การพัฒนาตำรับยาเม็ดนำส่งทางกระพุ้งแก้มที่ประกอบด้วยไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคติน

นางสาวชลวิภา ยารังษี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชอุตสาหกรรม ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม
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FORMULATION DEVELOPMENT OF BUCCAL TABLETS CONTAINING MUCOADHESIVE NICOTINE MICROCAPSULES

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy
Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmaceutical Sciences
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Thesis Title	FORMULATION DEVELOPMENT OF BUCCAL TABLETS CONTAINING MUCOADHESIVE NICOTINE MICROCAPSULES
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ชลวิภา ยารังษี : การพัฒนาตำรับยาเม็ดนำส่งทางกระพุ้งแก้มที่ประกอบด้วยไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคติน. (FORMULATION DEVELOPMENT OF BUCCAL TABLETS CONTAINING MUCOADHESIVE NICOTINE MICROCAPSULES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ.ดร.นฤพร สุตัณฑวิบูลย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ.ดร.พรรณเพ็ญ วัฒนาอาษากิจ, 122 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาและประเมินยาเม็ดนำส่งทางกระพุ้งแก้มที่ประกอบด้วยไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคติน ไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคตินเตรียมโดยเครื่องเคลือบฟลูอิดไดซ์เบดชนิดสเปรย์ด้านล่าง ไฮดรอกซีโพรพิลเมธิลเซลลูโลส อีสิบห้า (HPMC E15) ใช้เป็นพอลิเมอร์ชนิดยึดติดเยื่อเมือกโดยปรับปริมาณในสารละลายเคลือบ สารละลายเคลือบที่ประกอบด้วยปริมาณของพอลิเมอร์ร้อยละ 5 โดยน้ำหนักที่เพิ่มขึ้น ส่งผลต่อพฤติกรรมการยึดติดเยื่อเมือกได้เหมาะสมที่สุด แม้ว่าปริมาณของ HPMC E15 เพิ่มขึ้นมากกว่าร้อยละ 5 โดยน้ำหนักที่เพิ่มขึ้นไม่สามารถเพิ่มเวลาในการยึดติดเยื่อเมือกได้ การตรวจสอบคุณสมบัติเชิงของแข็งของนิโคตินไฮโดรเจนทาร์เทรตไดไฮเดรท (nicotine hydrogen tartrate dihydrate) ในไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคติน ด้วยโซลิดสเตท 13 คาร์บอนนิวเคลียร์แมกเนติกเรโซแนนซ์ (solid state ^{13}C nuclear magnetic resonance) พบว่าการเพิ่มเติมสารเติมแต่งและกระบวนการเคลือบด้วยฟลูอิดไดซ์เบดไม่มีผลต่อการเปลี่ยนแปลงคุณสมบัติเชิงของแข็งของนิโคตินไฮโดรเจนทาร์เทรตไดไฮเดรทในไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคติน ยาเม็ดนำส่งทางกระพุ้งแก้มที่ประกอบด้วยไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคตินเตรียมในรูปแบบยาเม็ดสองชั้นด้วยการตอกอัดโดยตรง ตำรับยาเม็ดที่เหมาะสมในชั้นที่มีนิโคตินประกอบด้วยไมโครแคปซูลชนิดยึดติดเยื่อเมือกของนิโคติน 140 มิลลิกรัม, ดี-แกรนูล 510 มิลลิกรัม, ซูคราโลส 1 มิลลิกรัม, เมนทอล 12 มิลลิกรัม, แมกนีเซียมสเตียเรทร้อยละ 0.5 และทาลคัมร้อยละ 2 การทดสอบคุณสมบัติทางเคมีกายภาพของยาเม็ด แสดงผลที่น่าพอใจจากความแปรปรวนของน้ำหนักยาเม็ดเท่ากับ 0.08±0.56%, ความหนา 5.59±0.01 มิลลิเมตร, ความแข็ง 12.95±0.68 กิโลกรัมต่อตารางเซนติเมตร และความกร่อนร้อยละ 0.14 ยาเม็ดถูกประเมินสำหรับการปลดปล่อยแบบนอกกายของนิโคตินโดยใช้เซลล์แพร่ฟรานซ์แบบดัดแปร พบว่าสามารถปลดปล่อยนิโคตินได้สูงสุดเท่ากับ 61.38% ในระยะเวลาหกชั่วโมง โดยอัตราการปลดปล่อยเป็นไปตามอัตราจลนศาสตร์อันดับศูนย์

ภาควิชา...วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม.....ลายมือชื่อนิสิต.....
สาขาวิชา…….เภสัชอุตสาหกรรม…….. ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก…….....
ปีการศึกษา……..2552……. ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม....……..

4976565833 : MAJOR INDUSTRIAL PHARMACY
KEYWORDS: NICOTINE/ FLUIDIZED BED/ MUCOADHESIVE/ MICROCAPSULE/ BUCCAL TABLET

CHONWIPA YARANGSEE: FORMULATION DEVELOPMENT OF BUCCAL TABLETS CONTAINING MUCOADHESIVE NICOTINE MICROCAPSULES. THESIS ADVISOR: NARUEPORN SUTANTHAVIBUL, Ph.D., THESIS CO-ADVISOR: PHANPHEN WATTANAARSAKIT, Ph.D., 122 pp.

The present study aim to develop and evaluate buccal tablets containing mucoadhesive nicotine microcapsules. The mucoadhesive nicotine microcapsules were prepared by bottom spray fluidized-bed coater. HPMC E15 was used as the mucoadhesive polymer by varying the amount in the coating solution. Coating solution of 5% weight gain of HPMC E15 resulted in the most desirable mucoadhesive behavior. Although, the amounts of HPMC E15 were increased to more than 5% weight gain, it could not further extend the mucoadhesion time. Solid state characterization of nicotine hydrogen tartrate dihydrate (NHT) in mucoadhesive nicotine microcapsules was carried out by solid state ^{13}C nuclear magnetic resonance and revealed that the addition of additives and the fluidized-bed coating process did not affect the solid state modification of NHT within the mucoadhesive nicotine microcapsules. Buccal tablets containing mucoadhesive nicotine microcapsules were prepared as a bilayer tablet by direct compression. Optimum formulation of nicotine-containing layer consisted of mucoadhesive nicotine microapsules 140 mg, D-granules 510 mg, sucralose 1 mg, menthol 12 mg, magnesium stearate 0.5% and talcum 2%. Various physicochemical evaluation of optimal formulation showed satisfactory results as seen from weight variation (0.08±0.56%), thickness (5.59±0.01mm), hardness (12.95±0.68 kg/cm^2) and friability (0.14%). Tablets were evaluated for *in vitro* release of nicotine using modified Franz diffusion cells which illustrates a maximum nicotine release of 61.38% after 6 hours. The release rate followed zero-order rate kinetics.

Department: Pharmaceutics and Industrial Pharmacy… Student's signature:.....
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LIST OF ABBREVIATIONS

%	Percentage
μg	Microgram (s)
μm	Micrometer (s)
μl	Microliter (s)
°C	Degree celcius (centrigrade)
cm	Centrimeter (s)
CV	Coefficient of variation
Cu	Curium
et al.	*et alii,* 'and others'
EtOH	Ethanol
g	Gram (s)
h	Hour (s)
HCl	Hydrochloride
HPLC	High performance liquid chromatographic
HPMC	Hydroxypropylmethylcellulose
kg	Kilogram(s)
kV	Kilovolt (s)
M	Molar (mole/litre)
MeOH	Methanol
MHz	Megahurtz
mM	Millimolar
mg	Milligram (s)
min	Minute (s)
ml	Milliliter (s)
mm	Millimeter (s)
NHT	Nicotine hydrogen tartrate dihydrate
pH	The negative logarithm of the hydrogen ion concentration
q.s.	*Quantum sufficit,* 'sufficient quantity'
rpm	Revolutions per minute
RH	Relative humidity

RSD	Relative standard deviation
s	Second (s)
SD	Standard deviation
SEM	Scanning electron microscopy
UV	Ultraviolet
v/v	Volume by volume
w/w	Weight by weight

CHAPTER I
INTRODUCTION

Cigarette smoking is the leading cause of health harm and the use of tobacco is still increasing globally (Mackay and Eriksen, 2002). Smoking cessation is essential to the health of the current smokers, but hard to achieve because of nicotine dependence. This dependence results in a withdrawal syndrome when stop smoking, leading to early relapse in significant number of cases (Kochak, Sun, Choi and Piraino, 1992). Smokers who quit smoking reduce their risk of tobacco-related disease, prolong their lives substantially and increase the national income.

Nicotine is the main alkaloid in tobacco, it is not a direct cause of most tobacco-related diseases, but it is reliable for causing dependence. The addictiveness of nicotine is the cause of continuing use of tobacco products (Hukkanen et al, 2005). Hence, the most widely used for smoking cessation is nicotine replacement therapy (NRT) which offers an effective treatment to act in smoking cessation. The primary goal of nicotine replacement is to relieve smoking withdrawal symptoms, which include craving for cigarettes, irritability, anxiety, restlessness, depressed mood, difficulty concentrating, insomnia, increased appetite and weight gain (Rose, 1996).

Nicotine replacement therapy helps the smokers to defeat these withdrawal symptoms by providing nicotine in therapeutic doses in a tapering manner over a period of time. Currently, the commercially available forms of NRT for example chewing gum, transdermal patch, nasal spray, inhaler, sublingual tablet and lozenge, which have specific advantages (Cummings and Hyland, 2005). In Thailand, there are only two commercially forms of NRT, nicotine gum and transdermal patch. The nicotine in the gum is either 2 mg and 4 mg in strength and the patches range in strength from 7 mg to 21 mg of nicotine.

Nicotine is extensively first pass metabolized by the liver resulting in low bioavailability. Therefore, NRT products are preferably designed to deliver nicotine via the route that avoid hepatic first pass metabolism. Nicotine gum contains nicotine

in a resin base that the absorption is through buccal mucosa. However, nicotine chewing gum has drawbacks: its taste is objectionable, it is difficult to chew, and it causes jaw ache, hiccup and dyspepsia (Mitrouska et al, 2007; Rose, 1996). Such common adverse effects of nicotine gum may reduce patient compliance. Others disadvantages include that chewing gum is probably socially unacceptable to some smokers or under some social circumstances and that it is contraindicated for people with dentures or other dental appliances (Lane et al., 1993). The residual nicotine retained in the gum caused nicotine absorbed is inadequate. These limiting factors leading to treatment failure. In addition, cost of NRT products is the most frequently cited reason for smokers who have never used any nicotine medications. In Thailand, there is only the generic nicotine chewing gum nowadays. Nicotine transdermal patches have to import and the price for full course treatment is expensive.

Drug delivery via the buccal route provides an alternative to oral administration. On the other hand, the buccal mucosa is relatively permeable with a rich blood supply. Buccal delivery provides direct entry into the systemic circulation, thus avoiding the hepatic first pass metabolism, ensuring ease of administration, and the ability to produce a systemic effect with a rapid onset of action. Moreover, the route provides reasonable patient acceptance and compliance and the dosage form can be removed at any time (Park and Munday, 2002; Patel et al, 2007). Consequently, buccal delivery of nicotine was chosen for development in this study due to the fact that the system could bypass hepatic first pass metabolism, as mentioned above.

However, nicotine provides acrid burning taste when dissolved and swallowed. Therefore, the mucoadhesive microcapsule system was chosen to mask undesirable taste and prolong contacts of nicotine to buccal mucosa through mucoadhesion in order to prevent swallowing. Mucosal adhesive materials have been identified and investigated in previous work (Smart, 1993; Eouani et al., 2001; Miller, Chittchang, and Johnston, 2005). The mucosal adhesive materials widely used are hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC) and poly(acrylic acids) (Fabregas and Garcia, 1995; Han, Fang, Sung and Hu, 1999; Park and Munday, 2002; Ìkinci, Senel, Wilson and Sumnu, 2004). The mechanisms of mucoadhesion between adhesive materials and mucus layer are adsorption, diffusion,

electronic, fracture and wetting (Peppas and Buri, 1985; Duchene, Touchard and Peppas, 1988; Mortazavi and Smart, 1995; Miller, Chittchang and Johnson, 2005).

From the recent studied, buccal nicotine bioadhesive matrix tablet was produced. To our knowledge, formulation of the buccal tablet containing mucoadhesive nicotine microcapsules has never been examined. Nicotine hydrogen tartrate (NHT) is more stable than nicotine base. Thus, it was selected to be used in this study. Nicotine was designed as mucoadhesive nicotine microcapsules using fluidized bed coating technique and coating agent was HPMC E15. The buccal tablet containing mucoadhesive nicotine microcapsules that equivalent to 2 mg of nicotine were prepared by direct tablet compression.

The study was aimed to develop the mucoadhesive nicotine microcapsules and to formulate the buccal tablets containing mucoadhesive nicotine microcapsules that provided drug release gradually and expect to have no buccal side effect.

Objectives of the present study were:

1. To develop the mucoadhesive nicotine microcapsules by microencapsulation technique.
2. To develop the buccal tablets containing mucoadhesive nicotine microcapsules.

