayer a structured amide function. Superhydrophobic performance with a Cassie state was obtained after the covalent grafting of semifluorinated amides in two steps. This work led to a performing nanorough surface with only one monolayer of covalently grafted fluorinated active chemicals. Its covalent anchorage to the surface ensures the toughness of the coating [37].

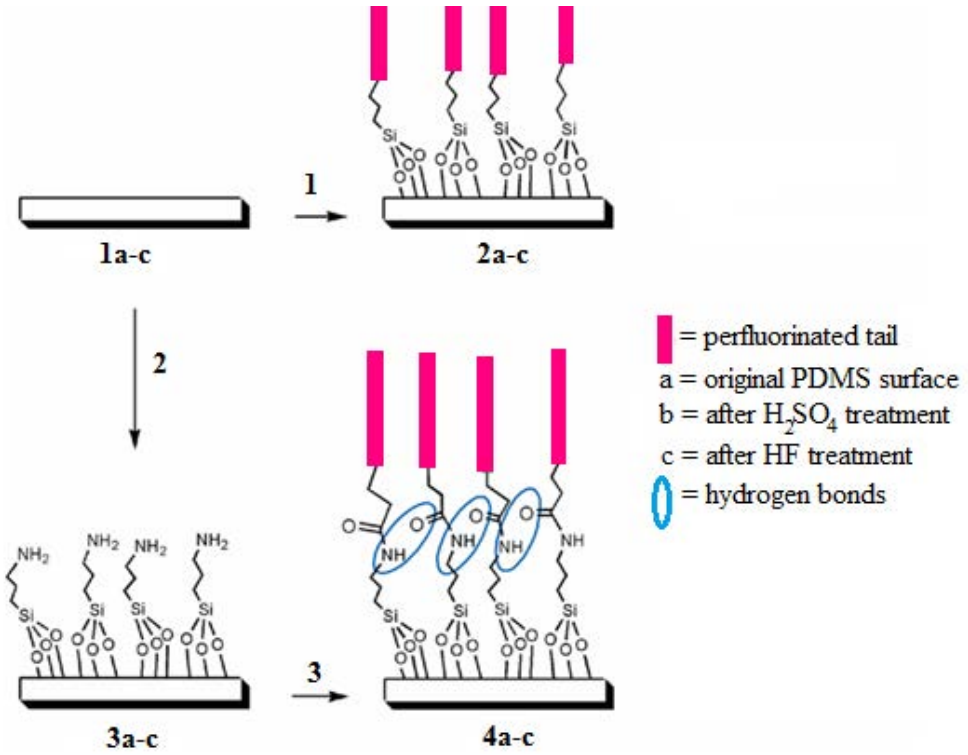


Figure 1.8 General synthetic scheme: (1) O_2 plasma activation then perfluoro decyltriethoxysilane, EtOH, acetic acid; (2) O_2 plasma activation then N,N –dimethylaminopropyltriethoxysilane, EtOH, acetic acid (3) 3-perfluorooctylpropanoic acid chloride, AcOEt [37].

1.6.2 Oxygen plasma treated PDMS surface

In 2006, Hong et al. demonstrated the efficacy of the atmospheric oxygen RF plasma in surface cleaning and modification of PDMS stamps for simple press bonding to the glass surfaces. The water contact angle, XPS, and AFM analysis showed the increase of hydrophilic surface of the PDMS stamps after the plasma treatment [35].

(a) (b)

Figure 1.9 Contact angles of (a) before and
(b) after oxygen-plasma-treated PDMS surfaces [35].

In 2010, Tan et al. produced hydrophilic PDMS devices through the use of a second extended oxygen plasma treatment. The method could maintain the hydrophilicity of the devices for several weeks. At 70 W plasma power and treatment time longer than 300 sec, the PDMS surface can remain hydrophilic for more than 6 h after the exposure to ambient air. The reasonably low contact angle between 50° and 60° is sufficient for most microfluidic applications. The time span of 6 h is also enough for most experiments in microfluidics. Surface analysis revealed that a longer plasma treatment time produced a much smoother surface [38].

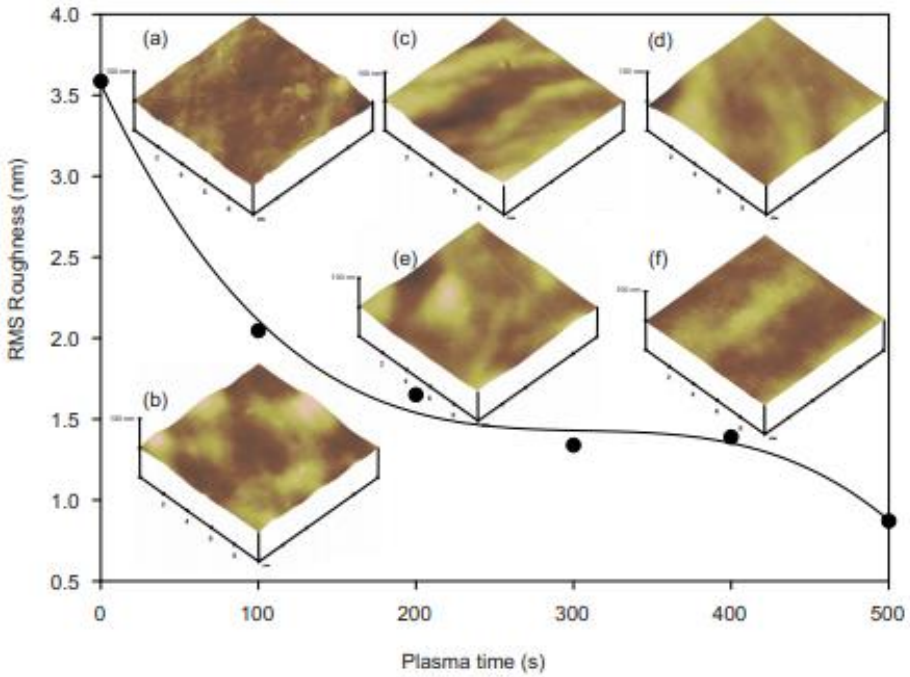
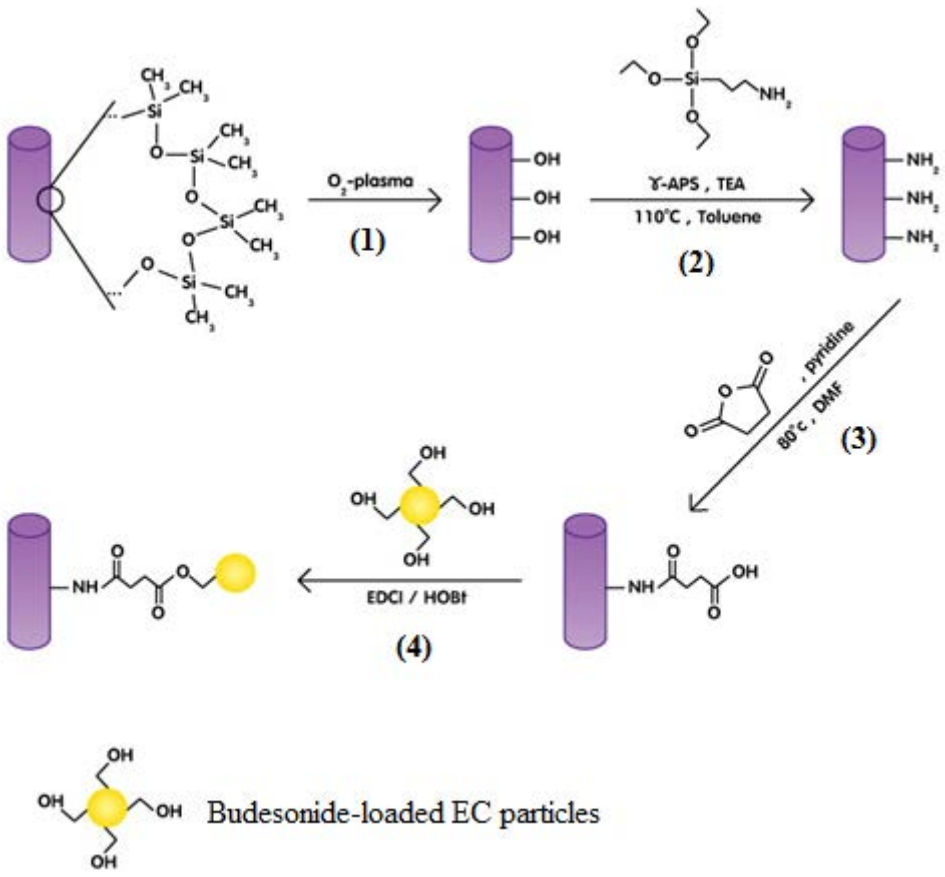


Figure 1.10 Root mean square roughness as a function of treatment time with oxygen plasma. Respective AFM images are taken of specimen with oxygen plasma exposure of (a) 0 s, (b) 100 s, (c) 200 s, (d) 300 s, (e) 400 s and (f) 500 s [38].

Therefore, our PDMS tubes were treated with oxygen plasma several times to obtain smooth and active surface for chemical modification.

1.7 Research goal

The aim of this study is to fabricate Polydimethylsiloxane (PDMS) tube which can slowly release anti-inflammatory drug, budesonide. The scheme of this study is as follow: (1) oxygen plasma treatment of PDMS surface, (2) coupling of γ-APS onto plasma-treated PDMS, (3) coupling reaction of γ-APS-grafted PDMS with succinic anhydride and (4) grafting of budesonide-loaded EC particles onto PDMS tube. The detail of each step is shown in the following diagram.



Scheme 1.1 Surface functionalization of the PDMS tube and grafting of budesonide-loaded EC particles onto the surface of the functionalized PDMS tube

This research, we incorporate budesonide into the PDMS tube in order to add anti-inflammatory activity for lessening the inflammation problem during tracheostomy. The release profile of the budesonide from the tube was studied under liked-trachea environment condition; at pH value closed to an attending of infection in mucosa (pH 5.8).

CHAPTER II

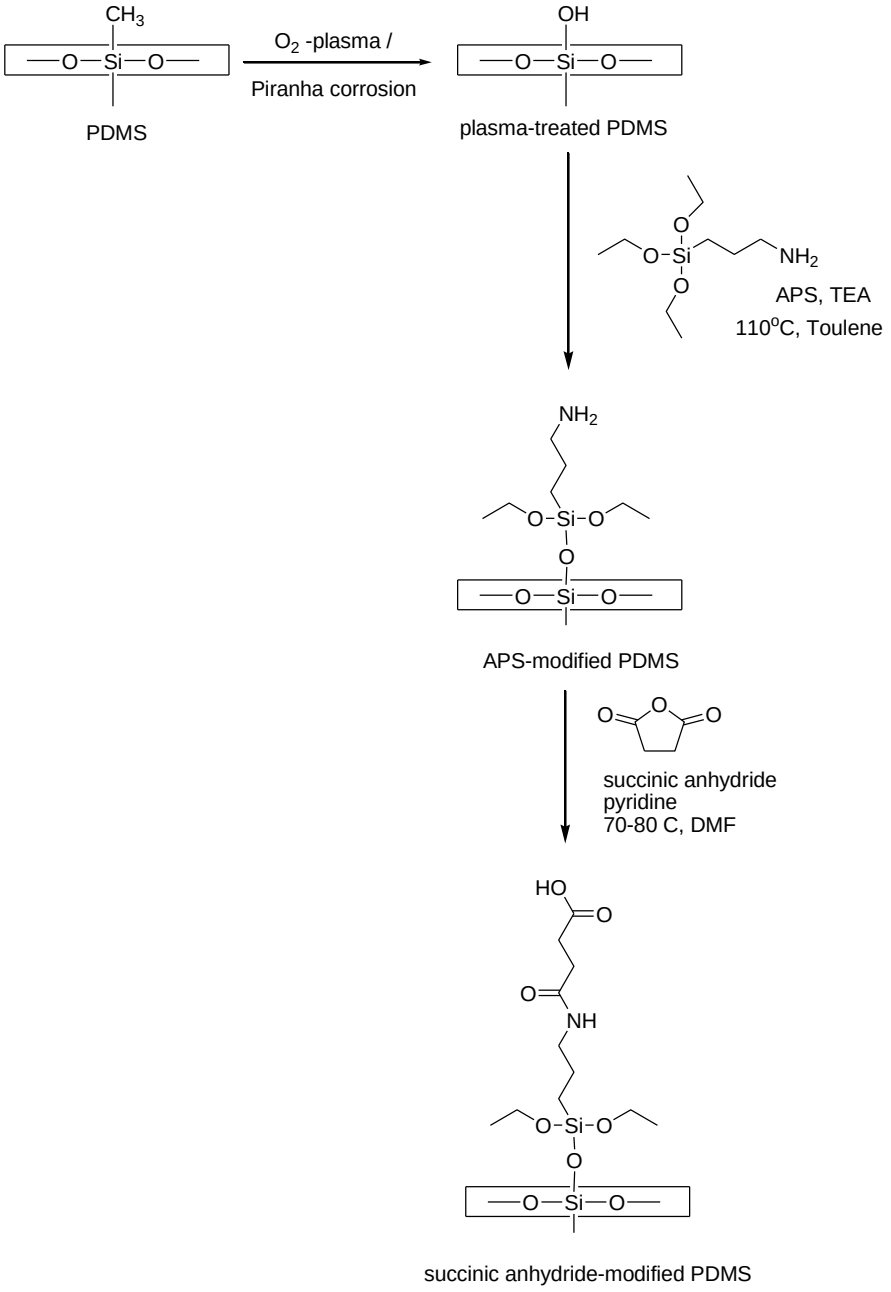
EXPERIMENTAL

2.1 Materials and Chemicals

Polydimethylsiloxane (PDMS) tubes were purchased from Koken (Tokyo, Japan). Hydrogen peroxide (59% H_2O_2) was purchased from Johnson & Johnson (California, USA). Ethylcellulose (EC, ethyl 48% w/w ethoxy content, viscosity 300 cp) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Sigma Aldrich (Steinheim, Germany). Budesonide (MW 430.5, analytical grade) was purchased from Fluka (Seelze, Germany). Succinic anhydride (analytical grade), 3-aminopropyltriethoxysilane (γ-APS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) were purchased from Acros Organics (Geel, Belgium). 1-Hydroxybenzotriazole (HOBt) was recrystallized in MeOH. Lissamine Rhodamine B sulfonyl chloride (Rhodmine B) was purchased from Invitrogen (USA). Disodium hydrogen phosphate (Na_2HPO_4), triethanolamine and pyridine were purchased from Carlo Erba Reagents (Val de Reuil, France). Ethanol, ethyl acetate, hexane, tolulene, dimethyl formamide (DMF) and acetonitrile (HPLC grade) were purchased from ACI Labscan Limited (Bangkok, Thaiand). Polysorbate 20 (Tween 20) was purchased from Honghuat (Bangkok, Thaiand). Hydrochloric acid and sulfuric acid were purchased from Merck (Darmstadt, Germany). Other reagents were of analytical grade and were used without further purification. Membranes used for dialysis experiments were dialysis tubing cellulose membranes with molecular weight cut off (MWCO) 12,000-14,000 Daltons, 75 mm flat width, 17.9 ml/cm volume capacity, Membrane Filtration Products, Seguin, TX, USA.