CHAPTER II
LITERATURE REVIEW

1. The hazards of smoking

Cigarette smoking is very dangerous and widely prevalent in both sexes, men and women. Most people continue to smoke because they are addicted to nicotine. Smoking killed 100 million people in the 20th century, and is predicted to kill one billion in the 21st century (Britton and Edwards, 2008). The most harmful of smoking due to it delivers nicotine in combination with many other toxins and carcinogens. These toxins and carcinogens such as tar and carbon monoxide, play a major role for adverse health effects of smoking.

Smoking leads to development of many serious health risks that caused human death. Some are listed below (Benowitz, 1986).

(a) Chronic lung disease

According to the American Lung Association, smoking is directly responsible for about 90% of the deaths due to lung cancer. Smoking is the major risk factor for development of chronic obstructive pulmonary disease (COPD), which includes emphysema and chronic bronchitis. It is possible that nicotine promotes the inflammatory response and contributes to the development of chronic lung disease.

(b) Coronary and peripheral vascular disease

Nicotine could conduce to atherosclerotic disease by actions on lipid metabolism, coagulation, and hemodynamic effects. Smoking and being exposed to secondhand smoke greatly increase the risk of a heart attack.

The blood of smokers is known to coagulate more easily and nicotine can promote thrombosis. Nicotine increases heart rate and blood pressure and, therefore, myocardial oxygen consumption. Carbon monoxide inhaled in cigarette smoke

reduces the oxygen-carrying capacity of the blood. Furthermore, the sympathomimetic effects or prostacyclin inhibition of nicotine induce coronary spasm. All these factors contribute to cause acute myocardial infarction in a person with preexisting coronary atherosclerosis.

(c) Cancer

Nicotine is not in itself carcinogenic, but has been shown to be co-carcinogenic with benzo-a-pyrene. The mechanism of co-carcinogenic is not established. Nicotine can be nitrosated in tobacco smoke to form nitrosonornicotine and other related compounds. Tobacco-specific *N*-nitrosamines are highly carcinogenic.

(d) Peptic ulcer disease

Smoking also has been shown to decrease pancreatic fluid and bicarbonate secretion, resulting in greater and more prolonged acidity of gastric fluid. It also reduces blood flow and production of compounds that protect the stomach lining.

In addition, women who smoke generally have earlier menopause than nonsmoker. Smoking during pregnancy increases risk of stillborn or premature infants or infants with low birth weight. Children of women who smoked while pregnant have an increased risk for developing behavior disorders (http://health.nytimes.com/health/guides/disease/nicotine-withdrawal/health-risks.html).

2. Nicotine Replacement Therapy (NRT)

Smoking is a highly addictive behavior that causes the chronic diseases. The success rate of quit attempt is quite low due to the nicotine withdrawal symptoms. In order to improve smoking cessation rate, more effective treatment are required. Nicotine replacement therapy (NRT) was the first successful pharmacologic interventions for nicotine addiction and is now widely employed (Mitrouska, Bouloukaki and Siafakas, 2007). Therefore, according to UK and US Public Health

Service guidelines, all smokers should be considered for pharmacotherapies and NRT is also recommended as a first-line treatment (Hatsukami, Stead and Gupta, 2008).

Medicinal nicotine is mainly used to replace nicotine in tobacco smokers to maintain some of nicotine effects while also reducing the addiction potential. The actions of NRT underlie the concept of stimulation of nicotine receptors in the ventral tegmental area of the brain and the consequence release of dopamine in the nucleus accumbens. These actions of nicotine lead to decrease in withdrawal symptoms (Benowitz, 1996).

Table 1 FDA-approved nicotine replacement therapies (Cummings and Hyland, 2005).

Nicotine medication	Year approved	Dose	Advantages	Disadvantages
Gum	1984 (2mg Rx) 1992 (4mg Rx) 1996 (OTC)	2 or 4 mg per piece	Oral administration; comes in different flavors	Low compliance; under dosing is common
Patch	1992 (Rx) 1996 (OTC)	16-hour patch: 15, 10, 5 mg; 24-hour patch: 21, 14, 7 mg	Once a day administration	Fixed dose; slow delivery not conducive to treating acute
Nasal spray	1996 (Rx)	10 mg/ml, 0.5 mg per spray	Fast delivery of nicotine	Unpleasant side-effects discourage repeated use
Inhaler	1997 (Rx)	10 mg per cartridge	Hand-to-mouth action simulates smoking habit; comes in menthol flavor	Low compliance; under dosing is common
Lozenge	2003 (OTC)	1, 2, or 4 mg per piece	Oral administration; faster nicotine delivery than gum	Low compliance; under dosing is common

OTC, over the counter; Rx, prescription

Two-milligram prescription-only nicotine gum was first approved in the United States in 1984 (Table 1). Prescription-only nicotine patches were introduced in 1992, followed by different nicotine dose and medication formulations including 4-mg nicotine gum (1992), a nasal spray (1996), inhaler (1997), and lozenges (2003). Nicotine medications appear to aid smokers in quitting by providing relief from nicotine withdrawal symptoms typically experienced during the first few days and weeks of abstinence from tobacco.

Nicotine replacement products differ in their patterns, rates, and quantities of dosing and in resultant pharmacological effects. Different types of NRT may be more useful for different smokers. The U.S. Food and Drug Administration (FDA) has approved nicotine patches, gum and lozenge available over the counter (OTC) in order to increase access to these medications. Nicotine chewing gum and lozenge formulations deliver nicotine through buccal mucosa, where the nicotine is rapidly absorbed. Nicotine lozenges are easy to use and the amount of nicotine absorbed per lozenge is somewhat higher than that deliver by nicotine gum which is probably due to the residual nicotine retained in the gum (Henningfield et al., 2005; Mitrouska, Bouloukaki and Siafakas, 2007).

Shiffman et al. (2002) and Shiffman (2005) reported that nicotine lozenge significantly increased quit rates relative to placebo among light smokers and its efficacy among light smoker did not differ from that among heavier smokers. Furthermore, nicotine lozenge treatment was significantly effective for smokers with past failure in pharmacological treatment (Shiffman, Dresler and Rohay, 2004).

3. Nicotine

3.1 Chemical properties of nicotine

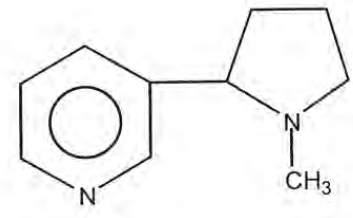


Figure 1 Chemical structure of nicotine (Canney, 2006)

Chemical name:	S-3-(1-methyl-2-pyrrolidinyl) pyridine
Molecular formula:	$C_{10}H_{14}N_2$
Molecular weight:	162.23
Solubility:	Miscible with water. Soluble in alcohol, chloroform, ether, light petroleum and fixed oils.

Nicotine is a tertiary amine composed of pyridine and pyrrolidine rings. It was determined to be the major constituent of tobacco. It a highly purified extract obtained from the dried leaves of the tobacco plant, *Nicotiana tobacum*. The structure of nicotine is shown in Figure 1. Pure nicotine is a clear to pale yellow oily liquid whereas it turns brown on exposure to air or light. It is volatile and hygroscopic liquid with a characteristic odor and an acrid burning taste. It can mix with water however, it partitions preferentially into organic solvents. Nicotine is a strong base and has a boiling point of 274.5 °C (partial decomposition) at 760 Torr (Yildiz, 2004).

Nicotine is highly toxic substance and is acute poisoning. Death may occur within a few minutes due to respiratory failure arising from paralysis of the muscle of respiration. The lethal dose (LD_{50}) of nicotine for adult human beings is from 40 to 60 mg (http://www.inchem.org/documents/pims/chemical/nicotine.htm).

3.2 Pharmacokinetics

3.2.1 Absorption of nicotine (Yildiz, 2004; Mitrouska, Bouloukaki and Siafakas, 2007)

Absorption of nicotine through the biological membrane such as oral cavity, skin, lung, urinary bladder and gastrointestinal tract is a pH dependent process. The presence of both pyridine and pyrrolidine nitrogen means that nicotine is dibasic with p*K*a of 3.04 and 7.84 at 25°C. The proportion of uncharged and charged nicotine depends on the pH of the aqueous solution. The principal route of nicotine

absorption for smokeless tobacco user is through the oral mucosa. The pH of cigarette tobacco is about 5.5 and nicotine at this pH is primarily ionized. Thus, nicotine is little absorbed via the buccal mucosa and the absorption of nicotine through the alveoli of lung has been shown to be the major route of absorption for smokers. The pH of alveoli is about 7.4 and nicotine at this pH is unionized form and therefore easily transfers across membranes into the blood circulation. Nicotine is poorly absorbed through the gastrointestinal tract because it is ionized in the acidic gastric fluid. Nicotine is well absorbed through the skin. That is a reason for the risk of nicotine in tobacco harvesters and also a basis for transdermal nicotine delivery.

Nicotine absorption from all NRT products is slower and the increase in nicotine blood level more gradual than from cigarette smoking (Figure 2). This slow increase in blood and especially brain levels results in low abuse of NRT. Compare to various of NRT products, nicotine is rapid absorbed from the formulation, such as nicotine gum, lozenge, inhaler or nasal spray. These faster actions appear to be helpful in satiating the positive effect of nicotine intake through smoking and reduce acute craving, while the slow acting of nicotine transdermal patch provides low but constant levels of nicotine, can relieve nicotine withdrawal symptoms.

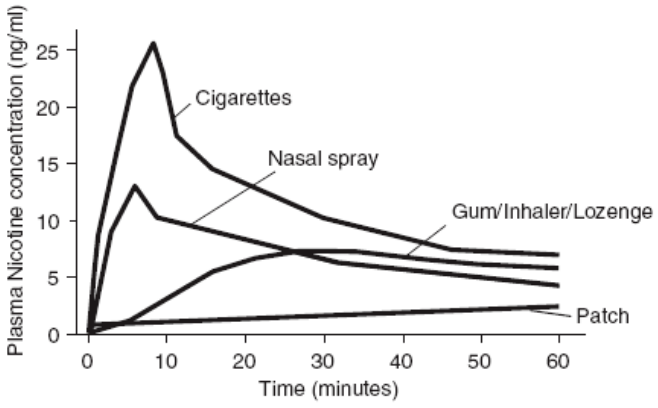


Figure 2 Plasma (venous blood) concentration of nicotine after cigarette smoking and after using different NRT products (Mitrouska, Bouloukaki and Siafakas, 2007).

3.2.2 Distribution of nicotine in body tissue (Hukkanen, Jacob III and Benowitz, 2005)

After absorption, nicotine enters the bloodstream and the drug is distributed extensively to body tissues. The distribution of nicotine to tissue is pH dependent, with the highest concentrations of nicotine found in the brain, stomach, kidney and liver, and the lowest concentrations in adipose tissue.

Nicotine binds to brain with high affinity, and the receptor binding capacity is increased in smokers compared with nonsmokers. Nicotine easily crosses the placental barrier and is accumulated in fetal serum. Nicotine also accumulates in breast milk, gastric fluid and saliva.

3.2.3 Metabolism and excretion of nicotine

Nicotine is extensively metabolized to a number of metabolites, all of which are less active than the parent compound. The metabolism of nicotine primarily occurs in the liver, but also in the lung and kidneys. The major metabolite of nicotine is cotinine, a product of oxidation of the pyrrolidine ring, but is also metabolized to nicotine-N-oxide. Cotinine is further oxidized to *trans*-3′-hydroxycotinine, which is the most abundant metabolite of nicotine in the urine (Figure 3). Both nicotine and cotinine undergo glucuronidation (Hukkanen, Jacob III and Benowitz, 2005).

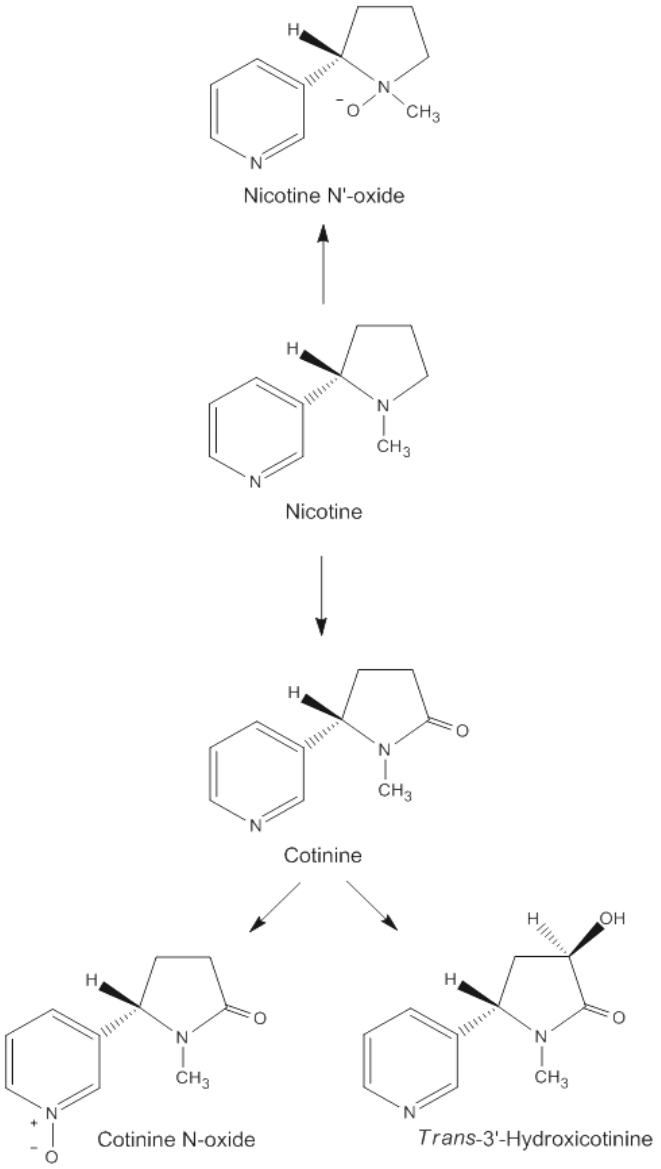


Figure 3 Metabolites of nicotine by C-oxidation (Yildiz, 2004)

Cotinine has a much longer elimination half-life than nicotine (average 16 h versus 2 h for nicotine) and its blood levels are on average 15-fold higher than levels of nicotine during regular smoking or NRT (Benowitz, 1996).

Conjugates of nicotine and its metabolites with glucuronide are excreted in urine (Figure 4). It has been indicated that nicotine could be excreted through urine, feces, saliva, sweat and breast fluid (Yildiz, 2004). The rate of nicotine

excretion depends on the pH of urine. Excretion of nicotine is increased in acid urine where nicotine is ionized, thus less nicotine is reabsorbed and more nicotine is excreted.

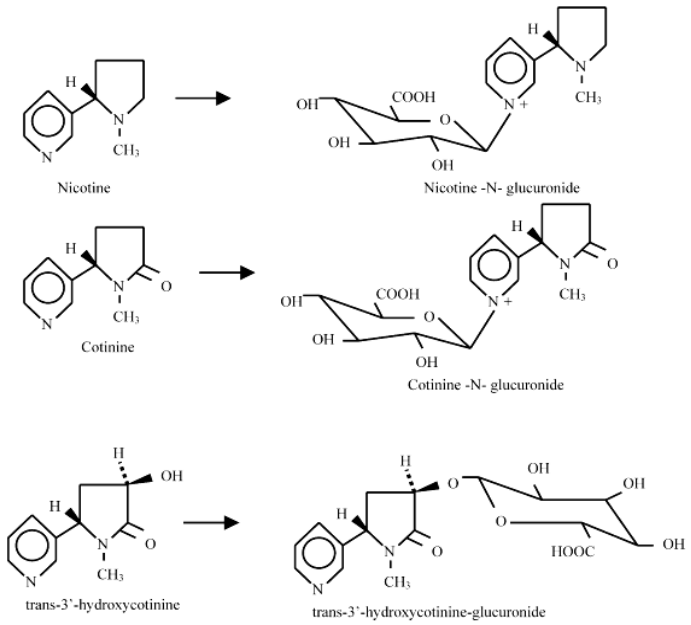


Figure 4 N- and O-glucuronidation of nicotine and its metabolites (Yildiz, 2004)

3.3 Pharmacodynamics of nicotine (Benowitz, 1986; Benowitz, 1996)

Of its two steroisomers, (S)-nicotine is the more prevalent form in tobacco, binds stereoselectively to nicotinic cholinergic receptors. (R)-nicotine, found in small quantities in cigarette smoke due to racemization during the pyrolysis process, is a weak agonist at cholinergic receptors.

Nicotinic cholinergic receptors are found in the brain, autonomic ganglia, and the neuromuscular junction. Diversity of nicotinic cholinergic receptors may explain the multiple effects of nicotine in humans.

Nicotine acts on the nicotinic acetylcholine receptors in the peripheral and central nervous system, and has pronounced CNS and cardiovascular effects. In small concentrations it increases the activity of these receptors, leading to an increased flow of adrenaline. The release of adrenaline causes an increase in heart rate, blood

pressure and respiration, as well as higher glucose levels in the blood (Table 2). In high doses, nicotine will cause a blocking of the nicotinic acetylcholine receptor, which is the reason for its toxicity.

Table 2 Actions of nicotine in human (Benowitz, 1986)

Human system	**Effects of nicotine**
Cardiovascular	Increased heart rate
	Increased cardiac contractility
	Increased blood pressure
	Cutaneous vasoconstriction – decreased skin temperature
	Catecholamine release (norepinephrine, epinephrine)
Metabolic	Increased free fatty acid, glycerol and lactate concentrations
Central nervous system	Arousal or relaxation
	Electroencephalographic changes
	Tremor
Endocrine	Increased growth hormone, adrenocorticothrophic, cortisol, hormone, vasopressin, beta endorphins
	Inhibition of prostacyclin synthesis

In addition, nicotine increases dopamine levels in the reward circuits of the brain. In this way, it generates feelings of pleasure, the behavioral effects of nicotine, thus causing the addiction associated with the need to sustain high dopamine levels.

3.4 Nicotine addiction

In the brain, nicotine acts as an agonist of neuronal nicotinic acetylcholine receptors (nAChRs) which are found presynaptically in the central nervous system and postsynaptically in the autonomic nervous system.

Cigarette smoking delivers nicotine rapidly into the arterial blood circulation that reaches the brain within 10-16 seconds (Mitrouska, Bouloukaki and Siafakas, 2007). Nicotine activates nicotinic acetylcholine receptors located in ventral tegmental area (VTA), which induces the release of dopamine in the nucleus accumbens resulting in a feeling of pleasure, an important step in the process of nicotine addiction (Figure 5). Furthermore, nicotine also facilitates the neurotransmitters such as acetylcholine, serotonin, norepinephrine and vasopressin, implicated in positive reinforcing effects.

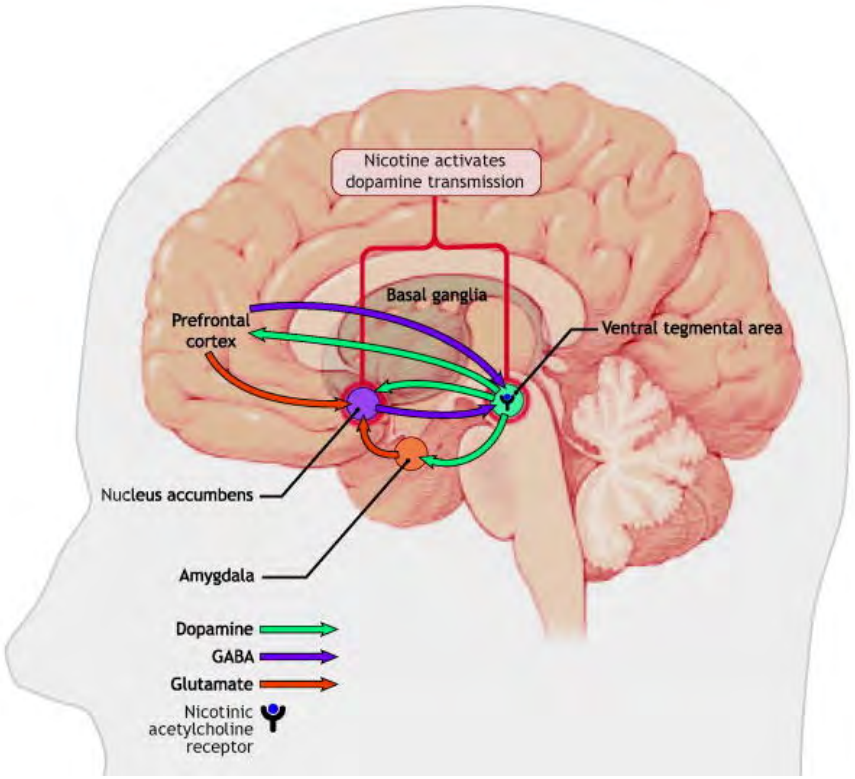


Figure 5 Areas in the brain involved in nicotine addiction (Foll and George, 2007)

Transiently high nicotine levels in the brain, which subsequently fall between puffing, allow time for resensitization of brain nicotinic receptors. Thus, nicotine from continued cigarettes is capable of overcoming tolerance to produce further pharmacological effects. Eventually, rapid delivery nicotine to the brain allows the smoker to titrate the dose of nicotine from a cigarette to achieve a

particular desired pharmacologic effect, further reinforcing drug self-administration and enhancing the development of addiction (Hukkanen, Jacob III and Benowitz, 2005).

3.5 Therapeutic use of nicotine

The primary therapeutic use of nicotine is in nicotine dependence therapy. Controlled levels of nicotine are given to a patient through gums, lozenges, dermal patches, or nasal sprays. Nicotine is being investigated as a treatment for ulcerative colitis, Alzheimer's disease, Parkinson's disease, sleep apnea, attention deficit disorder, and other medical diseases (Benowitz, 1996).

4. Pharmaceutical aspects of mucoadhesive system

4.1 Structure of the buccal mucosa

The primary function of the buccal mucosa is to protect underlying structure from foreign agents. Buccal mucosa composed of several layers of different cells as shown in Figure 6. The surface of the buccal mucosa consists of a stratified squamous epithelium. Below this lies a basement membrane, a lamina propria followed by the submucosa as the innermost layer (Shojaei, 1998). Lamina propria is rich with blood vessels and capillaries that open to the internal jugular vein.

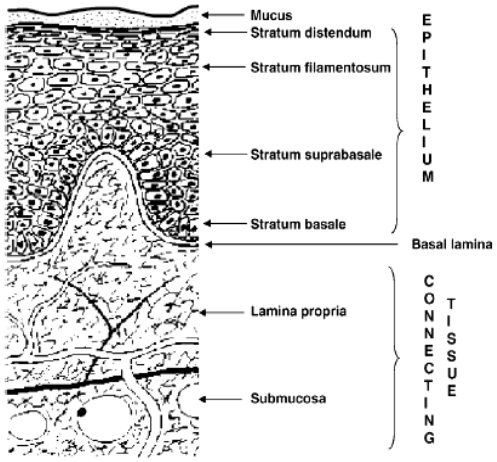


Figure 6 Cross-section of buccal mucosa (Sudhakar, Kuotsu and Bandyopadhyay, 2006)

The epithelium of the buccal mucosa is about 40-50 cell layers, resulting in a buccal mucosa which is 500-800 μm thick (Harris and Robinson, 1992), while that of the sublingual epithelium contains somewhat fewer. This stratified squamous epithelium composes of differentiating layers of cell (keratinocytes) which change in size, shape, and content. Their size increase and become flatter as they travel from the basal layers to the superficial layers (Nicolazzo, Reed and Finnin, 2005).

The turnover time for the buccal epithelium has been estimated at 5-6 days (Sudhakar, Kuotsu and Bandyopadhyay, 2006), and this is probably representative of the oral mucosa as a whole. The composition of the epithelium also varies depending on the site in the oral cavity. The mucosae of areas subject to mechanical stress (the gingivae and hard palate) are keratinized similar to the epidermis. The mucosae of the soft palate, the sublingual, and the buccal regions, are non- keratinized regions (Shojaei, 1998).

The keratinized epithelia contain neutral lipids like ceramides and acylceramides which have been associated with the barrier function. These epithelia are relatively impermeable to water. In contrast, non-keratinized epithelia do not contain acylceramides and only have minimal amounts of ceramides. They also contain small amounts of neutral but polar lipids, mainly cholesterol esters and glycosylceramides. These epithelia have been found to be considerably more permeable to water than keratinized epithelia (Shojaei, 1998; Nicolazzo, Reed and Finnin, 2005).

4.2 Barriers to permeability of buccal mucosa

4.2.1 Membrane coating granules (MCG)

In general, the permeabilities of the oral mucosae decrease in the order of sublingual greater than buccal, and buccal greater than palatal (Harris and Robinson, 1992). This rank order is based on the relative thickness and degree of keratinization of these tissues, with the sublingual mucosa being relatively thin and

non-keratinized, the buccal thicker and non-keratinized, and the palatal intermediate in thickness but keratinized.

It is currently believed that the permeability barrier in the oral mucosa involves the membrane coating granules (MCG), which are spherical or oval organelles with diameter of 100-300 nm, and are found in the intermediate cell layers of many stratified epithelia (Chen, Chetty and Chien, 1999). When cells go through differentiation, MCG start forming, concentrated close to the distal cell membrane, and in the third quarter of the epithelium they appear to fuse with the cell membrane, and their contents are discharged into the intercellular spaces of the epithelium. This barrier exists in the outermost 200μm of the superficial layer (Shojaei, 1998).

The MCG of keratinized epithelium are composed of lamellar lipid stacks which contain polar lipid (glycolipids and phospholipids), glycoprotein and considerable number of hydrolytic enzymes. It is believed that these polar lipids are precursors of the nonpolar lipids which constitute the permeability barrier of the keratinized epithelia. In non-keratinized epithelia, the MCG are almost nonlamellated in appearance. However, there is a good circumstantial evidence for a role of MCG in the formation of the permeability barrier of the non-keratinized oral mucosa (Harris and Robinson, 1992).