2.2 Preparation of chemically functionalized PDMS surface

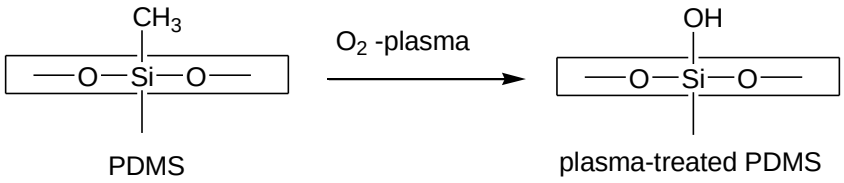
The surface functionalization of PDMS tube was carried out as outlined in Scheme 2.1. In the first step, the PDMS surface was activated by oxygen plasma or acidic corrosion. The second step was the coupling of γ-APS onto plasma-treated PDMS and the last step was the coupling reaction of γ-APS-grafted PDMS with succinic anhydride. The details of each step are shown in the following diagram.



Scheme 2.1 Surface functionalized of the PDMS tube

2.2.1. Surface treatment of PDMS by oxygen plasma or piranha corrosion

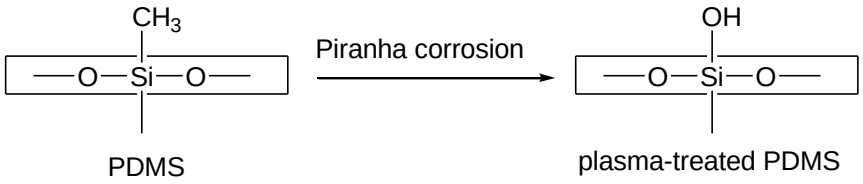
2.2.1.1 Plasma treatment of PDMS surface



Scheme 2.2 Surface treatment of PDMS tube with oxygen plasma

Oxygen-plasma treatment was carried out by a STERRAD sterilization system (ASP, STERRAD100S, California, USA) at Faculty of medicine, Chulalongkorn University. In this work, the PDMS tube (100 cm in length) was treated with oxygen plasma at 55-60 °C with radio wave (RF) and 59% (v/v) H_2O_2 (18 ml) for 10 cycles (Scheme 2.2). The cycle was composed of vacuum stage (pressure 397 mtorr, 20 min 16 sec), injection stage (pressure 6.34 torr, 6 min 2 sec), diffusion stage (pressure 15 torr, 2 min), plasma stage (pressure 552 mtorr, 7 min 23 sec), injection stage (pressure 7.67 torr, 6 min 2 sec), diffusion stage (pressure 15 torr, 2min), plasma stage (pressure 630 mtorr, 7 min 11 sec) and vent stage. The treated tube obtained from oxygen plasma treatment was then analyzed by contact angle.

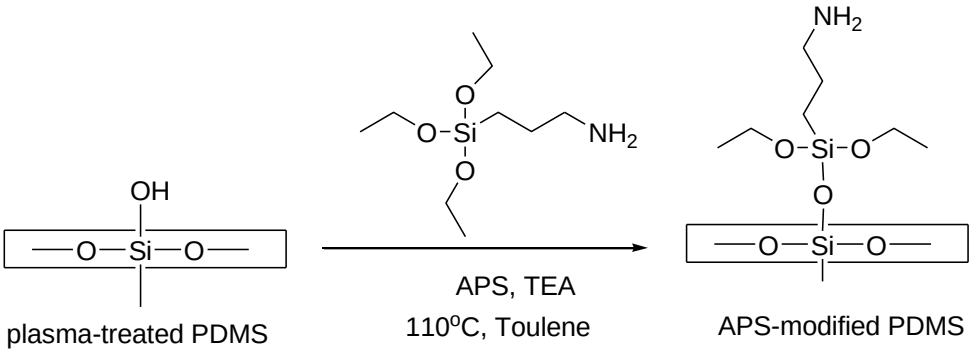
2.2.1.2 Piranha corrosion of PDMS surface



Scheme 2.3 Surface treatment of PDMS tube with piranha corrosion

The PDMS tube was cut into 1 cm length for this experiment. The PDMS tube was treated with piranha solution (a mixture of 3:1 v/v 75% sulfuric acid and 30% hydrogen peroxide) at 90 °C for 30 minutes, following by a triple rinse with deionized water (Scheme 2.3). The treated tube from piranha corrosion was then analyzed by contact angle.

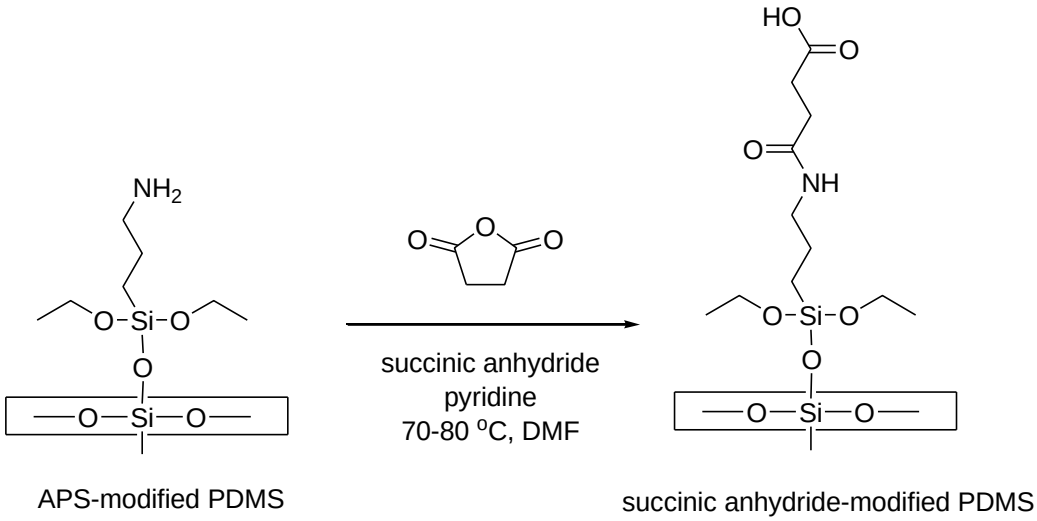
2.2.2 γ-APS-grafted plasma-treated PDMS



Scheme 2.4 γ-APS-grafted plasma-treated PDMS

The oxygen-plasma-treated PDMS tubes, 30 tubes (4.40 cm^2 for each tube) were put into 250 ml of a round bottom flask. The mixture was refluxed with γ-APS in toluene (2% v/v) at 110 ^{o} C for 3 h under N_2 atmosphere. Triethanolamine (TEA) (0.5 ml) served as the catalyst was added to a reaction mixture (Scheme 2.4). Then the tubes were rinsed with 1000 ml of distilled water for three times, 40 ml of hexane and followed by 40 ml of EtOH. The washed tubes were dried in desiccator for 24 h to obtain γ-APS-modified on PDMS tubes. The product was characterized by ninhydrin test [37].

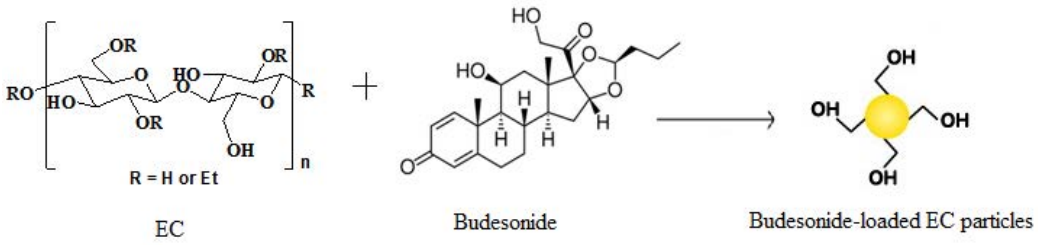
2.2.3 Coupling reaction of γ-APS-grafted PDMS with succinic anhydride



Scheme 2.5 Coupling reaction of γ-APS-grafted PDMS with succinic anhydride

To produce succinimide group on surface of the γ-APS-modified PDMS tubes (Scheme 2.5), the γ-APS-modified PDMS tubes, 30 tubes (4.40 cm^2 for each tube) were put into a round bottom flask and succinic anhydride solution (1 g, 0.01 mol in 50 ml dried DMF) and a catalytic amount of pyridine were put into the flask. The mixture was stirred at 80 °C overnight under N_2 atmosphere. Free succinic anhydride or succinic acid was eliminated by washing with 1000 ml of distilled water for three times. The product was then subjected to ninhydrin test.

2.3 Encapsulation of budesonide into the EC



Scheme 2.6 The budesonide-loaded EC by solvent displacement method

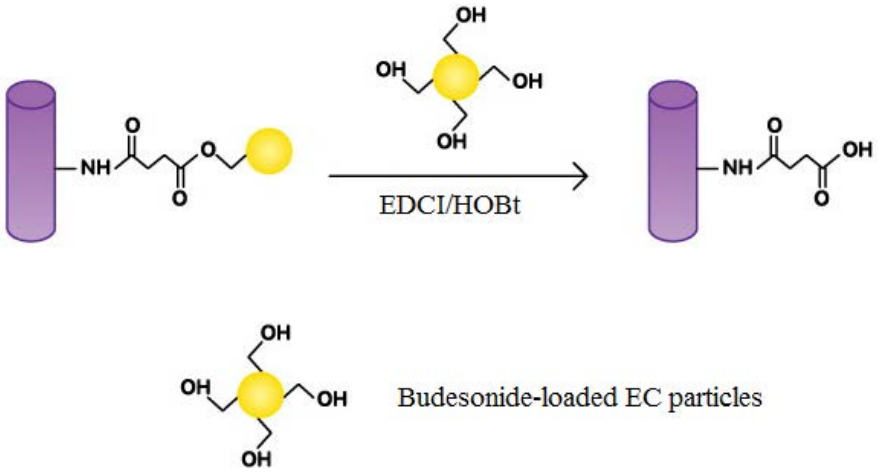
Budesonide was encapsulated into the EC at budesonide to polymer weight ratios of 1:1 by solvent displacement method (displacing ethanol with water) (Scheme 2.6). In brief, 250 mg of the budesonide and 250 mg of the EC were dissolved in ethanol (50 mL) to give EC and budesonide concentrations of 5,000 ppm each. The mixture (50 mL) was dialyzed against distilled water. Final concentration of the polymer in the obtained suspension was 2,500 ppm. The obtained budesonide encapsulated particles in aqueous suspension were subjected to scanning electron microscope (SEM), transmission electron microscope (TEM) and dynamic light scattering (DLS) analysis. The amounts of budesonide encapsulated in polymeric particles and in the dialysate water were determined using UV/VIS absorption spectroscopy at 246 nm with the aid of a calibration curve (5, 10, 15, 20, 25, 30 and 35 ppm standard solutions) (UV2500 spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The encapsulation efficiency (% EE) and loading capacity (% loading capacity) were calculated using equation (1) and (2) as follows:

$$\%\,EE = \frac{\text{weight of encapsulated budesonid}}{\text{weight of budesonide initially used}} \times 100 \quad (1)$$

$$\%\,LC = \frac{\text{weight of encapsulated budesonide}}{\text{weight of encapsulated budesonide + polymer}} \times 100 \quad (2)$$

2.4 Preparation of budesonide particles grafted tube

The budesonide particles grafted tube was carried out through esterification reaction using EDCI and HOBt as coupling agents (Scheme 2.7).

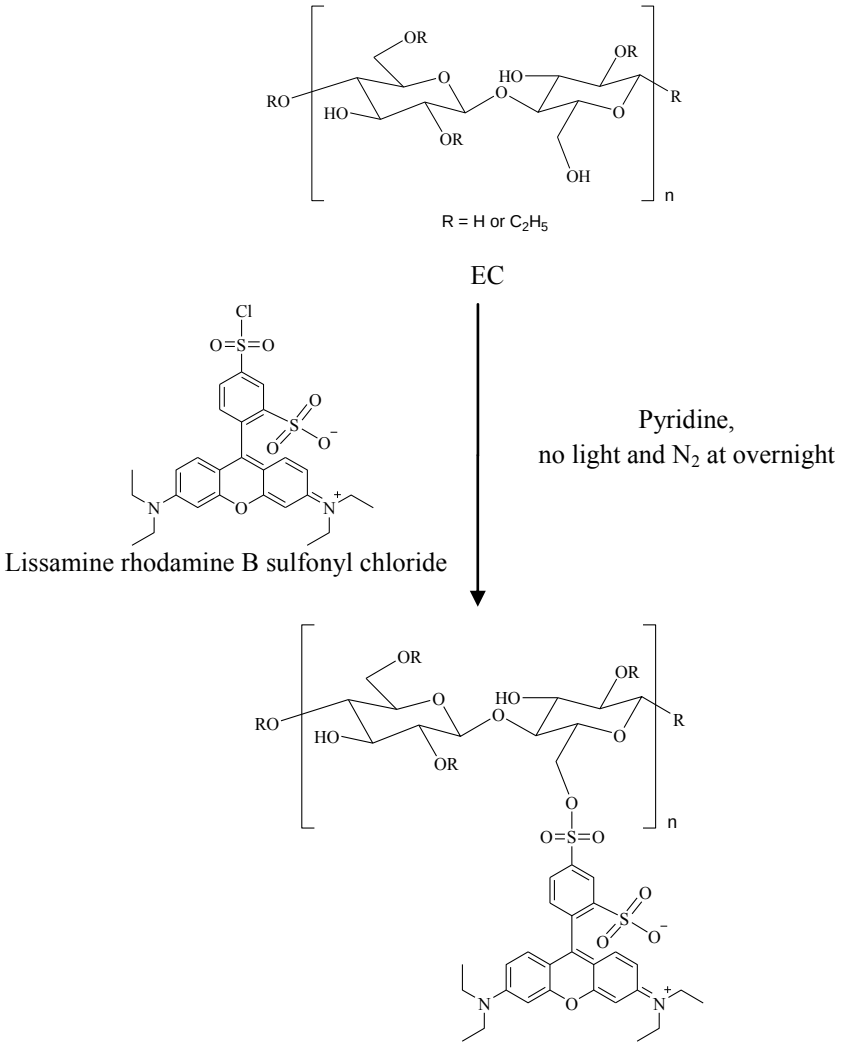


Scheme 2.7 The particle grafted succinimide PDMS tube

Aqueous suspension of budesonide-loaded particles (50 ml, 250 mg budesonide, 250 mg EC) was put into a round bottom flask. HOBt (0.88 g, 0.65 mol) in EtOH (5 ml) was added. Then, the succinimide PDMS tubes (0.5 mol, 4.40 cm^2 surface area on each tube, 30 tubes) and EDCI (1.25 g, 0.65 mol) were put into the flask and the mixture was stirred in ice bath at 0 ºC under N_2 atmosphere overnight. Then the tubes were rinsed with 1000 ml of distilled water for three times and repeatedly washed with 20 ml of EtOH. Morphology of the nanoparticles and the budesonide particles grafted on the succinimide PDMS tube was observed by SEM and fluorescent microscope (CLFM).