4.2.2 Basement membrane

Although the MCG of the superficial layers of the oral epithelium is still considered to be the primary barrier to the penetration of substances. It is thought that, the basement membrane may present some resistance to permeation as well. The charge on the constituents of the basal lamina may limit the rate of penetration of lipophilic compounds that can across the outer epithelium barrier relatively easily (Sudhakar, Kuotsu and Bandyopadhyay, 2006).

4.2.3 Mucus

The epithelial cells of buccal mucosa are surrounded by the intercellular ground substance called mucus. The thickness of this mucus layer varies

on different mucosal surface, from 50 μm to 450 μm in the stomach, to less than 1 μm in the oral cavity (Smart, 2005). Mucus is composed chiefly of mucin and inorganic salts suspended in water. Mucins are glycosylated proteins composed of oligosaccharide chains attached to a protein core (Sudhakar, Kuotsu and Bandyopadhyay, 2006).

At physiological saliva pH the mucus network carries a negative charge, due to the sialic acids and sulfate residues, which may play a role in mucoadhesion. At this pH mucus can form a strongly cohesive gel structure that binds to the epithelial cell surface as a gelatinous layer.

4.3 Routes of drug transport

There are two pathways of drug transport through the buccal mucosa: paracellular (between the cells) and transcellular (across the cells) routes. Since the intercellular spaces and cytoplasm are hydrophilic in character, lipophilic compounds would have low solubilities in this environment. The cell membrane, however, is rather lipophilic in nature and hydrophilic solutes will have difficulty permeating through the cell membrane due to a low partition coefficient. Therefore, the intercellular spaces pose as the major barrier to permeation of lipophilic compounds and the cell membrane acts as the major transport barrier for hydrophilic compounds (Shojaei, 1998; Nicolazzo, Reed and Finnin, 2005). Since the oral epithelium is stratified, solute permeation may involve a combination of these two routes. The route that predominates, however, is generally the one that provides the least amount of hindrance to passage.

4.4 Mechanism of mucosal adhesion

The term “bioadhesion” referred when interaction occurs between polymer and epithelial surface and “mucoadhesion” when occurs with the mucus layer covering a tissue. However, these two terms seem to be used exchangeably.

Mucoadhesion is proposed to occur in three stages. The first step, intimate contact must form between the mucoadhesive material and mucus. This contact result either from a good wetting of the mucoadhesive surface, or from the swelling of the mucoadhesive material. When contact is established, the penetration of mucoadhesive macromolecules with the mucus then takes place. Finally, the molecule interact with each other by secondary non-covalent bonds (Smart, 1993).

The mucosal surfaces are covered with a mucus layer, which mucin is the major component. Mucins are negative charge at physiological saliva pH. Therefore, the positive charged polymers can bind to mucins via electrosatatic interactions with the negatively charged sialic acid moieties. In addition, other mechanisms, including hydrophobic interactions, hydrogen bonding, and Van der Waal's interactions which probably involve other parts of the mucin molecules. Hydrogen bonds and hydrophobic interactions are typical types of interactions that are desirable for mucoadhesion (Miller, Chittchang and Johnston, 2005).

Mechanism of polymer attachment to mucosal surfaces are not yet fully understood. There are five general theories have been suggested to play a major role in bioadhesion (Miller, Chittchang and Johnston, 2005; Smart, 2005).

(1) The adsorption theory describes the attachment of adhesives on the basis of the covalent and non-covalent bond (electrostatic, Van der Waals' forces, hydrogen and hydrophobic bonds). Most of the initial interfacial bonding forces are attributed to non-covalent forces. Although these forces are individually weak, it has been proposed that they are the main contributors to the adhesive interaction. The formation of secondary chemical bonds chiefly depends on the properties of the polymer.

(2) The diffusion theory bases on inter-diffusion and entanglement of polymer chains and mucus polymer, which produce semi-permanent adhesive bonds. Subsequence initial contact, diffusion of the polymer chain into the mucus network creates an entangled network between the two polymers. Sufficient polymer chain

flexibility, adequate time of contact and the diffusion coefficient of the polymer are among the factors which influence the inter-diffusion of the macromolecule network.

(3) The electronic theory suggests that electron transfer occurs upon contact of adhering surfaces due to different electronic properties of the mucoadhesive polymer and the mucus glycoproteins. Electron transfer contributes to formation of a charged double layers at the interface of the mucus and the polymer, with subsequent adhesion due to attractive force in this region.

(4) The fracture theory relates the force required for the detachment of polymers from the mucus to the strength of their adhesive bonds.

(5) The wetting theory describes the ability of a bioadhesive polymer to spread on biological surfaces as a prerequisite for the development of adhesion.

4.5 Buccal adhesive polymers

Mucosal adhesive polymers have been investigated and identified in several previous works. These polymers are generally hydrophilic macromolecules that contain numerous hydrogen bond-forming groups (Smart, 1993). In most cases these polymers required moisture leading to adhesive interactions. The ideal characteristics of mucoadhesive polymer for buccal adhesive formulations as following:

- Polymer and its degradation products should be non-toxic and non-irritant.
- Polymer should have good spreadbility, wetting, swelling and solubility properties and biodegradability property.
- pH should be biocompatible.
- Polymer should adhere quickly to buccal mucosa and poss sufficient mechanical strength.
- Polymer should show bioadhesive properties in both dry and liquid state.
- Polymer must be easily available and its cost should not be high.
- Polymer should not aid in development of secondary infections such as dental caries.

Table 3 Polymers used in buccal adhesive formulation (Miller et.al., 2005)

Criteria	Categories	Examples
Source	Semi-natural/natural	Agarose, chitosan, gelatin, hyaluronic acid, various gums (guar, hakea, xanthan, gellan, carragenan, pectin, and sodium alginate)
	Synthetic	**Cellulose derivatives** [CMC, thiolated CMC, sodium CMC, HEC, HPC, HPMC, MC, methylhydroxyethylcellulose] **Poly(acrylic acid)-based polymers** [CP, PC, PAA, polyacrylates, poly(methylvinylether-co-methacrylic acid), poly(2-hydroxyethyl methacrylate), poly(acrylic acid-co-ethylhexylacrylate), poly(methacrylate), poly(alkylcyanoacrylate), poly(isohexylcyanoacrylate), poly(isobutylcyanoacrylate), copolymer of acrylic acid and PEG] **Others** Poly(N-2-hydroxypropyl methacrylamide) (PHPMAm), polyoxyethylene, PVA, PVP, thiolated polymers
Aqueous solubility	Water-soluble	CP, HEC, HPC (waterb38 8C), HPMC (cold water), PAA, sodium CMC, sodium alginate
	Water-insoluble	Chitosan (soluble in dilute aqueous acids), EC, PC
Charge	Cationic	Aminodextran, chitosan, dimethylaminoethyl (DEAE)-dextran, trimethylated chitosan
	Anionic	Chitosan-EDTA, CP, CMC, pectin, PAA, PC, sodium alginate, sodium CMC, xanthan gum
	Non-ionic	Hydroxyethyl starch, HPC, poly(ethylene oxide), PVA, PVP, scleroglucan
Potential bioadhesive forces	Covalent	Cyanoacrylate
	Hydrogen bond	Acrylates [hydroxylated methacrylate, poly(methacrylic acid)], CP, PC, PVA
	Electrostatic interaction	Chitosan

Table 4 Characteristics of some representative mucoadhesive polymers (Adapted from: Sudhakar, Kuotsu and Bandyopadhyay, 2006)

Bioadhesives	Properties	Characteristics
Polycarbophil (polyacrylic acid crosslinked with divinyl glycol)	• Mw 2.2×10^5 • η 2000–22,500 cps (1% aq. soln.) • κ 15–35 mL/g in acidic media (pH 1–3), 100 mL/g in neutral and basic media • φ viscous colloid in cold water • Insoluble in water, but swell to varying degrees in common organic solvents, strong mineral acids, and bases.	• Synthesized by lightly crosslinking of 0.5–1% w/w divinyl glycol. • Swellable depending on pH and ionic strength. • Swelling increases as pH increases. • At pH 1–3, absorbs 15–35 ml of water per gram but absorbs 100 ml per gram at neutral and alkaline pH. • Entangle the polymer with mucus on the surface of the tissue. • Hydrogen bonding between the nonionized carboxylic acid and mucin.
Carbopol/carbomer (carboxy polymethylene) empirical formula: $(C_3H_4O_2)_x$ $(C_3H_5–Sucrose)_y$	• Pharmaceutical grades: 934 P, 940 P, 971 P and 974 P. • Mw $1\times10^6–4\times10^6$ • η 29,400–39,400 cps at 25 °C with 0.5% neutralized aqueous solution. • κ 5 g/cm^3 in bulk, 1.4 g/ cm^3 tapped. • pH 2.5–3.0 • φ water, alcohol, glycerin • White, fluffy, acidic, hygroscopic powder with a slight characteristic odour.	• Synthesized by cross-linker of allyl sucrose or allyl pentaerythritol • Excellent thickening, emulsifying, suspending, gelling agent. • Common component in bioadhesive dosage forms. • Gel looses viscosity on exposure to sunlight. • Unaffected by temperature variations, hydrolysis, oxidation and resistant to bacterial growth. • It contributes no off-taste and may mask the undesirable taste of the formulation. • Incompatible with Phenols, cationic polymers, high concentrations of electrolytes and resorcinol.
Sodium carboxymethyl cellulose; SCMC (cellulose carboxymethyl ether sodium salt)	• It is an anionic polymer made by swelling cellulose with NaOH and then reacting it with monochloroacetic acid.	• Emulsifying, gelling, binding agent. • Sterilization in dry and solution form, irradiation of solution loses the viscosity.

Mw; Molecular weight, η; viscosity, κ; compressibility, φ; solubility

Bioadhesives	Properties	Characteristics
empirical formula: $[C_6H_7O_2(OH)_{3x}(OCH_2–COONa)_x]_n$	• Grades H, M, and L • Mw 9×10^4–7×10^5 • η 1200 cps with 1.0% solution. • ρ 0.75 g/cm^3 in bulk • pH 6.5–8.5 • φ water • White to faint yellow, odorless, hygroscopic powder or granular material having faint paper-like taste.	• Stable on storage. • Incompatible with strongly acidic solutions • In general, stability with monovalent salts is very good; with divalent salts good to marginal; with trivalent and heavy metal salts poor, resulting in gelation or precipitation. • CMC solutions offer good tolerance of water miscible solvents, good viscosity stability over the pH 4 to pH 10 range, compatibility with most water soluble nonionic gums, and synergism with HEC and HPC. • Most CMC solutions are thixotropic; some are strictly pseudoplastic. • All solutions show a reversible decrease in viscosity at elevated temperatures. CMC solutions lack yield value. • Solutions are susceptible to shear, heat, bacterial, enzyme, and UV degradation. • Good bioadhesive strength. • Cell immobilization via a combination of ionotropic gelation and polyelectrolyte complex formation (e.g., with chitosan) in drug delivery systems and dialysis membranes.
Hydroxypropyl cellulose partially substituted polyhydroxy propylether of cellulose; **HPC** (cellulose 2-hydroxypropyl ether) empirical formula: $(C_{15}H_{28}O_8)_n$	• Grades: Klucel EF, LF, JF, GF, MF and HF • Mw 6×10^4–1×10^6 • η 4–6500 cps with 2.0% aqueous solution. • pH 5.0–8.0 • ρ 0.5 g/cm^3 in bulk • Soluble in water below 38 °C, ethanol, propylene	• Best pH is between 6.0 and 8.0. • Solutions of HPC are susceptible to shear, heat, bacterial, enzymatic and bacterial degradation. • It is inert and showed no evidence of skin irritation or sensitization.

Mw; Molecular weight, η; viscosity, κ; compressibility, φ; solubility, ρ; density

Bioadhesives	Properties	Characteristics
	glycol, dioxane, methanol, isopropyl alcohol, dimethyl sulphoxide, dimethyl formamide etc. • Insoluble in hot water • White to slightly yellowish, odorless powder.	• Compatible with most water-soluble gums and resins. • Synergistic with CMC and sodium alginate. • Not metabolized in the body. • It may not tolerate high concentrations of dissolved materials and tend to be salting out. • It is also incompatible with the substituted phenolic derivatives such as methyl and propyl parahydroxy benzoate. • Granulating and film coating agent for tablet • Thickening agent,emulsion • Stabilizer, suspending agent in oral and topical solution or suspension
Hydroxypropylmethyl Cellulose HPMC (cellulose 2-hydroxypropylmethyl ether) empirical formula: $C_8H_{15}O_6 – (C_{10}H_{18}O_6)_n –C_8H_{15}O_5$	• Methocel E5, E15, E50, E4M, F50, F4M, K100, K4M, K15M, K100M. • Mw 8.6×10^4 • η E15–15 cps, E4M–400 cps and K4M–4000 cps (2% aqueous solution.) • φ Cold water, mixtures of methylene chloride and isopropylalcohol. • Insoluble in alcohol, chloroform and ether. • Odorless, tasteless, white or creamy white fibrous or granular powder. • ρ 0.6 g/mL • Solutions show only a fair tolerance with water • pH 6–8.5 miscible solvents (10 to 30% of solution weight). colorless solution.	• Mixed alkyl hydroxyalkyl cellulosic ether • Suspending, viscosity-increasing and film forming agent • Tablet binder and adhesive ointment ingredient • E grades are generally suitable as film formers while the K grades are used as thickeners. • Stable when dry. • Solutions are stable at pH 3.0 to 11.0 • Incompatible to extreme pH conditions and oxidizing materials. • Susceptible for bacterial and enzymatic degradation. • Polyvalent inorganic salts will salt out HEC at lower concentrations than monovalent salts.

Bioadhesives	Properties	Characteristics
	• φ in hot or cold water and gives a clear, • Compatible with most water-soluble gums and resins.	• Shows good viscosity stability over the pH 2 to pH 12 ranges. • Used as suspending or viscosity builder • Binder, film former.
Chitosan a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).	• Prepared from chitin of crabs and lobsters by N-deacetylation with alkali. • Φ dilute acids to produce a linear polyelectrolyte with a high positive charge density and forms salts with inorganic and organic acids such as glutamic acid, hydrochloric acid, lactic acid, and acetic acid. • The amino group in chitosan has a pKa value of 6.5, thus, chitosan is positively charged and soluble in acidic to neutral solution with a charge density dependent on pH and the % DA-value.	• Mucoadhesive agent due to either secondary chemical bonds such as hydrogen bonds or ionic interactions between the positively charged amino groups of chitosan and the negatively charged sialic of mucus glycoproteins or mucins. • Possesses cell-binding activity due to polymer cationic polyelectrolyte structure and to the negative charge of the cell surface. • Biocompatible and biodegradable. • Excellent gel forming and film forming ability. •Widely used in controlled delivery systems such as gels, membranes, microspheres. • Chitosan enhance the transport of polar drugs across epithelial surfaces. acid residues. Purified qualities of chitosans are available for biomedical applications. Chitosan and its derivatives such as trimethylchitosan (where the amino group has been trimethylated) have been used in non-viral gene delivery. Trimethylchitosan, or quaternised chitosan, has been shown to transfect breast cancer cells. As the degree of trimethylation increases the cytotoxicity of

Φ; solubility, DA; dalton

Bioadhesives	Properties	Characteristics
		the derivative increases. At approximately 50% trimethylation the derivative is the most efficient at gene delivery. Oligomeric derivatives (3–6 kDa) are relatively non-toxic and have good gene delivery properties.
Sodium Alginate consists chiefly of the alginic acid, a polyuronic acid composed of β-D-mannuronic acid residues. empirical formula: $(C_6H_7O_6Na)_n$ anionic polysaccharide extracted principally from the giant kelp Macrocystis Pyrifera as alginic acid and neutralized to sodium salt.	• Purified carbohydrate product extracted from brown seaweed by the use of dilute alkali. • Occurs as a white or buff powder, which is odorless and tasteless. • pH 7.2 • η 20–400 Cps (1% aqueous solution.) • φ Water, forming a viscous, colloidal solution. • Insoluble in other organic solvents and acids where the pH of the resulting solution and acids where the pH of the resulting solution falls below 3.0.	• Safe and nonallergenic. • Incompatible with acridine derivatives, crystal violet, phenyl mercuric nitrate and acetate, calcium salts, alcohol in concentrations greater than 5%, and heavy metals. • Stabilizer in emulsion, suspending agent, tablet disintegrant, tablet binder. • It is also used as haemostatic agent in surgical dressings • Excellent gel formation properties • Biocompatible • Microstructure and viscosity are dependent on the chemical composition. • Used as immobilization matrices for cells and enzymes, controlled release of bioactive substances, injectable microcapsules for treating neurodegenerative and hormone deficiency diseases. • Lacks yield value. • Solutions show fair to good tolerance of water miscible solvents (10–30% of volatile solvents; 40–70% of glycols) • Compatible with most water-soluble thickeners and resins.

Bioadhesives	Properties	Characteristics
		• Its solutions are more resistant to bacterial and enzymatic degradation than many other organic thickeners.
Poly (hydroxy butyrate), Poly (e-caprolactone) and copolymers	• Biodegradable • Properties can be changed by chemical modification, copolymerization and blending.	• Used as a matrix for drug delivery systems, cell microencapsulation.
Poly (ortho esters)	• Surface eroding polymers.	• Application in sustained drug delivery and opthalmology.
Poly (cyano acrylates)	• Biodegradable depending on the length of the alkyl chain.	• Used as surgical adhesives and glues. • Potentially used in drug delivery.
Poly (vinyl alcohol)	• Biocompatible	• Gels and blended membranes are used in drug delivery and cell immobilization.
Poly (ethylene oxide)	• Highly biocompatible.	• Its derivatives and copolymers are used in various biomedical applications.
Poly (hydroxytheyl methacrylate)	• Biocompatible	• Hydrogels have been used as soft contact lenses, for drug delivery, as skin coatings, and for immunoisolation membranes.

5. Fluidization

Fluidization is a process in which a bed of solid particles is suspended and agitated by a rising stream of gas which empowers a thorough gas-solid contact throughout the bed (Yang et al., 1992). Fluid bed technology has been increasingly utilized by the pharmaceutical industry in various unit operations, including drying, granulation and coating. Coating can be applied to fluidized particles by a variety of

techniques, including spraying from the top, bottom, or tangentially. The use of fluid bed equipment in applying coating systems has increased greatly due to (1) improved drying efficiency, (2) improved design and (3) increased experience (Mehta, 1997).

5.1 Stage of fluidization

The successive stages of fluidization are shown in Figure 7. Fluidization is usually carried out in a cylindrical container or column holding the powder, which is supported when at rest on a porous plate. As the upward flow of fluidizing gas is increased through the stationary powder bed (stage A), the particles are lifted upward and the bed expands (stage B). Suddenly, there is a break in the heretofore uniform relation between the pressure applied and the flow rate produced, and the bed quickly changes over from static to mobile (stage C). Further small increases in pressure cause large increases in flow, and the bed expands considerably with an increase of voidage and usually the formation of bubbles (stage D). Eventually, the lifting force of the upward gas flow causes particles to be blown out of the bed altogether and pneumatic transport occurs (stage E). It is generally desirable that the air velocity be controlled at the minimum fluidization level (Mathur, 1992). Thus the upward lifting force on the bed of particles is just equal to their weight and the bed is just fluidized.

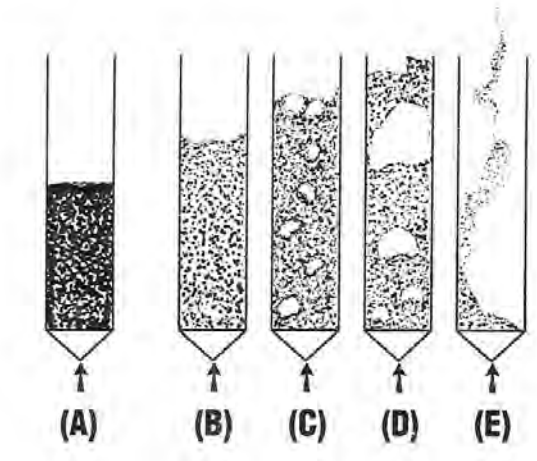


Figure 7 Stages of fluidization: (A) Static bed; (B) Expanded bed; (C) Mobile bed; (D) Bubble formation; (E) Pneumatic transport (Mathur, 1992).

5.2 Fluidized bed coaters

Fluidized bed coaters are another mechanical type of encapsulation process (Thies, 1996). They are used extensively to encapsulate many different solids. Pharmaceutical powders, granules, pellets, and tablets are often coated with polymeric materials to mask an objectionable taste or odor, protect unstable ingredient or improve appearance. A liquid coating is sprayed onto the individual particles, and the coated particles are cycled into a zone where the coating is dried by solvent evaporation or cooling. This coating and drying sequence is repeated until a desired coating thickness has been applied. Three types of fluidized bed are available: top spray, bottom spray or tangential spray. These units differ in location of the nozzle used to apply the liquid coating.

5.2.1 Top-spray coating

The top spray coater has been used to apply aqueous and organic solvent based film coatings, controlled-release coatings, and hot melts on granules and small particles. In a top spray coater (Figure 8), the expansion chamber is longer to allow powder to remain fluidized longer and move with a higher velocity so that reduce agglomeration tendencies, and has a conical shape to allow uniform deceleration of the air stream.

The most significant characteristic of the top spray method is that the nozzle sprays countercurrently or down into the fluidizing particles (Jones, 1988). The fluidization pattern is random and unrestricted. As a result, controlling the distance of the droplets travel before contacting the substrate is impossible. The top spray coater is simple and rapid coating process which can be scaled up to very large batches (up to 15 kg). Agglomeration of the particles should be observed closely and controlled by adjusting process variables such as spray rate, fluidization air velocity and temperature, nozzle position, and atomizing air pressure.

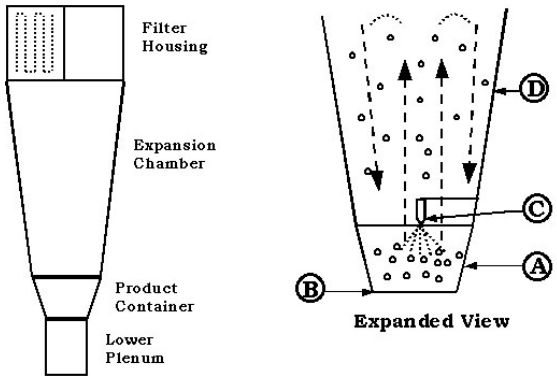


Figure 8 Top spray coater: (a) product container; (b) air distribution plate (c) spray nozzle; (d) expansion chamber (Mehta, 1997).

5.2.2 Bottom-spray coating (Wurster process)

The Wurster process was invented by Dr. Dale Wurster in 1959, then at the University of Wisconsin. In the bottom spray unit, the nozzle is placed in the center of the gas distributor plate and liquid is sprayed concurrently with the fluidization air. The majority of the air is diverted through the partition, causing fluidization and upward travel of the cores. The process chamber for laboratory size equipment has a narrow diameter in the product-containing area which facilitates well organized particle motion, and therefore, reproducible coating results can be obtained. However, as the chamber becomes larger, particle motion loses its regular and circulatory pattern and becomes disorganized.

Inside the Wurster chamber (Figure 9), a cylindrical coating partition is mounted in the center and raised slightly above the perforated plate. The plate is designed with many larger holes under the area of coating partition to allow more air enters the partition than the surrounding area. This design allows the solid particles to be pneumatically transported upward through the coating partition, and fell back outside this partition. This type of fluidization is characterized as a spouting bed. Particles circulate rapidly in this style and receive the layer of coating on each pass through the coating partition.

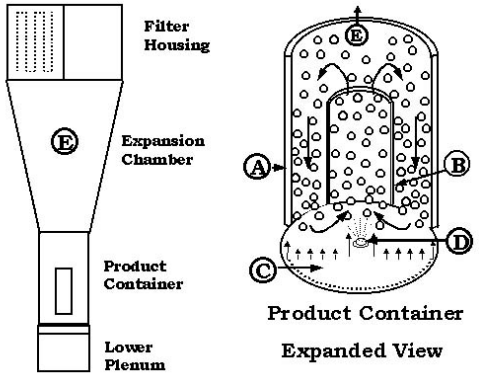


Figure 9 Wurster bottom spray coater: (a) coating chamber; (b) partition; (c) air distribution plate; (d) spray nozzle; (e) expansion chamber (Mehta, 1997).

The Wurster process provides a highly organized particle flow and high quality reproducible film. Hence, the Wurster system is used extensively for sustained-release coating, and has the widest application range of both water and organic solvents (Mathur, 1992). The certain disadvantages of this system are that it is somewhat complicated, it is the tallest of the three types of fluidized bed machines, and the nozzles are inaccessible during the processing. In addition, it has minimum volume limitation, and shows difficulty of loading and unloading (Parikh and Mogavero, 2005).

5.2.3 Tangential spray coating (Rotating disk coater)

The rotary or tangential spray system, also concurrent-spray technique similar to Wurster system. The basic design (Figure 10) employs a rotating disk in the product container. The disk can be moved up or down to create a variable slit opening between the outer perimeter of the disk and the side wall of the container. This allows independent control of air velocity over air volume. Air is drawn into the product container through the slit under negative pressure. This fluidizes the material along the circumferential surface of product chamber. At the same time the disk rotates at varying speeds and moves the product by centrifugal force to the outer portions where it is lifted by the fluidizing air stream into the expansion chamber. As the material decelerates, it descends to the center of the disk and repeats the sequence

(Mathur, 1992). The motion of the fluidized material is thus controlled by the forces of fluidization, centrifugal force, and gravity. The magnitude of each of these forces depends upon the fluidization air volume, the slit width and the rotating speed of the disk (Yang et al., 1992).