2.5 Preparation of fluorescence labeled particles

The fluorophore Rhodmine B was covalently linked to the EC by adding 1% of Rhodmine B into aqueous EC particle suspension in a presence of catalytic amount of pyridine under light-proof and N_2 atmosphere. The mixture was stirred at room temperature overnight. The Rhodamine B-labeled EC polymer was then purified from free Rhodamine B by dialysis under light-proof condition (Scheme 2.8). The obtained polymer was used to prepare particles as described above. The Rhodamine B-labeled EC particles were grafted onto the PDMS tubes as previously described and the product was subjected to CLFM analysis.



Lissamine rhodamine B-grafted EC (Rho B-labeled EC)

Scheme 2.8 Synthesis of Rho B-labeled EC

2.6 Morphology, Hydrodynamic diameter and Zeta potential of the nanoparticles

The morphology of the budesonide-loaded particles was determined with SEM and TEM. The SEM photographs were obtained from JEM-6400 (JEOL, Tokyo, Japan). The budesonide-loaded nanoparticle suspension (in water) was dropped on a glass slide and dried in the desiccator overnight. The sample was coated with gold under vacuum at 15kV for 90 sec, the gold coat and SEM visualization was conducted at an accelerating voltage of 15kV. The TEM analysis was carried out on a JEM-2100 (JEOL, Tokyo, Japan). The budesonide-loaded nanoparticle suspension (in water) was dropped onto carbon film coated on a copper grid and dried in the desiccator. Observation was performed at 100-120 kV with 15 kV accelerating voltage.

The average hydrodynamic diameter, polydispersity index (PDI), particle size distribution and zeta potential values of budesonide-loaded nanoparticles suspension (in water) were conducted by DLS technique using a zetasizer nanoseries model S4700 (Malvern Instruments, Worcestershire, UK). Before measurement, the freshly prepared colloidal suspension was appropriately diluted and ultrasonicated for five minutes. All measurements were carried out in triplicate and average size was reported.

2.7 Surface characterization

The Kaiser reaction was used to confirm the presence of primary amino terminal groups on the sample tube [37]. Ninhydrin solution (0.1% in EtOH, 0.5 ml) was dropped on the sample tube (4.40 cm^2). After that, the sample tube was heated for 1 min to activate reaction of ninhydrin.

The contact angle measurement was determined by Ramé-hart, Model 200, Standard Contact Angle Goniometer (Succasunna, NJ, USA). The sample tube was attached on a glass slide. Deionized water was dropped on the sample tube during measurement (4.40 cm^2 for each sample tube). All measurements were carried out in five repeats. The obtained contact angle data from five individual drops were averaged.

To determine topography of reflecting surfaces and indicated nanospherical morphology of budesonide-loaded particles-graft onto the PDMS tube, SEM and CLFM were used. The sample tube was attached on a glass slide. Then, coated with a gold layer under vacuum at 15kV for 90s then SEM visualization was carried out at an accelerating voltage of 15kV.

The Rhodamine B-labeled EC particles-graft onto the PDMS tube were attached on a glass slide and were subjected to CLFM analysis on a Nikon Digital Eclipse C1si Confocal Microscope system (Tokyo, Japan).

2.8 Determination of release profiles

Seven tubes, all grafted with budesonide-loaded particles (4.40 cm^2 for each tube) were soaked in 5 ml of 5% (v/v) of 1.55 mM phosphate buffer saline solution (PBS) (pH 5.8) together with tween 20 and various concentrations of EtOH (5, 20 and 50% v/v) at 37 °C. Aliquots of the release medium (500 µL each) were transferred into micro centrifuge tubes, at the indicated times and were subjected to budesonide quantification. During each time the volume of the suspension was kept constant by adding PBS as required (500 µL each). The withdrawn aliquot was filtered by nylon syringe filters (13 mm, 0.2µm, 100/PK), following a nitrogen gas flow for preconcentrate. Then, ethyl acetate were added with 200 µL, the suspension of the withdrawn aliquot were extracted with ethyl acetate [40]. The ethyl acetate layer was subjected to budesonide quantification using high performance liquid chromatography (HPLC) analysis. The HPLC experiment were conducted using a waters 1525 binary HPLC (pump), connected to a waters 2489 (UV/VIS detector). UV–Vis spectroscopy was obtained from UV-2550 (SHIMADZU, Japan). The stationary phase was 100 mm × 4.6 mm column packed with Hypersil C18 (Thermo Fisher Inc, Waltham, Massachusetts, USA) and the mobile phase consisting of ethanol : acetonitrile : 25 mM phosphate buffer pH 3 (2:30:68, v/v/v) [40]. The flow rate was at 1.5 ml/min, UV detection was at 240 nm and injection volume was 10 µL. Calibration curve was created from a series of budesonide solutions freshly prepared in ethyl acetate at

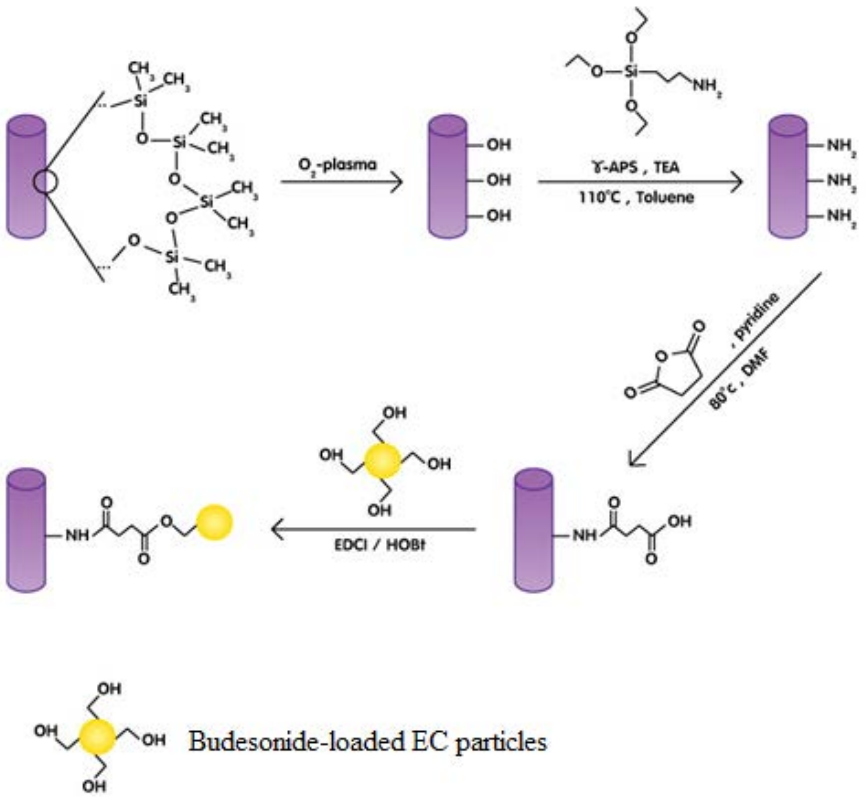
concentrations of 10, 20, 30, 40 and 50 ppm. The experiment was repeated three times.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Preparation and characterization of chemically functionalized PDMS surface

The surface functionalization of PDMS was prepared via 4 steps: In the first step, PDMS surface was activated by oxygen plasma. Under the condition used (see chapter II), long treatment time was required to generated enough hydrophilicity to the surface. In the second step, the amino functional groups were introduced by the attachment of 3-aminopropyltrimethoxysilane (γ-APS) onto plasma-treated PDMS surfaces. In the third step, the succinic anhydride was employed to react with the amino units on the γ-APS-modified PDMS to generate carboxylic groups. The carboxylic group is then used for the attachment of budesonide-loaded particles.



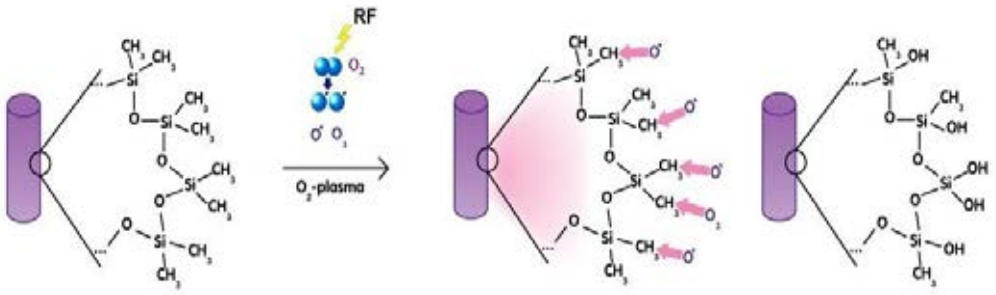
Scheme 3.1 Surface functionalization of the PDMS tube and grafting of budesonide-loaded nanoparticles onto the surface of the functionalized PDMS tube

With some modification of Roth and coworkers procedure [36], we firstly attached 3-aminopropyltriethoxysilane (γ-APS) onto the oxygen-plasma-treated PDMS tube. Then, γ-APS-modified PDMS tubes were reacted with succinic anhydride to produce succinimide group on surface of the γ-APS-modified PDMS tubes.

However, before chemical modification, inert surface of PDMS tubes needs to be treated with oxygen-plasma to activate its surface.

3.1.1 Surface treatment of PDMS by oxygen plasma or piranha corrosion

3.1.1.1 Treatment of the PDMS surface by oxygen-plasma



Scheme 3.2 Surface treatment of PDMS tube with oxygen plasma

The plasma system combined both radio wave (RF) energy to produce the reactive plasma and hydrogen peroxide to generate gas plasma. The RF energy broke the hydrogen peroxide into free radicals that were reactive components of the plasma (O_2^+, O_2^-, O_3, O, O^+, O^-, ionised ozone and free electrons). Reactive oxygen radicals can attack the methyl groups (Si-CH_3) and change them was into hydroxyl groups (Si-OH). At the end of the process, the surface of PDMS tube changed to silanol groups (Si-OH). This treatment provides the hydrophilic surface of PDMS tubes (Scheme 3.2). In this research mild condition was applied in plasma treated process similar to the condition used in sterrad system in medication. Therefore several cycles of treated process were needed. Usually the PDMS surface is hydrophobic and the contact angle of water on PDMS is usually high. After oxygen plasma treatment, the contact angle was reduced as shown in Table 3.1 and Figure 3.1.

Table 3.1 Water contact angles of before and after oxygen plasma treated PDMS tube

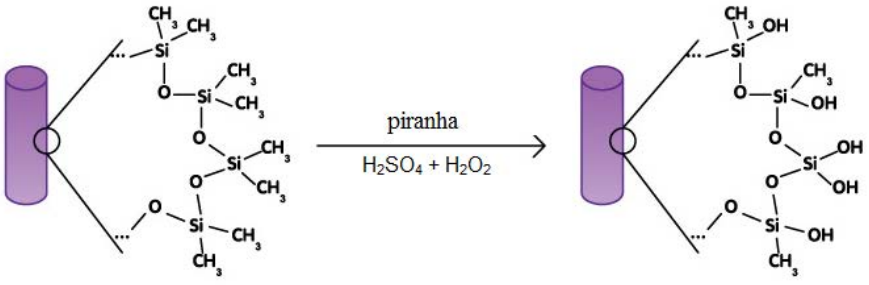
Oxygen-plasma (cycle)	Contact angles	
	Before oxygen plasma treated	After oxygen plasma treated
1	107.28 ± 1.9°	99.20 ± 5.9°
5	107.28 ± 1.9°	90.88 ± 1.5°
10	107.28 ± 1.9°	71.60 ± 6.5°

Table 3.1 indicates that modification the surface of PDMS tubes by oxygen-plasma increased the surface's hydrophilicity (see also Table A1-A4 in appendix). The more the number of cycles, the better the surface functionalization. The oxygen-plasma treatment for 10 cycles, leaded to an acceptable decrease in the contact angles (Figure 3.1). Therefore, 10 cycles treatment was chosen for the further experiment.

(a) (b)

Figure 3.1 Contact angles of (a) untreated PDMS and (b) oxygen plasma-treated PDMS surface.

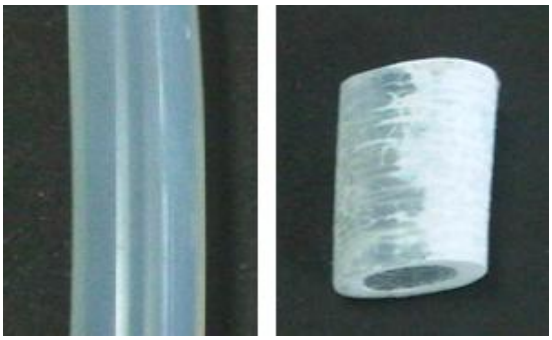
3.1.1.2 Treatment of the PDMS surface by piranha corrosion



Scheme 3.3 Surface treatment of PDMS tube with piranha solution

Piranha solution, a strong oxidizing agent, was a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) (Scheme 3.3). This mixture is able to hydroxylate most surfaces by adding hydroxyl groups (-OH) on its surface, making the surface highly hydrophilic.

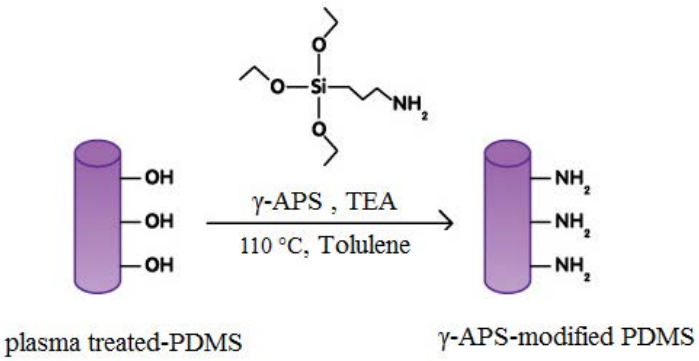
The contact angle of PDMS after piranha treatment was reduced from 105.88 ± 4.36° to 97.98 ± 7.06° (Table A5 and Table A6). However, a difference of physical property between modified tube and the original one, such as the decreases of flexibility due to surface cracking (Figure 3.2) was also observed. Because of these observable physical changes, the piranha corrosion was not used further.



(a) (b)

Figure 3.2 Surface of (a) untreated PDMS tube (b) piranha-treated PDMS tube

3.1.2 γ-APS-grafted plasma-treated PDMS

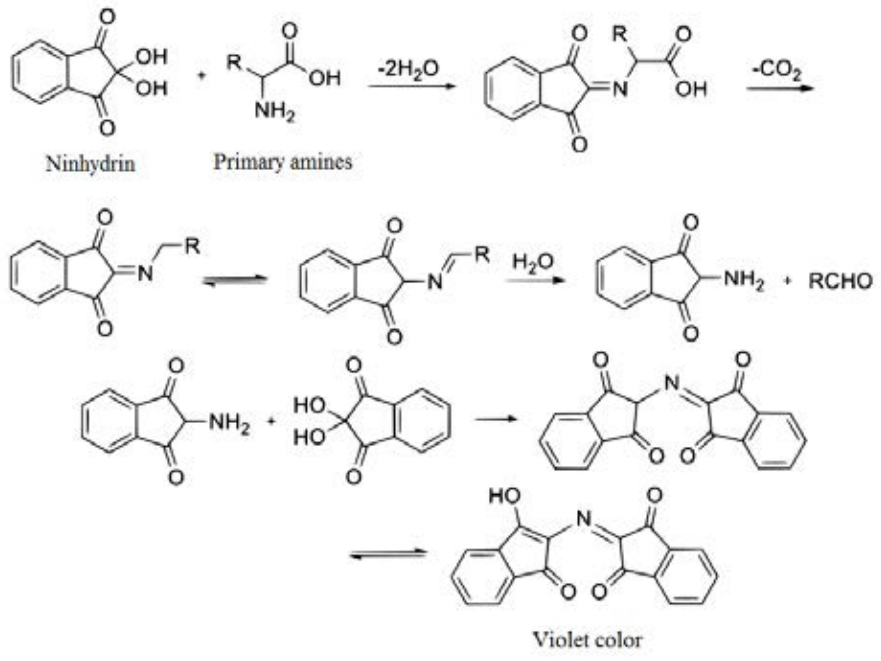


Scheme 3.4 γ-APS-grafted plasma-treated PDMS

The reaction of γ-APS with silanol groups of oxygen plasma-treated PDMS was carried out in tolulene or EtOH to introduce primary amino groups onto the surface of the γ-APS-modified PDMS.

Modification carried out in EtOH gave less amount of reactive amino groups comparing to that in tolulene. The reason is likely take water molecules in EtOH. Which could hydrolyzed γ-APS. Ninhydrin test also showed lighter violet color for tube functionalized in EtOH (Figure 3.4). Therefore tolulene was selected as a solvent for the reaction.

Ninhydrin (2,2-Dihydroxyindane-1,3-dione) was a chemical used to detect primary and secondary amines, the amine was condensed with a molecule of ninhydrin to give a Schiff base. Thus only ammonia and primary amines can proceed past this step (Scheme 3.5). At this step, there must also be an alpha proton for Schiff base transfer, so an amine adjacent to a tertiary carbon cannot be detected by the ninhydrin. When reacting with ammonia or primary amines, ninhydrin gives a deep blue or purple color. Here plasma-treated PDMS was grafted with γ-APS, and the presence of primary amino terminal groups on the tube could be confirmed through a deep violet color from ninhydrin test in solid phase [37] (Figure 3.3).



Scheme 3.5 Ninhydrin test of primary amines

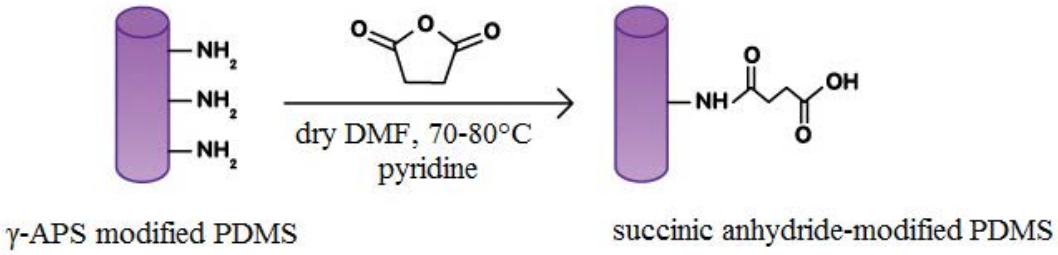
(a) (b) (c)

Figure 3.3 Colorimetric revelation of the amine on (a) the reference PDMS tube, (b) the aminopropylsilanized PDMS tube and (c) tube b after ninhydrin test.

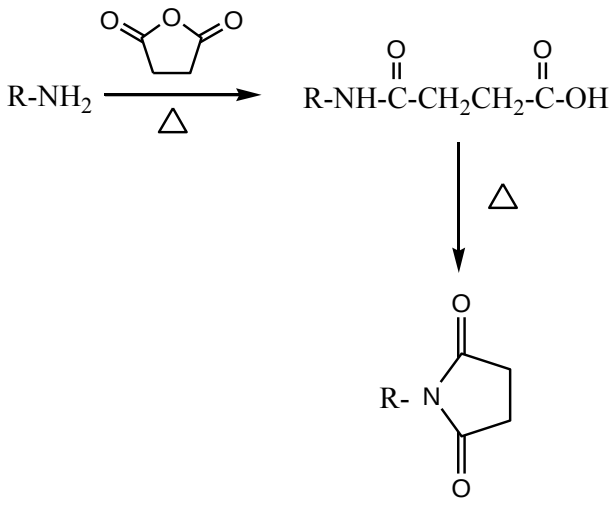
(a) (b)

Figure 3.4 Colorimetric revelation of the amine after ninhydrin test
(a) The aminopropylsilanized PDMS carried out in EtOH and
(b) The aminopropylsilanized PDMS carried out in tolulene

3.1.3 Coupling reaction of γ-APS-grafted PDMS with succinic anhydride



Scheme 3.6 Coupling reaction of γ-APS-grafted PDMS with succinic anhydride



Scheme 3.7 Cyclization reaction of primary amine with succinic anhydride [36]

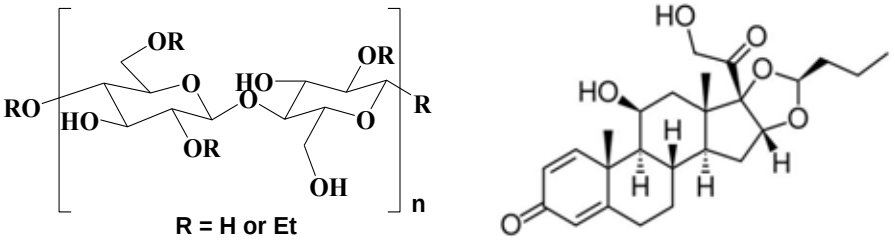
In this research, 70-80 °C was used as our condition to modify γ-APS PDMS with succinic anhydride. This condition was chosen to avoid side reaction that may occur. At high temperature (120 °C), cyclization reaction would take place and provide imide instead of desired product (Scheme 3.7). Therefore, to generate carboxylic group for coupling reaction in the next step, 70-80 °C was chosen.

Succinic anhydride was allowed to react with amino units on the γ-APS-modified PDMS, to change from amino groups into succinimide (Scheme 3.6). The use up of amino groups on the tube then resulted in the fading of violet color when tested with ninhydrin test (Figure 3.5).

(a) (b)

Figure 3.5 Colorimetric (Ninhydrin test) revelation of the amine in (a) aminopropyl silanized PDMS and (b) PDMS succinimide.

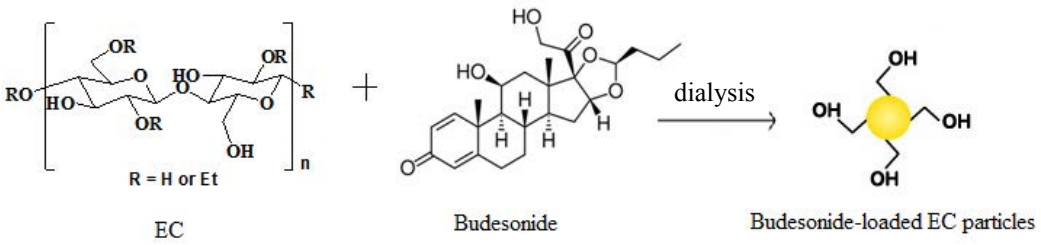
3.2 Encapsulation of budesonide into the EC particles



(a) (b)

Figure 3.6 Molecular structure of the (a) EC and (b) corticosteroid budesonide

Budesonide was encapsulated into the EC particles at budesonide to polymer weight ratios of 1:1 by solvent displacement method (Scheme 3.8). EC, water insoluble polymer, was dissolved in ethanol and placed inside a dialysis membrane with proper molecular weight cut off and dialysis against deionized water (Figure 3.7). The displacement of the solvent inside the membrane was followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of particles. During the particles formation, the hydrophobic nature of budesonide move into the hydrophobic core of the sphere, while the hydrophilic domains of the EC (hydroxyl groups) arranged themselves at the outer surface of the sphere to have maximal interaction with the hydrophilic water molecules, leading to spontaneous particle formation. Self-assembing of the budesonide-encapsulated particles was observed as the formation of milky white aqueous particle suspension as shown in Figure 3.8.



Scheme 3.8 The budesonide-loaded EC by solvent displacement method

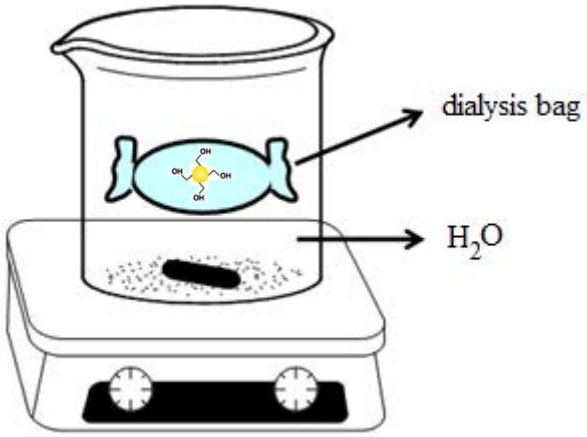


Figure 3.7 Schematic representation of budesonide-loaded EC

Figure 3.8 Suspension of budesonide encapsulated particles

3.3 Encapsulation efficiency and loading capacity

Drug encapsulation efficiency is an important index indicating the effectiveness of the process to encapsulate the drug molucules into the selected delivery system. The encapsulation efficiency and loading capacity were evaluated by measuring amount of budesonide which was loaded in the particles. The amounts of budesonide encapsulated into polymeric nanoparticles and in the dialysate water were determined using UV/VIS absorption spectroscopy at 246 nm, with the aid of a calibration curve.

The encapsulation efficiency (EE) and loading capacity (LC) were calculated as follows:

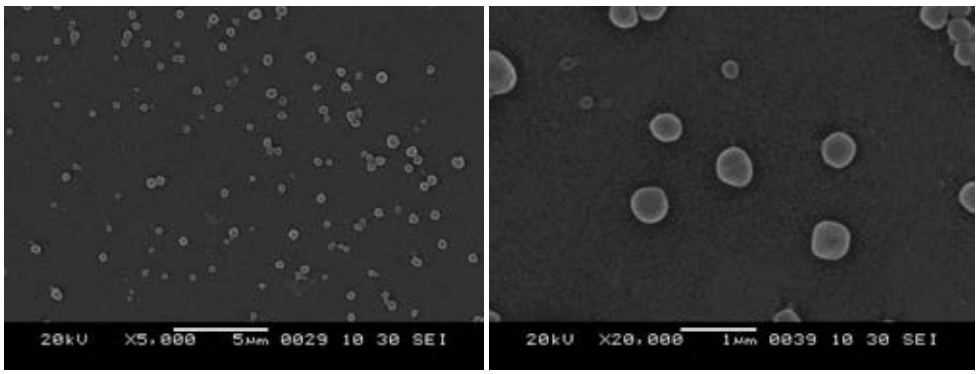
$$\%\,EE = \frac{\text{weight of encapsulated budesonid}}{\text{weight of budesonide initially used}} \times 100$$

$$\%\,LC = \frac{\text{weight of encapsulated budesonide}}{\text{weight of encapsulated budesonide + polymer}} \times 100$$

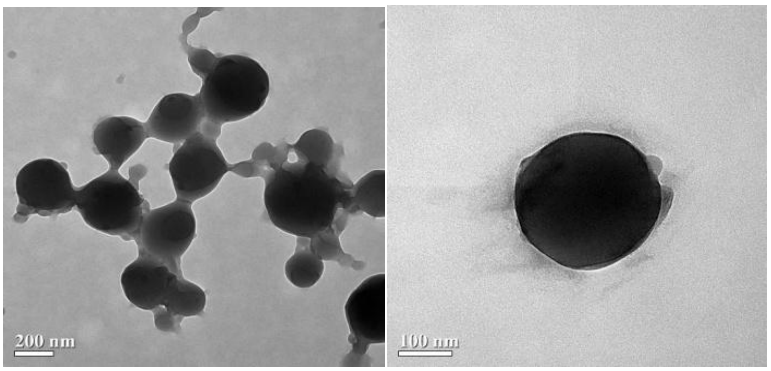
Budesonide-loaded EC showed 33.26 ± 0.23 % (w/w) loading capacity and the encapsulation process gave 49.83 ± 0.11 % encapsulation efficiency, indicating high loading capacity and moderated efficiency of the process (Appendix B).