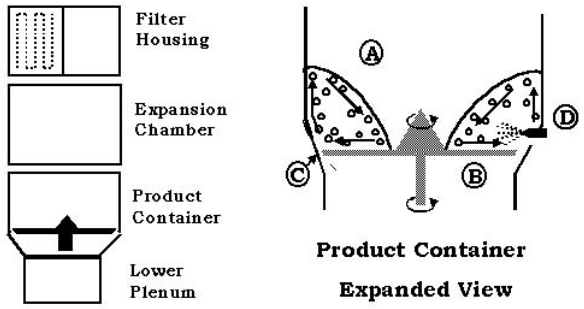


Figure 10 Tangential spray coater: (a) produce chamber; (b) variable speed disc (c) disc gap or slit; (d) spray nozzle (Mehta, 1997).

Different from the Wurster system, the rotary tangential spraying system has a relatively wide application range. It is the shortest machine in height of the three, and allows nozzle accessible during processing. Its primary disadvantage is that it exerts the greatest mechanical stress of the three methods and, thus, is discouraged for use with friable substrates.

Since the spray mode determines not only the spray pattern of the coating formulation, but also how the sprayed droplets impinge and spread on the substrates, it is expected to have a significant impact on the film structure (Mehta and Jones, 1985).

The evaporative efficiency of fluidized bed apparatus and the ability to apply a film to particles have resulted in widespread use of this technique. The three fluidized bed methods are not functionally equivalent, but offer a broad variety of applications. These methods have some common features and process variables, but each has unique advantages and limitations.

5.3 Film coating

The first coated pharmaceutical dosage forms were medication with sugar coatings for the purpose of masking unpleasant tastes and imparting a more elegant appearance. Nowadays the purpose of coating as the following categories (Radebaugh, 1992):

- Protection of drugs from environment factors such as light, moisture and air in order to improve chemical and physical stability.
- Masking of unpleasant taste, texture or odor.
- Controlled or modified release of drugs.
- Increased mechanical stability during manufacture, packing and shipment.
- Modification of product appearance to enhance marketability or hide undesirable color changes of the substrate.
- A mechanical barrier to avoidance of side effects or the interaction of incompatible ingredients.

The significant ingredients used in film coating are polymers, plasticizers and additives such as colorants, flavors or sweeteners, surfactants, antioxidants and antimicrobial agents. Coating pans and fluidized bed equipment are generally used for processing.

The process of film coating involves the application of a thin film onto the surface of a solid substrate. The substrate can be tablets, capsules, pellets, granules or particles. Typically, the coating is applied to improve the physical and chemical properties of the substrate (non-functional film coating) or modified film coating, controlled release coating (functional film coating).

Application of a film to a solid is very complex. A layer of coating does not occur during a single pass through the coating zone, but relies on many such passes to produce complete coverage of the surface. Droplet formation, contact,

spreading, coalescence, and evaporation, as displayed in Figure 11, are occurring almost simultaneously during the process (Jones, 1988; Porter and Bruno, 1990)

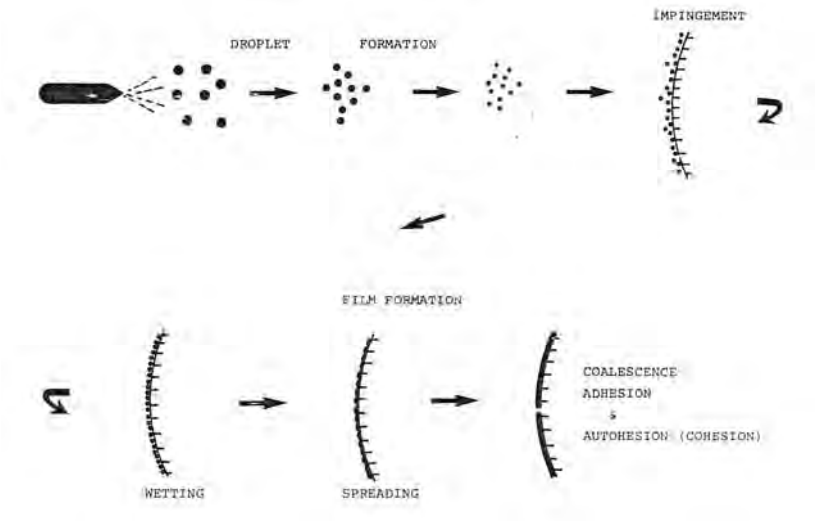


Figure 11 Schematic representation of film coating process

5.3.1 Core or substrate for film coating (Bauer, 1998)

The various core or substrate can be used in film coating. Whether or not a core is suitable for coating depends on the following common properties:

Hardness

Coating process requires core of adequate strength or abrasion-resistant substrate that is less susceptible to impact stress, because the formation of a coherent film coating takes some time.

Shape

Slightly curved tablet cores are preferred for film coating. Small cores like pellets, crystals and granules show pronounced fluctuation in size, shape and surface. A narrow particle size distribution can be obtained by screening, which then avoids batch to batch fluctuations in material consumption and irreproducible results.

Surface

The surface has a major influence on the buildup of the first few coating layers. Film coating requires smooth and dust-free surfaces because they are relatively small thickness. Surface which are poorly wettable make it difficult to achieve adhesion between layers.

Size

Typically, coating technique serves for cores from about 0.2 mm diameter upwards. If the core are in the diameter range of 0.2 to 2 mm, they relatively large surface and small mass. The adhesion between the particles then has adverse influence on coating process, since they tend to stick together. The envisaged application dictates the upper limit of the core size. The pharmaceutical dosage form must still be swallowable and occasionally chewable.

Heat sensitivity

The process heat can have an adverse effect on sensitive drugs or excipients in the core. If the heat in the process is not properly controlled or critical temperature is exceeded, this may result in drug decomposition and changes in drug release or dissolution profile, in some cases even from batch to batch.

5.3.2 Polymeric solutions and Polymeric dispersions

Film coatings consist mainly of polymers, which are applied to the cores in the form of solution or dispersion in which the excipients are dissolved or suspended. After drying of the solvents or dispersing agents, the polymer and other excipients remain on the cores as a coherent, uniform film. The other excipients may account for up to 60% of the coating layer, depending on the pigment-binding capacity of the polymers (Bauer et al., 1998). Pharmaceutical film coating formulations including organic solutions and aqueous dispersions. Organic solution produces good results but suffers from serious drawbacks such as pollution, fire and safety hazards. However, the technique is still widely used particularly when specialized polymers are used for coating.

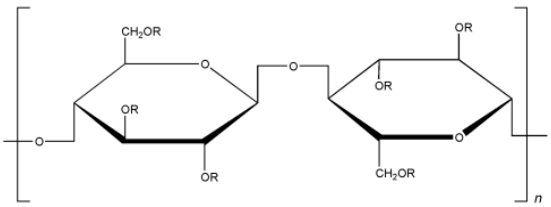
Generally, film formation from an aqueous or organic solvent solution of polymer involves conversion of a viscous liquid into a viscoelastic solid. Based on the solvation process itself, the particles involved are individual polymer molecules, extended as long chains and separated from each other only by molecular distances in the solvent. As solvent rapidly evaporates, the coating liquid will increase in concentration and contrast in volume and hence increment in the viscosity. Further loss of solvent at a slower rate is now controlled by the diffusion rate of solvent through the polymer matrix. Then, concentration of the polymer in coating increases to the point where the extended polymer chains ultimately become immobilized, so-called solidification point. As the remaining solvent is gradually lost, beyond the solidification point, resulting from the slow diffusion of residual solvent through the "dry" coating, the gelled solution forms a continuous film and produces a three-dimentional dried gel (Banker and Peck, 1981; Porter and Bruno, 1990).

6. Drug and excipients used in microencapsulation process and buccal tablet formulation

6.1 Nicotine hydrogen tartrate dihydrate; NHT (O'Neil, 2006)

Synonym:	Nicotine bitartrate dihydrate
Molecular formula:	$C_{10}H_{14}N_2(C_4H_6O_6).\ 2H_2O$
Molecular weight:	498.44
Appearance:	White to off-white crystalline powder
Solubility:	Very soluble in water and methanol

6.2 Hydroxypropyl methylcellulose; HPMC



n = degree of polymerization, where R is H, CH_3, or $[CH_3CH(OH)CH_2]$

Figure12 Chemical structure of HPMC (Rowe et al., 2003)

The first application of hypromellose, also known as Hydroxypropyl methylcellulose, for film coating appeared in a patent by Singiser of Abbott Laboratories in 1962. The employing of HPMC for film coatings have become popular due to the fact that they give a superior appearance, acts as protection for fragile tablet, and mask the unpleasant taste of drug substances (Obara and Kokubo, 2008).

The chemical structure of HPMC is shown in Figure 12. HPMC is classified according to the content of substituents and its viscosity. It is available in several grades that may be distinguished by appending a number indicative of the apparent viscosity, in mPa sec, of a 2% w/w aqueous solution at 20°C (Rowe et al., 2003). Labeled viscosity (nominal viscosity) does not mean the exact viscosity value of a product lot. In the monograph, the apparent viscosity of the low-viscosity HPMC product is specified to be form 80-120% of the labeled viscosity.

Commercially available HPMC includes several substitution types such as those shown in Table 5. Of the four digits in each number, the first two digits refer to the approximate percentage content of the methoxy group (OCH_3). The second two digits refer to the approximate percentage content of the hydroxypropoxy group ($OCH_2CH(OH)CH_3$), calculated on a dried basis. Molecular weight of HPMC is approximately 10,000–1,500,000.

Table 5 Contents of substitutions of HPMC (Obara and Kokubo, 2008)

	Methoxy (%)		Hydroxypropyl (%)	
Substitution type	Minimum	Maximum	Minimum	Maximum
1828	16.5	20.0	23.0	32.0
2208	19.0	24.0	4.0	12.0
2906	27.0	30.0	4.0	7.5
2910	28.0	30.0	7.0	12.0

HPMC of low-labeled viscosity (3-15 mPa sec) is commonly used in film coating. The low viscosity grades of HPMC are typically produced by depolymerization of high-viscosity grades. Commercially available products of HPMC for film coating widely used such as are Pharmacoat 603, 645, 606 and 615 (Shin-Etsu Chemical Co. Ltd.,) and Methocel E3, E5, E6 and E15 (Dow Chemical Company).

The typical properties of HPMC is exhibited in Table 6. HPMC forms transparent, tough and flexible films from aqueous solutions. HPMC is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption may have a laxative effect. The WHO has not specified an acceptable daily intake for HPMC due to the levels consumed were not considered to represent a hazard to health.

Table 6 Typical properties of HPMC (Rowe et al., 2003)

Appearance	An odorless and tasteless, white or creamy-white fibrous or granular powder
Acidity/alkalinity	pH = 5.5–8.0 for a 1% w/w aqueous solution.
Density (true)	1.326 g/cm^3
Melting point	Browns at 190–200°C; chars at 225–230°C. Glass transition temperature is 170–180°C.
Solubility	Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95%), and ether but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol.
Incompatibility	Incompatible with some oxidizing agents. Since it is nonionic, hypromellose will not complex with metallic salts or ionic organics to form insoluble precipitates.

Seitz (1988) suggested that film prepared with HPMC generally will need another polymer or plasticizers to improve their binding to tablet surfaces and avoid the problem of bridging or filling of tablet engraving.

High-viscosity grades of HPMC generally provide better films than the lower-viscosity grades. Furthermore, films produced from HPMC are clear but have excellent capacity for binding pigment, and thus can be easily colored (Miller and McGinity, 2008).

6.3 Polyethylene glycol; PEG

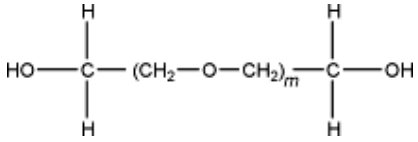


Figure 13 Chemical structure of polyethylene glycol (Rowe et al., 2003)

Polyethylene glycol grades 200–600 are liquids; grades 1000 and above are solids at ambient temperatures.

Liquid grades (PEG 200–600) occur as clear, colorless or slightly yellow-colored, viscous liquids. Solid grades (PEG>1000) are white or off-white in color, and range in consistency from pastes to waxy flakes. They have a faint, sweet odor. Grades of PEG 6000 and above are available as free-flowing milled powders.

In film coatings, solid grades of PEG are widely used as plasticizer in combination with film forming polymers. Polyethylene glycols are useful as plasticizers in microencapsulated products to avoid rupture of the coating film when the microcapsules are compressed into tablets.

The melting point of PEG6000 is 55-63 °C. Its density is 1.15-1.21 g/cm^3. Solid grade soluble in water (solubility of PEG6000 = 1,900 g/ml at 25 °C), acetone, dichloromethane, ethanol and methanol. Slightly soluble in aliphatic hydrocarbons and ether but insoluble in fats, fixed oils and mineral oil.