Morphology, Hydrodynamic diameter and Zeta potential of the nanoparticles

The budesonide-loaded EC particles could be dispersed in water as shown in Figure 3.7. Morphology of particles was characterized with scanning electron microscope (SEM) and transmission electron microscope (TEM). The suspension of the sample was left to dry before being subjected to SEM and TEM analysis. SEM (Figure 3.9 (a and b)) and TEM (Figure 3.9 (c and d)) images of budesonide-loaded particles indicated nanospherical morphology. The dry size of 342.10 ± 85.04 nm could be estimated from the SEM images. In water, the hydrodynamic diameter of approximately 356 nm was obtained from the dynamic light scattering analysis with a narrow size distribution (PDI of 0.256), as shown in Figure 3.10. The mean zeta potential of budesonide-loaded EC particles in water of pH 6.5 was -57.40 ± 1.27 mV. Hydroxyl groups in the polymer structure were speculated as the cause of the negative charge at the particle surface. This indicates unagglomorated nature of the particles and good stability.



(a) (b)



(c) (d)

Figure 3.9 SEM image of budesonide-loaded EC nanoparticles prepared at polymer concentration of 2500 ppm. (a) 5,000×magnification and (b) 20,000×magnification. TEM image of budesonide-loaded EC nanoparticles at polymer concentration of 2500 ppm in (c) and (d).

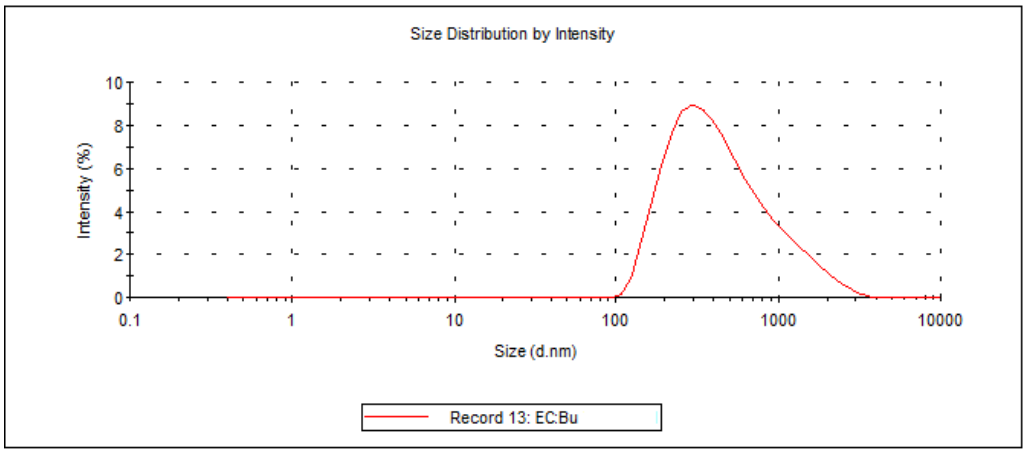
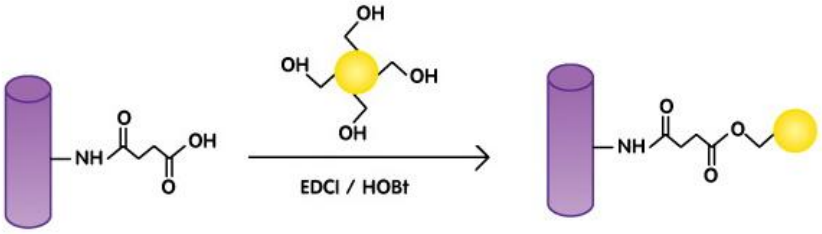


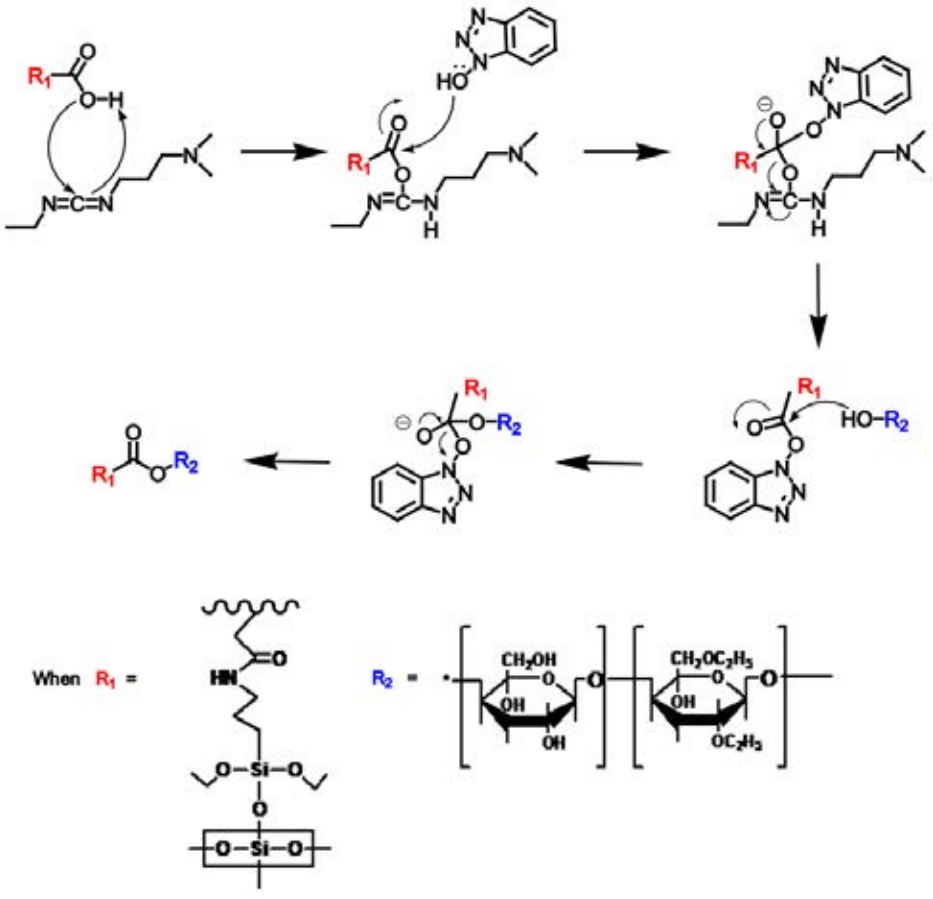
Figure 3.10 Size distribution of budesonide-loaded EC nanoparticles.

3.4 The budesonide particles grafted tube

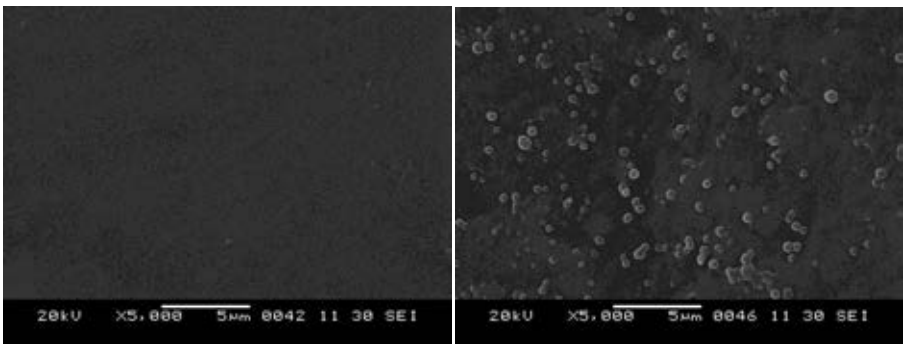
The budesonide particles were grafted onto the PDMS tube (Scheme 3.9) through the esterification between hydroxyl groups of the particles and carboxyl groups on the functionalized surface of the tube (Scheme 3.10). SEM images clearly indicated the presence of particles on the surface of the tube (Figure 3.11).



Scheme 3.9 Grafting of particles onto the functionalized tube.



Scheme 3.10 Mechanism of the coupling reaction

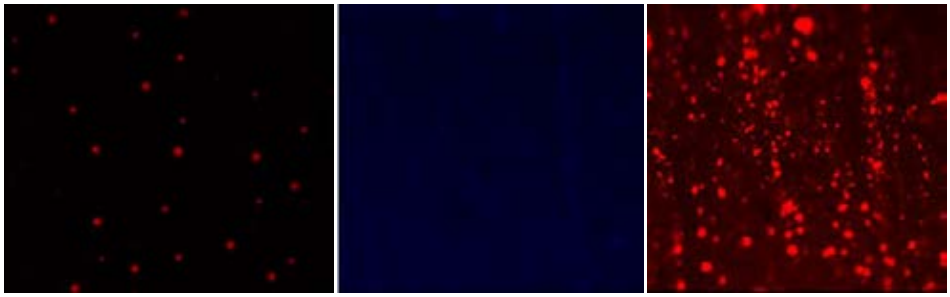


(a) (b)

Figure 3.11 SEM images of the surface of (a) PDMS tube and (b) the budesonide-loaded particles-grafted-PDMS tube.

3.5 Distribution of fluorescence-labeled particles onto the surface of the PDMS

The EC particles are not fluorescent, therefore, to track the location of particles on the surface of the PDMS tube, fluorophore Rhodamine B was first covalently linked to the EC polymeric chains by adding 1% of Rhodamine B into EC solution. The obtained EC was then used to prepare particles. Figure 3.12 (a) showed the fluorescent signal from Rho B-labeled EC particles at 587 nm. Then Rhodamine-labeled EC nanosparticles were grafted onto the tube and the distribution of the particles on the surface of tube could be obtained from fluorescent images (Figure 3.12 (c)). The fluorescent signals from Rhodamine-labeled EC nanoparticles confirmed that the EC nanoparticles were good distributed on the PDMS tube surface.



(a) (b) (c)

Figure 3.12 Fluorescent images of (a) Rho-labeled EC nanoparticles, (b) PDMS tube and (c) Rho-labeled EC nanoparticles on PDMS tube.

3.6 Release of budesonide

As described in the introduction, tracheotomy may cause tracheal inflammation and infection, an increased of bacterial contamination of the airway in patients with tracheotomy, thus increasing the risk of lower airway infections. Here, we demonstrated the grafting of the budesonide-loaded particles onto the PDMS tube in order to make the tubes with anti-inflammatory activity for lessening the inflammation problem during tracheostomy. The release profile of budesonide from the tube was evaluated. However release medium probably directly influence the release of the budesonide. Thus release medium conditions were varied with concentration of EtOH (Table 3.2).

Table 3.2 Conditions of release medium.

Temp. (°C)	pH	%(v/v) of PBS together with tween 20	%(v/v) of EtOH in release medium
37	5.8	5	50
37	5.8	5	20
37	5.8	5	5

In vitro drug release studies were determined using HPLC equipped with UV detector at 240 nm, with the aid of a calibration curve.

Calibration curve was created from a series of budesonide solutions freshly prepared in ethyl acetate at concentrations 10, 20, 30, 40 and 50 ppm. The obtained calibration curve was linear, retention time of budesonide solutions was 10 min (Figure C.1-C.6, Appendix C). The release behavior of budesonide from particles grafted onto the PDMS tube could be influenced by several factors.

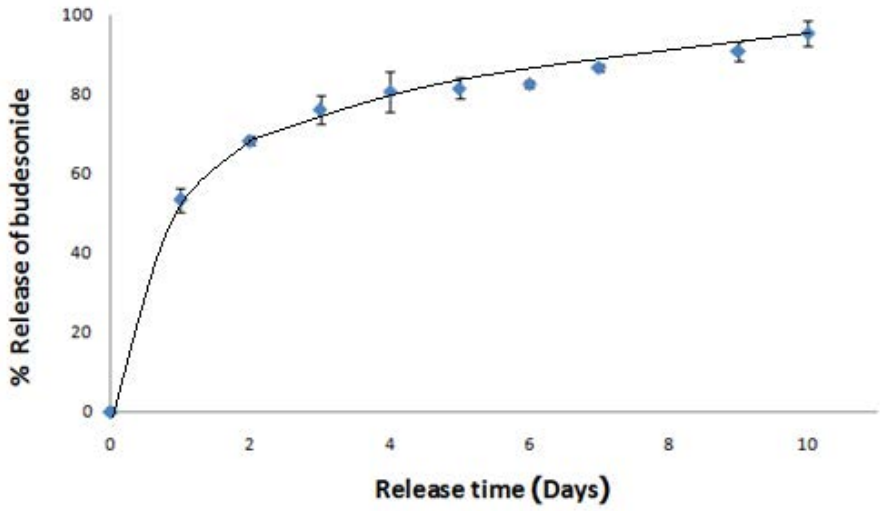


Figure 3.13 Release profile of budesonide from the surface of the PDMS tube in 5% (v/v) of PBS (pH 5.8) together with tween 20 and 50% (v/v) EtOH at the indicated times (0, 1, 2, 3, 4, 5, 6, 7, 9 and 10 days).

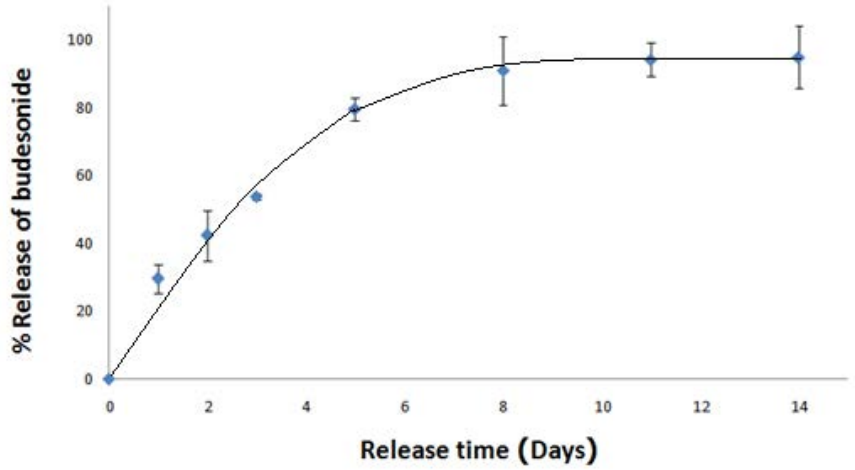


Figure 3.14 Release profile of budesonide from the surface of the PDMS tube in 5% (v/v) of PBS (pH 5.8) together with tween 20 and 20% (v/v) EtOH at the indicated times (0, 1, 2, 3, 5, 8, 11 and 14 days).

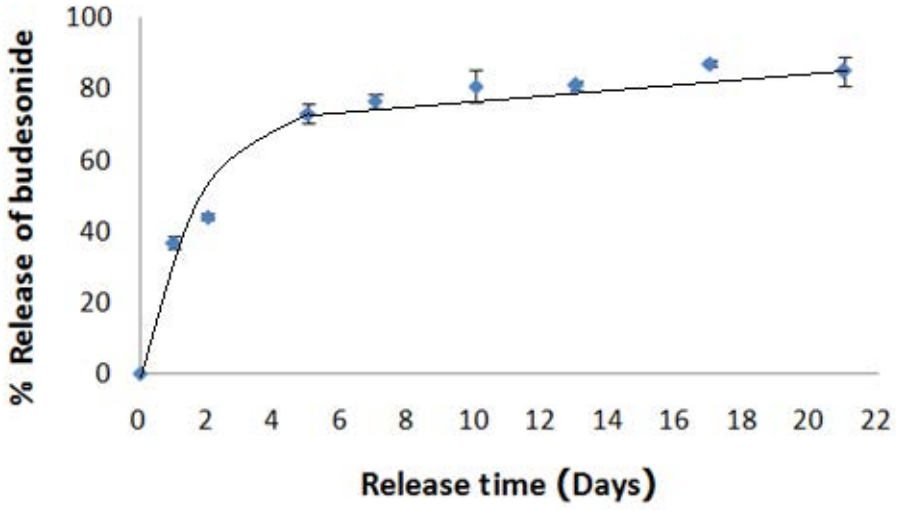


Figure 3.15 Release profile of budesonide from the surface of the PDMS tube in 5% (v/v) of PBS (pH 5.8) together with tween 20 and 5% (v/v) EtOH at the indicated times (0, 1, 2, 5, 7, 10, 13, 17 and 21).

Figure 3.13-3.15 showed the drug release behavior of the budesonide from budesonide-loaded particles grafted onto the PDMS tube into PBS measured *in vitro* at 37 ˚C. These Figures graph were plotted between % release of budesonide versus release time.

The results (Figure 3.13-3.15) indicated that the amount of released budesonide increased quite rapidly during the first five days. This could be interpreted as characteristic of the drug diffusion through the superficial layers of the polymeric particles. The fast release was likely a result of large drug concentration gradient between the sphere and the medium.

Table 3.3 Release medium system

Release medium system	Amount of EtOH % (v/v) in release medium	First day release (%)
1	50	53
2	20	30
3	5	35

As we expected, the controlled release profiles of different release medium were different.

From Table 3.2, we found that decreasing the amount of EtOH in the release medium led to the decrease of releasing rate in the first day. We concluded that the release of budesonide could be controlled by adjusting concentration of EtOH in release medium system.

In the present work, the 5% (v/v) of PBS (pH 5.8) with tween 20 and 5% (v/v) EtOH still showed controlled release of budesonide. This release medium although could not truly represent the tissue surrounding the tube, it closely represents the fluid around that area. Thus it was speculated that prolonged release of budesonide for more than 10 days could be achieved from our tube.

CHAPTER IV

CONCLUSION

The PDMS tube grafted with budesonide-loaded particles could be successfully fabricated. Slow release of budesonide from the modified tube was observed. Hydrophilic surface of PDMS was achieved by treating the tube with oxygen plasma. The treated surface was confirmed by contact angle measurement. Water contact angle of untreated and plasma treated PDMS surfaces were 107.28 ± 1.9° and 71.6 ± 6.5°, respectively. Grafting of γ-APS onto plasma-treated PDMS tube was confirmed by ninhydrin test. Budesonide was successfully encapsulated into EC using solvent displacement process. This process gave spherical shape of the particles with diameter of ~342.10 ± 85.04 nm. The loading capacity of budesonide was 33.26 ± 0.23% (w/w) and the encapsulation process gave 49.83 ± 0.11% of encapsulation efficiency. The budesonide-loaded EC particles were then grafted onto the modified PDMS tube. SEM images clearly indicated the presence of the particles on surface of the PDMS tube. The budesonide particles grafted tubes showed prolonged release for more than 10 days.

Suggestion for future work

1. Varying process parameters such as drug to polymer ratio
2. In vivo studies.

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APPENDICES

APPENDIX A

I. The contact angle data from

Table A1. Water contact angles of untreated PDMS tube

Liquid	Solid	No.	Left	Right	Mean	Height	Width
Water	PDMS	1	104.6	104.9	104.7	0.927	1.849
Water	PDMS	2	108.0	109.9	108.9	1.143	2.018
Water	PDMS	3	105.4	108.3	106.8	1.084	2.052
Water	PDMS	4	105.7	107.1	106.4	1.008	1.958
Water	PDMS	5	109.3	109.8	109.6	1.183	2.032

Table A2. Water contact angles of oxygen plasma treated PDMS tube for 1 cycle

Liquid	Solid	No.	Left	Right	Mean	Height	Width
Water	PDMS	1	89.8	95.1	92.4	0.94	2.436
Water	PDMS	2	97.8	97.4	97.6	1.866	2.369
Water	PDMS	3	101.6	101.5	101.5	1.065	2.22
Water	PDMS	4	108.5	107.5	108	1.144	2.129
Water	PDMS	5	97.6	95.3	96.5	1.006	2.272

Table A3. Water contact angles of oxygen plasma treated PDMS tube for 5 cycles

Liquid	Solid	No.	Left	Right	Mean	Height	Width
Water	PDMS	1	91.3	93.1	92.2	1.050	2.438
Water	PDMS	2	92.6	89.4	91.0	1.008	2.406
Water	PDMS	3	87.7	89.0	88.4	0.990	2.508
Water	PDMS	4	90.6	94.2	92.4	1.036	2.392
Water	PDMS	5	90.7	90.2	90.4	1.040	2.449

Table A4. Water contact angles of oxygen plasma treated PDMS tube for 10 cycles

Liquid	Solid	No.	Left	Right	Mean	Height	Width
Water	PDMS	1	76.3	75.6	76	0.747	2.663
Water	PDMS	2	73.8	76.8	75.3	0.795	2.665
Water	PDMS	3	78.8	75.4	77.1	0.652	2.037
Water	PDMS	4	66.5	56.8	61.6	1.514	2.920
Water	PDMS	5	69.0	66.8	67.9	0.488	1.838

Table A5. Water contact angles of untreated PDMS tube

Liquid	Solid	No.	Left	Right	Mean	Height	Width
Water	PDMS	1	99.6	103.4	101.5	1.555	2.315
Water	PDMS	2	102.2	101.8	102.0	1.107	2.115
Water	PDMS	3	108.7	109.1	108.9	1.231	2.082
Water	PDMS	4	108.7	102.2	105.4	1.174	2.057
Water	PDMS	5	112.3	110.9	111.6	1.201	1.940

Table A6. Water contact angles of piranha treated PDMS tube

Liquid	Solid	No.	Left	Right	Mean	Height	Width
Water	PDMS	1	86.9	90.5	88.7	1.033	2.519
Water	PDMS	2	92.6	93.9	93.2	1.168	2.481
Water	PDMS	3	103.8	102.9	103.3	1.263	2.178
Water	PDMS	4	101.9	109.8	105.8	1.348	2.178
Water	PDMS	5	99.3	98.5	98.9	1.240	2.252

APPENDIX B

I. Calculation of % encapsulation efficiency and loading content of budesonide into ethyl cellulose nanoparticles

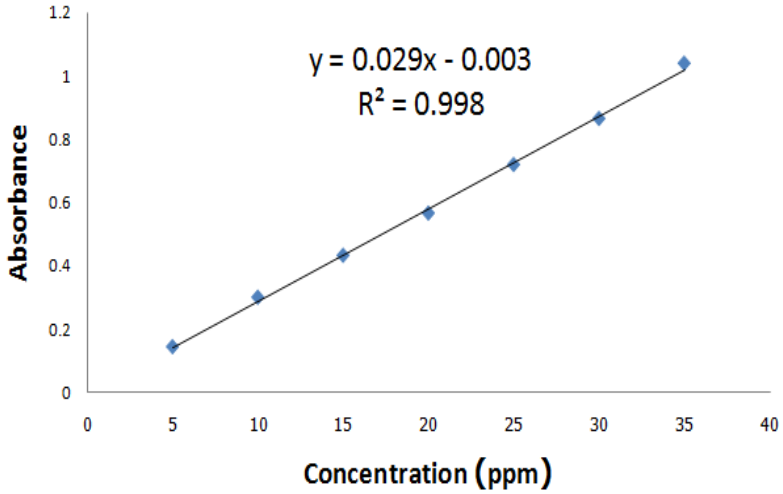

Figure B.1 Calibration curve of budesonide

By plotting a graph between absorbance and concentrations of budesonide solutions, a linear relationship was obtained and used for calculation of concentration of budesonide.

From the equation of calibration curve;

$$Y = 0.029X - 0.003,\ R^2 = 0.998 \quad (1)$$

The amount of budesonide at the outside of particles was calculated by equation (1);

$$0.290 = 0.029X - 0.003$$

$$X = 10.03 \text{ ppm} = 10.03 \text{ mg/L}$$

The budesonide diluted five-fold, so the amount of budesonide was 10.03 x 5 = 50.17 mg/L

In final volume of 1000 mL had budesonide of (50.17 x 1000)/1000 = 50.17 mg

Weight of employed budesonide and polymer were 100 and 100 mg

Weight of encapsulated budesonide = 100 – 50.17 = 49.83 mg

$$\% \text{ encapsulation efficiency (\% EE)} = \frac{\text{weight of encapsulated budesonid}}{\text{weight of budesonide initially used}} \times 100$$

$$= (49.83/100) \times 100$$

$$= 49.83\%$$

$$\% \text{ loading capacity (\% LC)} = \frac{\text{weight of encapsulated budesonide}}{\text{weight of encapsulated budesonide + polymer}} \times 100$$

$$= [49.83/ (49.83 + 100)] \times 100$$

$$= 33.26\ \%$$

Therefore, budesonide could be loaded and encapsulated into ethylcellulose nanoparticles with 49.83 and 33.26 %, respectively.