6.4 Mannitol

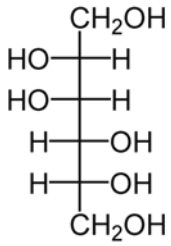


Figure 14 Chemical structure of mannitol (Rowe et al., 2003)

Empirical formula:	$C_6H_{14}O_6$
Molecular weight:	182.17
Heat of solution:	−120.9 J/g (−28.9 cal/g) at 25 °C
Melting point:	166–168 °C
Solubility (at 20 °C):	Soluble in water (1 in 55), glycerin (1 in 18), ethanol (1 in 83) and practically insoluble in ether.

Mannitol appears as a white, odorless, crystalline powder, or free-flowing granules. It has a sweet taste, approximately as sweet as glucose and half as sweet as sucrose, and imparts a cooling sensation in the mouth (Yoshinari et al., 2003).

Mannitol is widely used in pharmaceutical formulations and food products. In pharmaceutical preparations it is primarily used as a diluent (10–90% w/w) in tablet formulations, where it is of particular value since it is not hygroscopic and may thus be used with moisture-sensitive active ingredients (Peck et al., 1989).

Granulations containing mannitol have the advantage of being dried easily. Mannitol can function as both a nonnutritive sweetener and filler, thus it is commonly used as an excipient in the manufacture of chewable tablet formulations and lozenges.

6.5 Xylitol

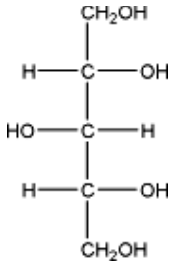


Figure 15 Chemical structure of xylitol (Rowe et al., 2003)

Xylitol appears as a white, granular solid comprising crystalline. It is odorless, with a sweet taste that imparts a cooling sensation. Xylitol is also commercially available in powdered form and several granular, directly compressible forms.

Xylitol is used as a noncariogenic sweetening agent in a variety of pharmaceutical dosage forms. Unlike sucrose, xylitol is not fermented into cariogenic acid end products and it has been shown to reduce dental caries by inhibiting the growth of cariogenic Streptococcus bacteria.

As xylitol has an equal sweetness intensity to sucrose, combined with a distinct cooling effect upon dissolution of the crystal, it is highly effective in enhancing the flavor of tablets and masking the unpleasant flavors of pharmaceutical actives and excipients. Granulates of xylitol are used as diluents in tablet formulations, where they can provide chewable tablets or lozenges with a desirable sweet taste and cooling sensation.

6.6 Microcrystalline cellulose; MCC

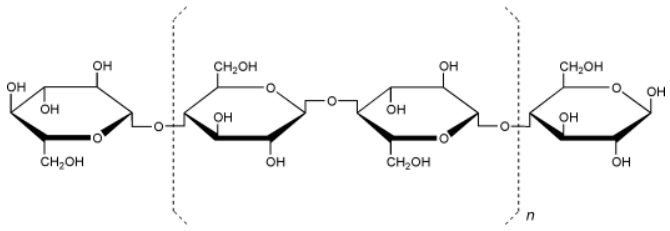


Figure Chemical structure of MCC (Rowe et al., 2003)

Synonyms: Avicel PH, Celex, Emocel

Microcrystalline cellulose is a purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications. MCC has proven to be stable, safe and physiologically inert.

Microcrystalline Cellulose exhibits excellent properties as an excipient for solid dosage forms. It compacts well under minimum compression pressures, has high binding capability, and creates tablets that are extremely hard, stable, disintegrate rapidly. These properties make MCC particularly valuable as a filler or binder for formulations prepared by direct compression, though it also is used in wet or dry granulation.

6.7 Sucralose

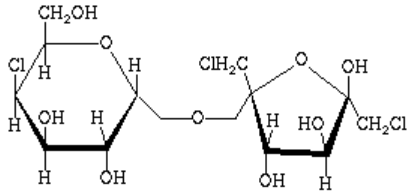


Figure 17 Chemical structure of sucralose

Sucralose is a zero-calorie sugar substitute artificial sweetener. Sucralose is approximately 600 times as sweet as sucrose twice as sweet as saccharin, and 3.3 times as sweet as aspartame. Unlike aspartame, it is stable under heat and over a broad range of pH conditions. Therefore, it can be used in products that require a longer shelf life. The commercial success of sucralose-based products stems from its favorable comparison to other low-calorie sweeteners in terms of taste, stability, and safety.

Sucralose is generally regarded as a nontoxic and nonirritant material. Following oral consumption, sucralose is mainly unabsorbed and is excreted in the feces. The WHO has set an acceptable daily intake for sucralose of up to 15 mg/kg body weight. (Rowe et al., 2003)

6.8 Menthol

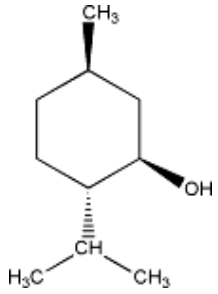


Figure 18 Chemical structure of menthol (Rowe et al., 2003)

Menthol occurs widely in nature as l-menthol and is the principal component of peppermint and cornmint oils. Commercially, l-menthol is mainly produced by extraction from these volatile oils. It may also be prepared by partial or total synthetic methods.

In regarding to its characteristic peppermint flavor, l-menthol, which occurs naturally, also exerts a cooling or refreshing. Unlike mannitol, which exerts a similar effect due to a negative heat of solution, l-menthol interacts directly with the body's coldness receptors. d-Menthol has no cooling effect, while racemic menthol exerts an effect approximately half that of l-menthol.

6.9 Magnesium stearate (Rowe et al., 2003)

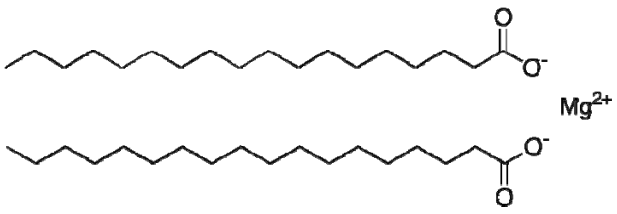


Figure 19 Chemical structure of magnesium stearate

Stuctural formula:	$[CH_3(CH_2)_{16}COO]_2Mg$
Empirical formula:	$C_{36}H_{70}MgO_4$
Molecular weight:	591.34

Magnesium stearate is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. It is generally regarded as being nontoxic following oral administration.

Magnesium stearate is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0% w/w. Blending times with magnesium stearate should be carefully controlled. Due to it may increase tablet friability or decrease dissolution rate as the time of blending increased.

6.10 Talcum

Talc is a purified, hydrated, magnesium silicate, approximating to the formula $Mg_6(Si_2O_5)_4(OH)_4$. It may contain small, variable amounts of aluminum silicate and iron. Talc is a very fine, white to grayish-white, odorless, impalpable, crystalline powder (Rowe et al., 2003).

Talc is also used as a lubricant in tablet formulations; in a novel powder coating for extended-release pellets; and as an adsorbant.

6.11 Tartrazine (FD&C Yellow #5)

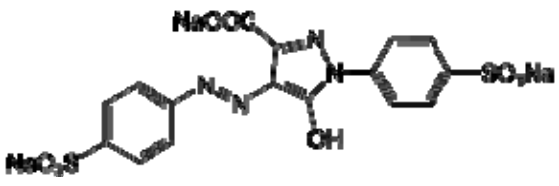


Figure 20 Chemical structure of tartrazine

Tartrazine is a synthetic lemon yellow azo dye used as a coloring agent. It is water soluble and has a maximum absorbance in an aqueous solution at 427±2 nm. Products containing tartrazine commonly include foods, cosmetics and medicines (Jain, Bhargava, and Sharma, 2003).

CHAPTER III
EXPERIMENTAL

1. Materials

The following materials obtained from commercial sources were used.

1.1. Drugs

- Dexamethasone (Lot No.116H0427, Sigma-Aldrich, Germany)
- Nicotine (Lot No.1218059, Fluka, Germany, purchased from A.C.S. Xenon Limited Partnership)
- Nicotine hydrogen tartrate dihydrate (Lot No.057K1521, Sigma-Aldrich, Germany, purchased from A.C.S. Xenon Limited Partnership)

1.2. Excipients

- Hydroxypropyl methylcellulose (Methocel® E15-L Lot no.UL24012404, Dow Chemical Company, LA, USA)
- Mannitol powder (Batch No.H100509006, Forbest Chemical Co.,Ltd., Bangkok, Thailand)
- Microcrystalline cellulose (Ceolus PH 102®, Lot no. 1556, ASAHI KASEI Chemicals Corporation, Tokyo, Japan)
- Magnesium stearate (Radiastar® 1100, Batch no. 1758, OLEON NV, Ertvelde, Belgium)
- Menthol crystal (Lot No. 0505061, Exp. 19/5/10 purchased from Srichand United Dispensary Co.,Ltd., Bangkok, Thailand)
- Peppermint oil (Lot No. 0706001, Exp. 7/3/10 purchased from Srichand United Dispensary Co.,Ltd., Bangkok, Thailand)
- Polyethylene glycol (PEG 6000, Lot no. 427124/1 33901, Fluka, Buchs, Switzerland)
- Sucralose (D-et max®, Lot no. 080503, purchased from U-Sing, Bangkok, Thailand)
- Talcum (Lot no. CH/294/07, China Chemical Industry, China)

- Tartrazine (Lot no.19140, Butterfield food ingredients Ltd., Norfolk, England)
- Xylitol C (Batch no. 0000204873, Danisco Sweeteners OY, Kotka, Finland, purchased from Rama Production, Bangkok, Thailand)

1.3 Chemicals

- Hydrochloric acid (Lot no.B40076, J.T.Baker Neutrasorb, USA)
- Ethanol absolute (Lot no. K37461883 726, Merck, Darmstadt, Germany)
- Methanol HPLC grade (Batch no.HAVG3H, Honeywell Berdick & Jackson, Ulsan, Korea)
- Mucin from porcine stomach type II (Batch no. 108K0010, Sigma-Aldrich, St. Louis, USA)
- Potassium phosphate, monobasic (Batch no. AF401428, Ajax Finechem, NSW, Australia)
- Di-sodium hydrogen phosphate anhydrous (Batch no. AF34152, Ajax Finechem, NSW, Australia)
- Sodium hydroxide (Lot no. B131198 214, Merck KGaA, Damstadt, Germany)
- Sodium chloride (Lot no. C25337, J.T.Baker, Malaysia)
- Triethylamine HPLC grade (Batch no. 0301299, Fisher Scientific, Leicestershire, UK)

2. Equipments

- Analytical Balance (Model PB3002, Mettler Toledo, Schwerzenbach, Switzerland and Model A200s, Sartorius Gbh, Goettingen, Germany)
- Desktop poly sealer (Model P-200, FIJI IMPULSE)
- Dialysis tubing cellulose membrane (D9777, 25 mm x 16 mm, Sigma-Aldrich, Germany)
- Differential Scanning Calorimeter (Model 822^e, Mettler Toledo, Schwerzenbach, Switzerland)
- Fluidized bed air suspension (Aeromatic Fielder AC, Model STRFA1, DWYER Instruments INC., USA)
- Friability tester (Erweka TAR 20, Heusenstamn, Germany)

- High performance liquid chromatograph (Model SCL-10A VP, Shimadzu, Kyoto, Japan) assembled with
    - System controller (Model SCL-10A VP, Shimadzu, Japan)
    - Liquid chromatograph (Model LC-10AD VP, Shimadzu, Japan)
    - Degasser (Model DGU-14A, Shimadzu, Japan)
    - Auto injector (Model SIL-10AD VP, Shimadzu, Japan)
    - Column oven (Model CTO-10AS VP, Shimadzu, Japan)
    - UV-VIS detector (Model SPD-10A VP, Shimadzu, Japan)
- High speed granulator (Pharmaceuticals and medical supply Ltd., Bangkok, Thailand)
- Hot air oven (Model UL80, Memmert, Munich, Germany)
- Hotplate magnetic stirrer (Model M6, CAT, Germany)
- Jolting volumeter (Modified by Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)
- Lazer diffraction particle sizer (Mastersizer 2000, Malvern instruments, Worcestershire, UK)
- Modified Franz cell diffusion (Modified by Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand)
- Moisture balance (Model HR83, Mettler Toledo, Schwerzenbach, Switzerland)
- Mortar and pestle (Obtained from Industrial Pharmaceutical Laboratory, Faculty of Pharmaceutical Sciences, Chulalongkorn university, Thailand)
- Oscillator (Erweka AR400, Heusenstamn, Germany)
- pH meter (Model 210A+, Thermo orion, Germany)
- Peristaltic pump (Roto Consulta, Serial Nr.03208, Luzern, Switzerland)
- Planetary mixer (Model 5K5SS, Kitchen aid, Michigan, USA and Model EB20F, Crypto-peerless Ltd., London, UK)
- Punch (EKO 16 mm, Tools parts mould CO.,Ltd., Bangkok, Thailand)
- Scanning electron microscope (Model JSM-5800LV, Joel Ltd., Tokyo Japan)

- Sieve shaker (Filtra, model FT-200M, Barcelona, Spain)
- Single punch tabletting machine (No.74, Viuhang engineering, Bangkok, Thailand)
- Solid-state nuclear magnetic resonance spectrometer (Bruker Biospin DPX-300, Switzerland)
- Tablet tester (Thermonik, model DHT-250, Labquip (Thailand) Ltd., Bangkok, Thailand)
- Texture analyzer (Model TA.XT plus, Stable micro systems, UK)
- Thermogravimetric analyzer (Model SDTA851^e, Mettler Toledo, Schwerzenbach, Switzerland)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Singen, Germany)
- X-ray diffractometer (Bruker AXS, model D8 Discover)

3. Methods

Part I. Characterization of nicotine hydrogen tartrate dihydrate (NHT) powder

1. Solid state characterization of NHT powder

1.1 Scanning electron microscopy

The shape and surface topography of nicotine hydrogen tartrate dihydrate were determined by scanning electron microscope (SEM). The samples were prepared by gold sputtering technique prior to SEM examination using scanning electron microscope (JSM-5800LV, Joel Ltd.).