APPENDIX C

I. Calculation amount of budesonide release at the indicated times

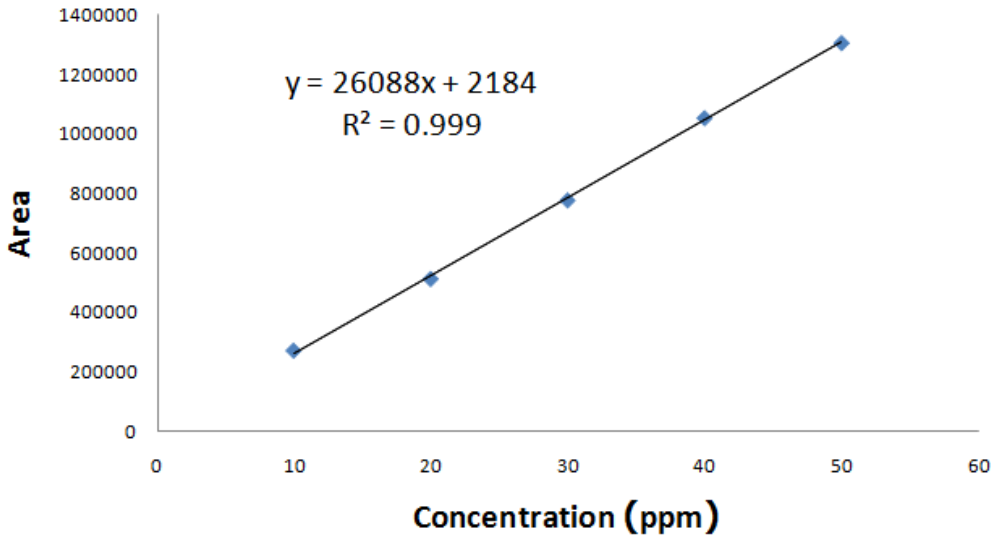

Figure C.1 Calibration curve of budesonide in EtOAc

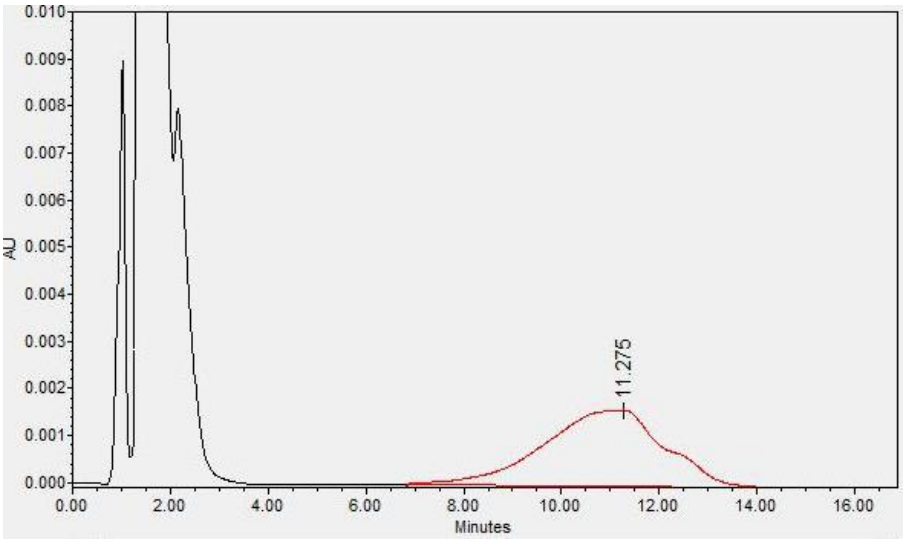

Figure C.2 Retention time and area of budesonide at 10 ppm

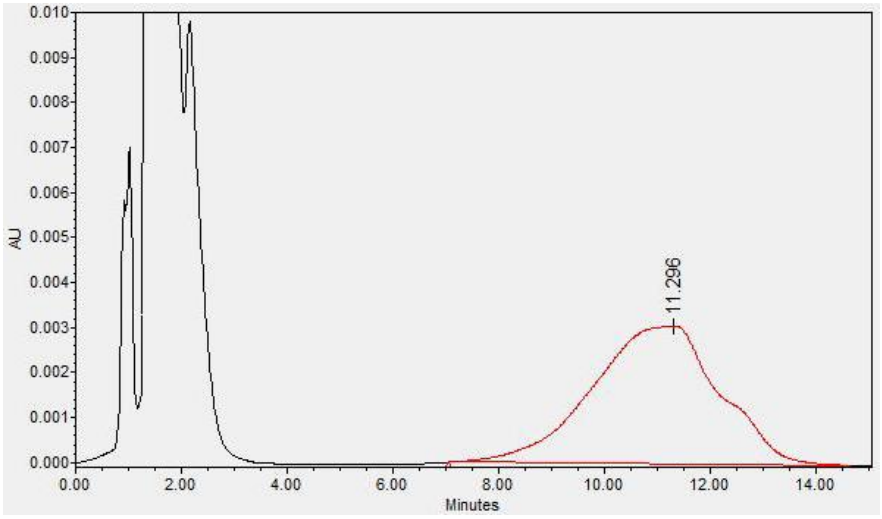

Figure C.3 Retention time and area of budesonide at 20 ppm

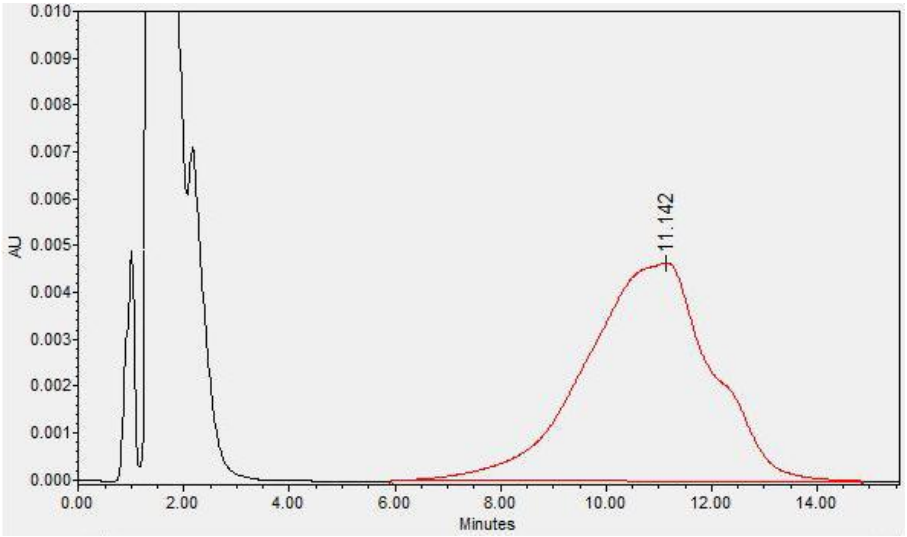

Figure C.4 Retention time and area of budesonide at 30 ppm

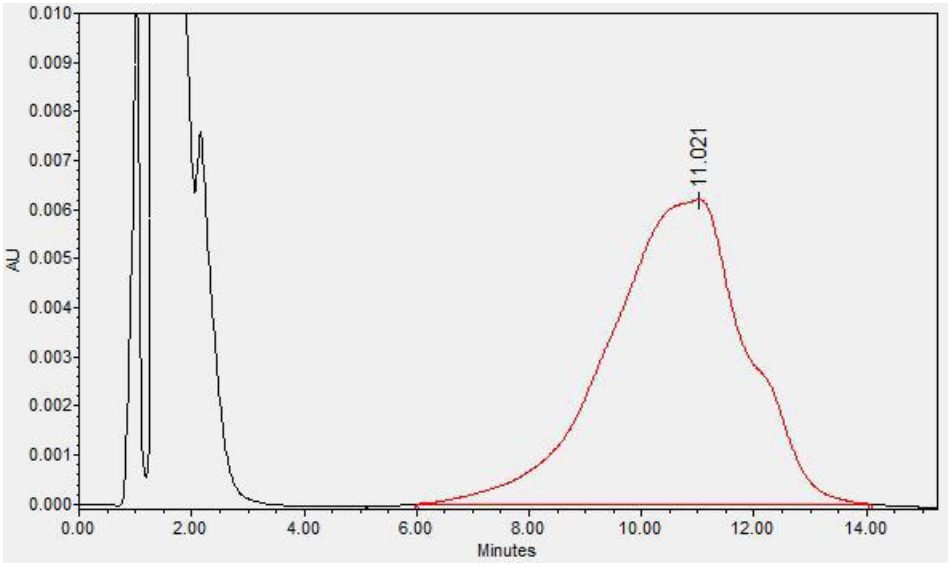

Figure C.5 Retention time and area of budesonide at 40 ppm

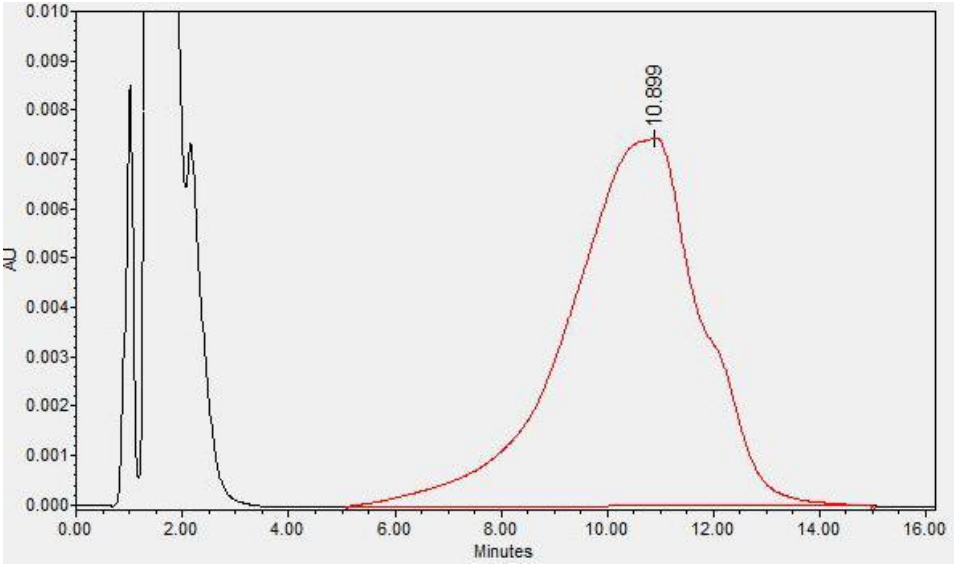

Figure C.6 Retention time and area of budesonide at 50 ppm

A) Amount of budesonide release at the indicated times

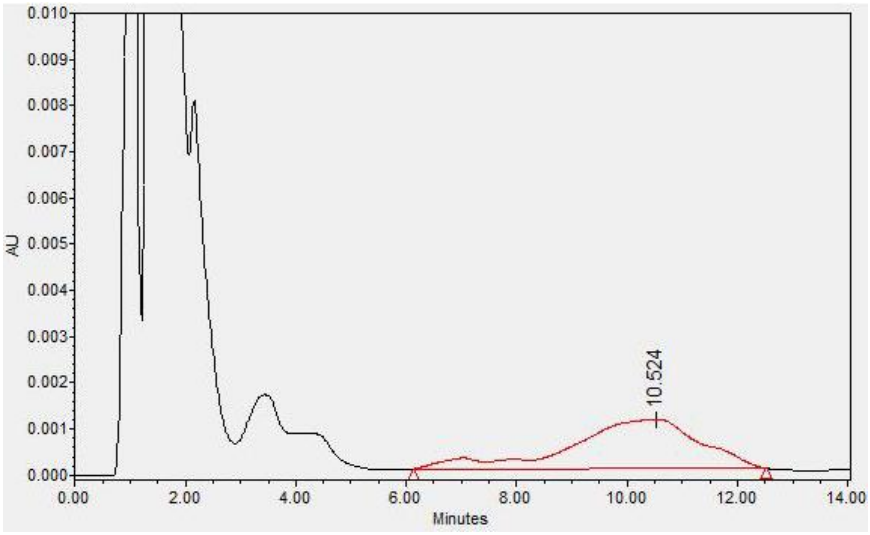

Figure C.7 Retention time and area of budesonide at day 1

Amount of budesonide release at day 1 was calculated by equation (1);

From the equation of calibration curve;

$$Y = 26088X + 2184,\ R^2 = 0.999\ (1);\ Y = \text{Area}$$

$$172850 = 26088X + 2184$$

$$X = 6.54$$

∴ Weight of budesonide in EtOAC extracted = 6.54 ppm

ppm → mg/L = 6.54 mg/L

Volumetric 1,000 ml gave amount of budesonide = 6.54 mg

In the 10×10^{-3} ml EtOAC extracted

Volumetric (inject into loop HPLC) 10×10^{-3} ml gave amount of budesonide

$$= 6.54\ (10\times10^{-3}) / 1{,}000 = 6.54\times10^{-5}\ \text{mg}$$

Total volume of 5 ml extracted medium gave total amount of budesonide

$$= \frac{(6.54\times10^{-5}) \times 5}{10\times10^{-3}} = 0.0327\ \text{mg}$$

Amount of budesonide release at day 2 was calculated by equation (1);

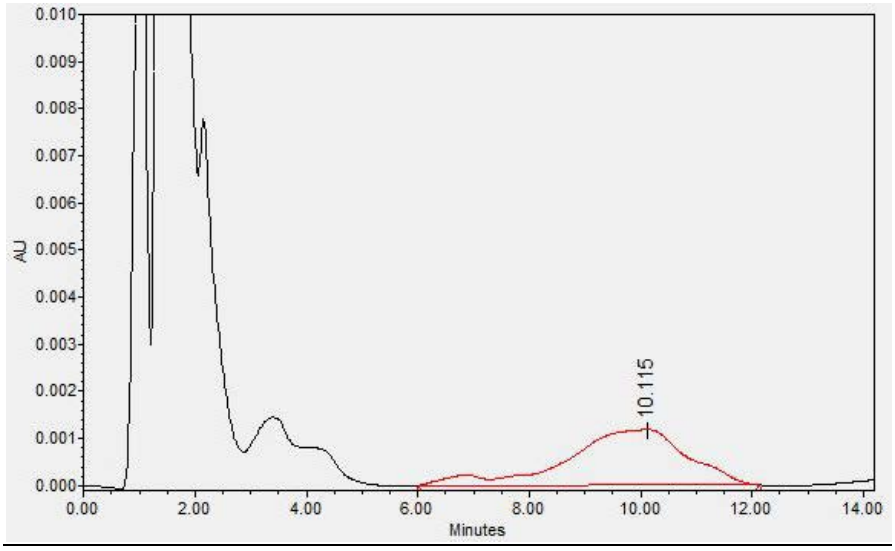

Figure C.8 Retention time and area of budesonide at day 2

From the equation of calibration curve;

$$Y = 26088X + 2184,\ R^2 = 0.999\ (1);\ Y = \text{Area}$$

$$178183 = 26088X + 2184$$

$$X = 6.75$$

∴ Weight of budesonide in EtOAC extracted = 6.75 ppm

ppm → mg/L = 6.75 mg/L

In the 10×10^{-3} ml EtOAC extracted

In volumetric (inject into loop HPLC) 10×10^{-3} ml gave amount of budesonide

$$= 6.75\ (10\times10^{-3}) / 1{,}000 = 6.75\times10^{-5} \text{ mg}$$

Total volume of 5 ml extracted medium gave total amount of budesonide

$$= \frac{(6.75\times10^{-5}) \times 5}{10\times10^{-3}} = 0.0338 \text{ mg}$$

Total Amount of budesonide release in day 2 = day2 + withdraw 0.5ml at day1

= 0.0338 + (6.54×0.5) / 1,000

= 0.0338 + 0.00327

= 0.0371 mg

Amount of budesonide release at day 3 was calculated by equation (1);

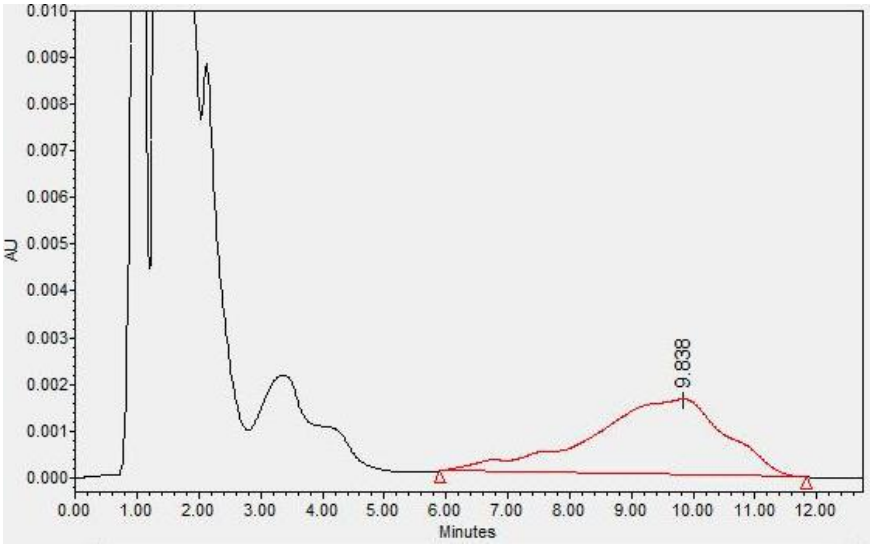

Figure C.9 Retention time and area of budesonide at day 3

From the equation of calibration curve;

$$Y = 26088X + 2184,\ R^2 = 0.999\ (1);\ Y = \text{Area}$$

$$245826 = 26088X + 2184$$

$$X = 9.34$$

∴ Weight of budesonide in EtOAC extracted = 9.34 ppm

ppm → mg/L = 9.34 mg/L

In the 10×10^{-3} ml EtOAC extracted

In volumetric (inject into loop HPLC) 10×10^{-3} ml gave amount of budesonide

$$= 9.34\ (10\times10^{-3}) / 1{,}000 = 9.34\times10^{-5}\ \text{mg}$$

Total volume of 5 ml extracted medium gave total amount of budesonide

$$= \frac{(9.34\times10^{-5})\times5}{10\times10^{-3}} = 0.0467\ \text{mg}$$

Total Amount of budesonide release in day 3

= day3 + withdraw 0.5ml at day1 + withdraw 0.5ml at day2

= 0.0467 + 0.00327 + (6.75×0.5) / 1,000

= 0.0467 + 0.00327 + 0.00338

= 0.0534 mg

Amount of budesonide release at day 5 was calculated by equation (1);

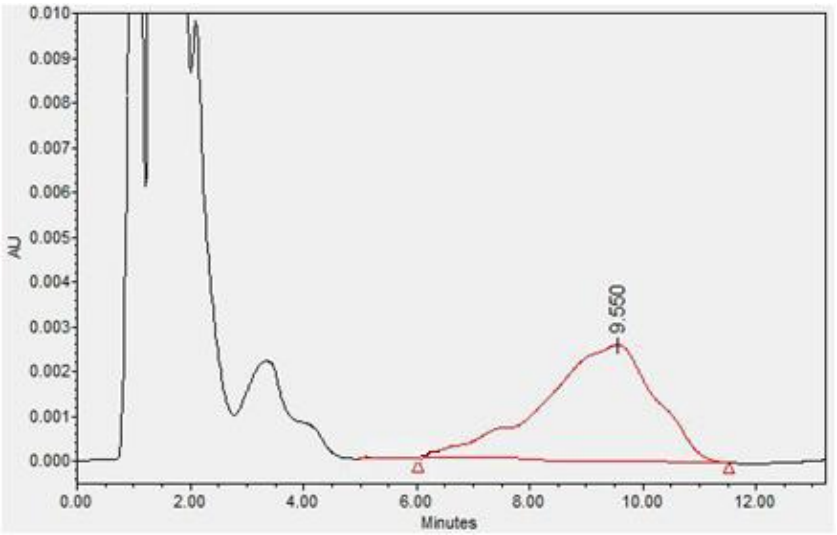

Figure C.10 Retention time and area of budesonide at day 5

From the equation of calibration curve;

$$Y = 26088X + 2184,\ R^2 = 0.999\ (1);\ Y = \text{Area}$$

$$371504 = 26088X + 2184$$

$$X = 14.16$$

∴ Weight of budesonide in EtOAC extracted = 14.16 ppm

ppm → mg/L = 14.16 mg/L

In the 10×10^{-3} ml EtOAC extracted

In volumetric (inject into loop HPLC) 10×10^{-3} ml gave amount of budesonide

$$= 14.16\ (10\times10^{-3}) / 1{,}000 = 1.42\times10^{-4}\ \text{mg}$$

Total volume of 5 ml extracted medium gave total amount of budesonide

$$= \frac{(1.42\times10^{-4})\times5}{10\times10^{-3}} = 0.0710\ \text{mg}$$

Total Amount of release budesonide in day 5

= day4 + withdraw 0.5ml at day1 + withdraw 0.5ml at day2 + withdraw 0.5ml at day3

$$= 0.0710 + 0.00327 + 0.00338 + (9.34\times0.5) / 1{,}000$$

$$= 0.0710 + 0.00327 + 0.00338 + 0.00467$$

$$= 0.0823\ \text{mg}$$

B) Amount of budesonide extract in EtOH

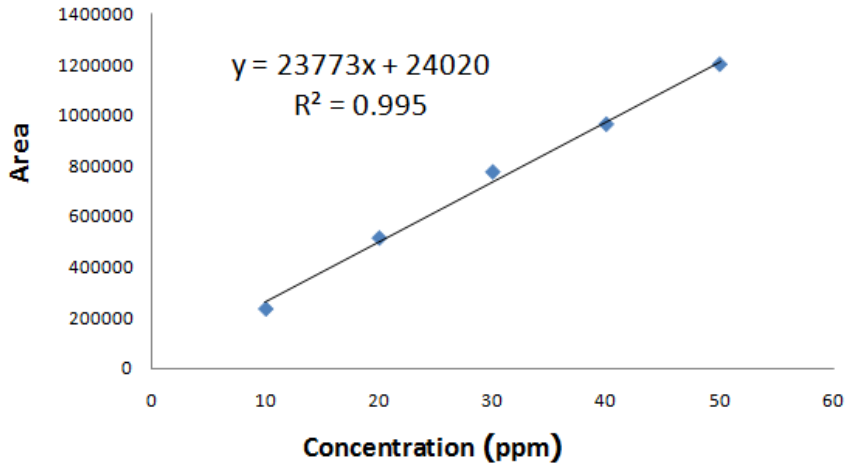

Figure C.11 Calibration curve of budesonide

From the equation of calibration curve;

$$Y = 23773X - 24020,\ R^2 = 0.995 \quad (1)$$

Amount of budesonide extract was calculated by equation (1);

$$657180 = 23773X - 24020$$

$$X = 28.65$$

∴ Weight of budesonide in EtOAC extracted = 28.65 ppm

ppm → mg/L = 28.65 mg/L

Total volume of 5 ml extracted medium gave total amount of budesonide

$$= (28.65 \times 5)/ 1{,}000$$

$$= 0.1433 \text{ mg}$$

II. Calculation % budesonide release at the indicated times

Amount of % budesonide release at day 1

Amount of budesonide extract 0.1433 mg	=	100
Amount of budesonide release 0.0327 mg	=	100(0.0327)/ 0.1433
% budesonide release at day 1	=	22.82 %

Amount of % budesonide release at day 2

Amount of budesonide extract 0.1433 mg	=	100
Amount of budesonide release 0.0371 mg	=	100(0.0371)/ 0.1433
% budesonide release at day 2	=	25.90 %

Amount of % budesonide release at day 3

Amount of budesonide extract 0.1433 mg	=	100
Amount of budesonide release 0.0534 mg	=	100(0.0534)/ 0.1433
% budesonide release at day 3	=	37.26 %

Amount of % budesonide release at day 5

Amount of budesonide extract 0.1433 mg	=	100
Amount of budesonide release 0.0823 mg	=	100(0.0823)/ 0.1433
% budesonide release at day 4	=	57.43 %

APPENDIX D

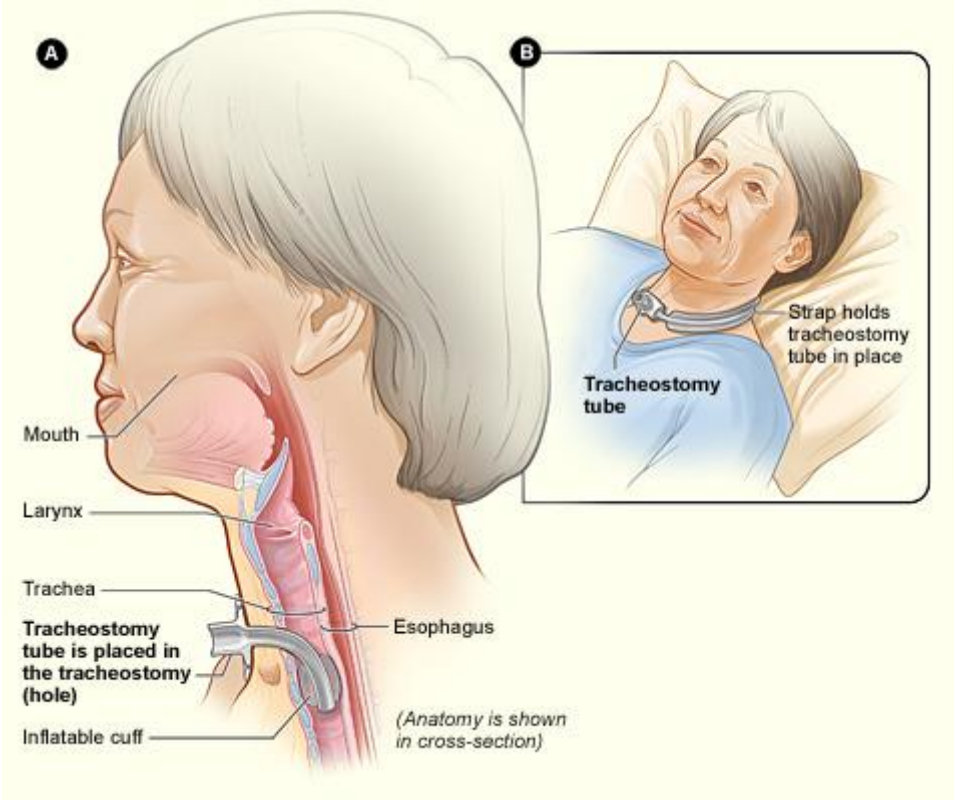

Figure D1. A) show a side view of the neck and the correct placement of a trach tube in the trachea, or windpipe B) show external view of a patient who has a tracheostomy [41]

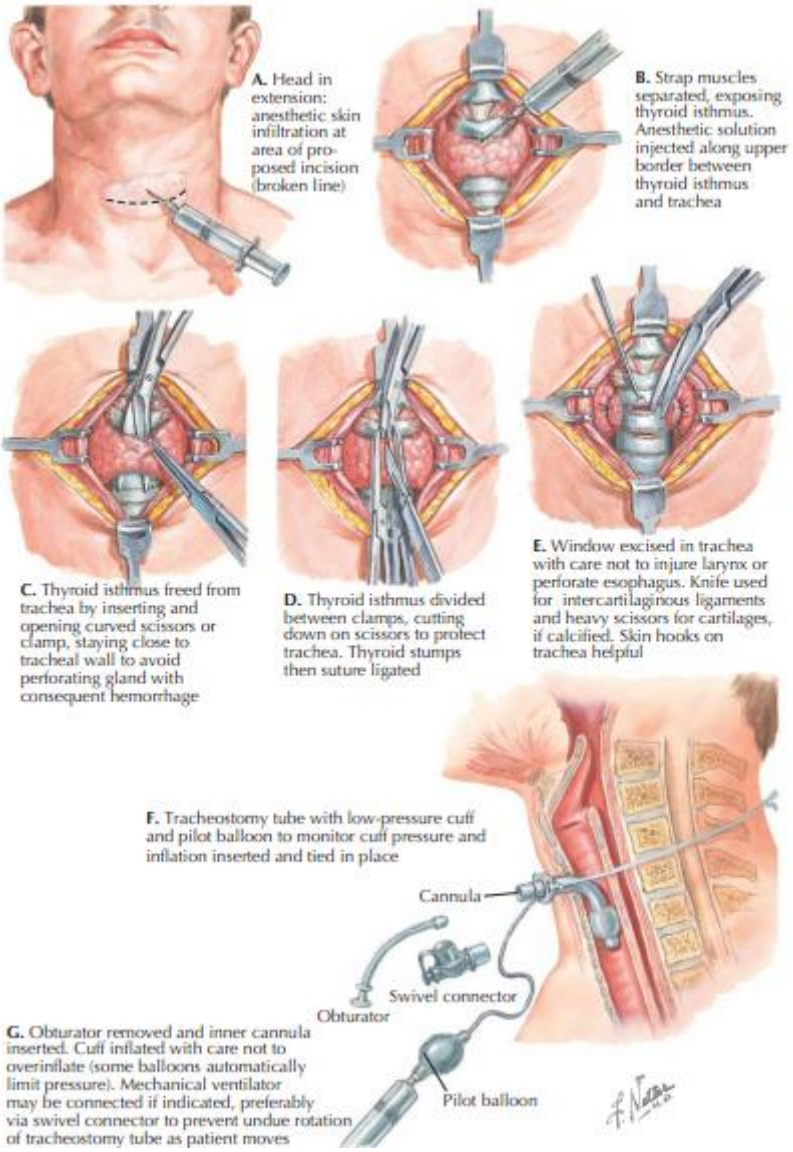

Figure D2. Schematic representation of tracheostomy [41]

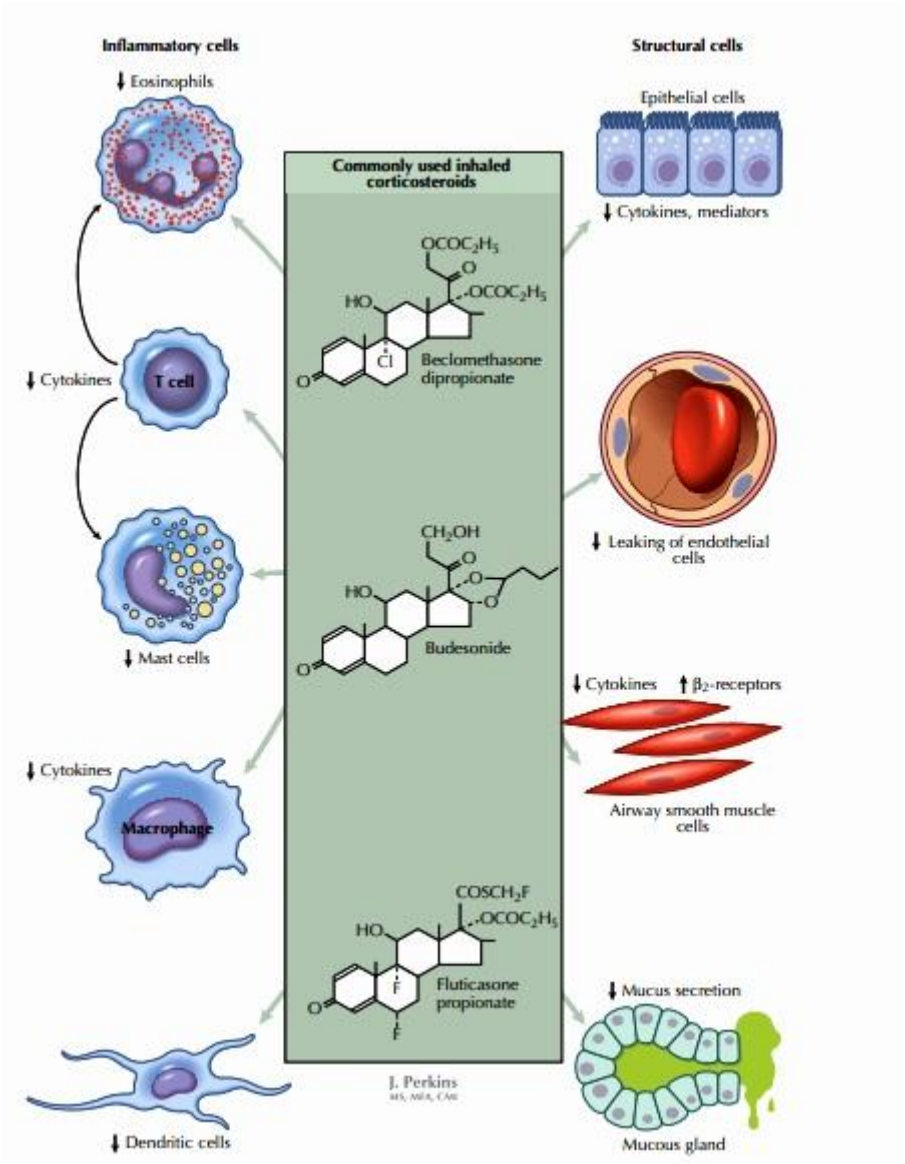

Figure D3. Mechanism of action of corticosteroids in asthma [41]

The mechanism of action of corticosteroids in asthma is most likely related to their anti inflammatory properties. Corticosteroids have widespread effects on gene transcription, increasing transcription of anti inflammatory genes and more importantly suppressing transcription of multiple inflammatory genes. At a cellular level, they have inhibitory effects on many inflammatory and structural cells that are activated in asthma. The inhibitory action of inhaled corticosteroids on airway epithelial cells may be particularly important; in a reduction in airway hyper responsiveness, but in long-standing asthma,

airway hyper responsiveness may not return to normal because of irreversible structural changes in airways.

VITAE

Miss Jiratchaya Mokkaphan was born on June 14, 1987 in Bangkok, Thailand. She got a Bachelor's Degree of Science in Chemistry from Silpakorn University in 2009. And then, she started her graduate study a Master's Degree in Program of Petrochemistry and Polymer Science, Faculty of Science at Chulalongkorn University in 2009.

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Academic Presentation

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