1.2 X-Ray powder diffractometry

Crystalline form of nicotine hydrogen tartrate dihydrate was examined using X-Ray powder diffractometry. Samples were filled in a zero-background quartz holder and exposed to CuKa radiation (40 kV, 40 mA) by a wide angle X-ray diffractometer (D8 Discover, Bruker AXS). The instrument was operated using the step-scan mode, in increments of 0.02 °2θ/step. The angular range was 5 to 40 °2θ and counts were accumulated for 0.2 s at each step.

1.3 Differential scanning calorimetry and thermogravimetric analysis

Thermal analyses were performed using differential scanning calorimetry (DSC; Model 822^e, Mettler Toledo) and thermogravimetric analysis (TGA; Model SDTA 851^e, Mettler Toledo). Weight loss profiles of sample were studied using a TGA equilibrated at 25°C then ramped to 250°C at 10°C/min under nitrogen purge. Sample sizes were approximately 3.0 mg. A DSC was used to evaluate phase transitions. Aluminum pans containing 3.0 mg NHT were heated under nitrogen purge using a thermal ramp of 10°C/min from 25°C to 250°C. All thermal experiments were performed in triplicate.

1.4 Solid state nuclear magnetic resonance spectrometry

Solid state nuclear magnetic resonance (SSNMR) was carried out using solid-state resolution ^{13}C CP/MAS NMR spectrometry (Bruker Biospin; DPX-300). All samples were characterized at room temperature (20±1°C). ^{13}C NMR spectra were recorded at a frequency of 75 MHz. The spectral parameters used were as follows: 1,600 number of scan (NS), relaxation delay of 4 sec, spin rate of 5 kHz and spectral size 2K with 4K domain size.

2. Particle size and size distribution of NHT powder determination

The particle size and shape were examined with a scanning electron microscope. The samples were mounted directly onto SEM sample holder with double-sided tape and were gold spray-coated.

The particle size distribution and mean diameters were determined using laser light scattering technique (Mastersizer 2000, Malvern instrument). Mineral oil was used as a medium and diffraction index value was 1.5. The samples were dispersed in the medium and measurement was made immediately to avoid agglomeration of the particles. The samples were determined in triplicate and the average of the mean diameters was calculated.

3. Compatibility studies

Differential scanning calorimetry (DSC) was used as a screening technique for evaluating the compatibility of NHT with excipients at a ratio of 1:1. Aluminum pans containing approximately 3.0 mg of sample were heated under nitrogen purge gas and heating rate of 10°C/min.

Part II. Preparation of mucoadhesive nicotine microcapsules

The mucoadhesive nicotine microcapsules were made by coating process using fluidized bed coater. Since there are three types of fluidized bed coater, then the widely used process between top-spray and bottom-spray coating were assessed before applied. In coating process, there are many parameters involved and these process parameters may affect the character of the final product. Therefore, the effects of these parameters were initially assessed.

Microcapsules preparation was separated into two steps. First, granules which contained NHT and mannitol were prepared by wet granulation with HPMC E15 as binding liquid. Second, the granules were further coated to produce mucoadhesive nicotine microcapsules via fluidized bed coating technique using HPMC E15 as coating material.

According to the most suitable system of fluidized-bed coating process, the granules were coated to form mucoadhesive microcapsules. HPMC E15 was used as coating material by varying the amounts as 4, 5, 6 and 7% weight gain of HPMC E15 using 6% w/w of coating solution. An *in vitro* mucoadhesive test and physical characterization of produced microcapsules were evaluated.

1. Core substrate formulation

NHT-mannitol granules were prepared as the core material by wet granulation using the formulation described in Table 7.

Table 7 Compositions of core material

Ingredients	Amount (g)		
	M1-A	**M1-B**	**M2**
NHT	5	5	12
Mannitol	250	250	240
Ratio of NHT:Mannitol	1:50	1:50	1:20
HPMC E15, dry powder (5% w/w of binding solution)	2.96	3.01	2.03

NHT and mannitol were mixed homogeneously by trituration in a mortar and pestel. Then liquid binder, HPMC E15 solution, was added and the damp mass was screened into granules. The granules were dried at 50 ºC for 2 hours and passed through a 0.425 mm sieve (mesh No.40).

2. Microencapsulation process

2.1 Compositions of coating solution

Hydroxypropylmethylcellulose (HPMC E15) was selected as a coating material with good mucoadhesive performance and could sufficiently form film with the core material. According to initially mucoadhesive test, 5% weight gain of HPMC E15 showed good mucoadhesive behaviors thus, it was used in the coating solution.

Table 8 Compositions of coating solution

Ingredients	Amount (g)	Functions
HPMC E15	12.5	Coating material
PEG 6000	2.5	Plasticizer
DI water	208	Solvent
Tartrazine	0.022	Coloring agent

Coating solution was prepared by dispersing hydroxylpropyl methylcellulose (HPMC) in 70 °C deionized water. Cold deionized water was added to obtain clear solution. Then, dissolve PEG6000 (20% of solid weight of HPMC E15) and dispersed tartrazine in the solution.

2.2 Preparation of mucoadhesive nicotine microcapsules

From preliminary study, the bottom-spray fluidized bed technique was selected to prepare the mucoadhesive nicotine microcapsules. The coating conditions used in microencapsulation process are shown in Table 9. Batch of 250 to 260 g of granules were allowed to fluidize in the coater until the inlet air had reached the required temperature. The coating solution was then sprayed via the spray nozzle using a peristaltic pump. On the completion of coating, the coated granules were fluidized for an additional 10 minutes to ensure complete drying.

Table 9 Coating conditions for bottom-spray coating

Parameters	Value
Pre-heating time (min), temperature (ºC)	10, 65
Inlet temperature (ºC)	65 ± 2
Outlet temperature (ºC)	50 ± 2
Fluidized air velocity (m/s)	7.0
Atomizing pressure (kg/cm^2)	1.2
Feed rate (ml/min)	2.0
Post-heating time (min), temperature (ºC)	10, 65

3. Characterization of mucoadhesive nicotine microcapsules

3.1 Physical characterization

3.1.1 Particle size and morphology

The surface and shape of microcapsules were determined by scanning electron microscope (SEM). The samples were gold spray-coated prior to SEM examination. SEM was also utilized to measure the film thickness of coated granules under magnification of x1000 at 15 kV.

Particle size of microcapsules were classified by sieve analysis which consisted of a set of US standard sieves, ranging from sieve no. 20, 30, 50 and 80 mesh (passing apertures of 850, 600, 300 and 180 µm, respectively) and a

collecting pan. Microcapsules were accurately weighed and placed on the top of the sieves. A set of sieves were placed on the sieve shaker and allowed to shake for 20 minutes. The retained microcapsules on each sieve size were weighed and calculated for the percentage of weight retained on each sieve by the following equation:

$$\textbf{\% Retained} = \frac{\text{Retained weight (g)}}{\text{Total microcapsule weight (g)}} \text{x } 100$$

3.1.2 Moisture content

The moisture content of microcapsules was determined by a moisture balance. Approximately 3 g of microcapsules were accurately weighed and uniformly distributed as thin layer on an aluminium plate. Microcapsules were exposed to high temperature of approximately 105°C until constant weight was obtained. The moisture content in terms of loss on drying was calculated automatically. The results were obtained from an average of three determinations.

3.1.3 Bulk, tapped densities and compressibility index

The bulk density (ρ_b) of microcapsules was determined by pouring 10 g of granules into a 25 ml graduate cylinder and measuring the volume of microcapsules. The graduate cylinder was tapped on a jolting volumeter until a constant volume was obtained. The tapped density (ρ_t) was then calculated. Both densities were average from three determinations. The Carr's compressibility, which expresses the flow property as presented in Table 10, was calculated from the following equation:

$$\text{\% Compressibility} = \frac{(\rho_t - \rho_b)}{\rho_t} x\,100$$

3.1.4 Angle of repose

The angle of repose was measured from a heap built up by a free powder flow of 20 g of microcapsules through a glass funnel with 0.65 cm internal stem diameter fixed on the clamp at 10 cm height from the smooth surface. Average

result from three determinations was reported. Angle of repose was calculated from the following equation:

$$\alpha = \tan^{-1} \frac{H}{R}$$

3.1.5 Flow rate

Accurately weighed 20 g microcapsules were filled in a glass funnel with 0.65 cm internal stem diameter fixed on a clamp. When the microcapsules started to flow until finished, the time was recorded. Flow rate was averaged from three determinations and reported in terms of g/s.

Table 10 Flow properties and corresponding flowability parameters (USP28)

Flow character	Angle of Repose (degree)	Compressibility index (%)
Excellent	25-30	≤ 10
Good	31-35	11-15
Fair	36-40	16-20
Passable	41-45	21-25
Poor	46-55	26-31
Very poor	56-65	32-37
Extremely poor	> 66	> 38

3.2 Determination of mucoadhesive performances

3.2.1 Mucoadhesion time

The mucoadhesion of nicotine microcapsules were carried out using freshly excised porcine buccal tissue according to Attama and Onuigbo (2007). Prior to the study, the buccal tissue was rinsed with simulated saliva pH 6.8 and attached on a microscope slide inclined at an angle of 60°. A coated granule were placed on the surface of the tissue and allowed to hydrate for 1 minute. Simulated saliva fluid was maintained at 37±0.5 ºC and allowed to flow over the tissue at the rate of 20 drops per minute, approximately 1 cm away from the tissue. The time for each microcapsule to detach from the porcine buccal tissue was recorded as the

mucoadhesion time. Mucoadhesion time for each formulation was an average of nine determinations.

3.2.2 Mucoadhesive force

Mucoadhesiveness of microcapsules was evaluated using a texture analyzer with a 5 kg load cell. A cellulose acetate membrane was first soaked in an aqueous mucin solution (porcine stomach type II, 10% w/w in water). The soaked membrane was then attached to the upper end of texture analyzer probe using double-sided adhesive tape. Ten mg of mucoadhesive microcapsules were weighed then swollen in simulated saliva pH 6.8 for 1 minute before attaching to the lower probe. The download force of 0.1 g was applied then the upper probe was lowered onto the surface of microcapsules with contact time of 90 s to ensure intimate contact between mucin and microcapsules. The upper probe was elevated upward in a vertical direction at a speed of 10 mm/s. The force required to detach the mucin from the microcapsules was determined as the mucoadhesive force. All measurements for each formulation were performed in triplicate.

Saliva fluid was simulated by using a solution of 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.0 g NaCl per litre of deionized water (Fábregas and García, 1995).

The data of mucoadhesion time and mucoadhesive force were subjected to the one-way analysis of variance (one-way ANOVA method).

3.3 Content of nicotine in mucoadhesive nicotine microcapsules

The nicotine content in the microcapsules was quantitatively determined by mean of absorption peak area using high performance liquid chromatography (HPLC) method.

Nicotine was analyzed by reversed phase HPLC. The design chromatographic conditions were previously mentioned (Tambwekar, Kakariya and Garg, 2003).

HPLC analysis

HPLC chromatographic conditions:

Column	: Hypersil® C18 BDS (Thermo Hypersil, UK) 250 x 4.6 mm, and 5 µm particle size
Mobile phase	: 10 mM Phosphate buffer (pH 6.8): methanol 35:65 %v/v. Phosphate buffer consisting triethylamine 0.1% v/v and adjust with HCl to final pH 6.8
Flow rate	: 1.0 ml/min
Injection volume	: 20 µl
Detector	: UV 259 nm
Retention times	: Nicotine 4.6 min

Validation of the HPLC method

The typical analytical characteristics used in method validation were specificity, accuracy, precision and linearity (USP30/NF25, 2007).

Preparation of internal standard solution for validation:

An accurately weighed 0.20 g of dexamethasone was placed into a 100 ml volumetric flask and diluted with methanol to volume. The final concentration of internal standard was 2.0 mg/ml.

Preparation of standard solutions for validation:

Liquid nicotine was accurately weighed about 50.10 mg (equivalent to 50 mg of nicotine) to 100 ml volumetric flask then diluted with methanol to volume. This solution was used as the standard stock solution and the final concentration was 0.5 mg/ml. The standard stock solution of 2.0, 3.0, 4.0, 5.0 and 6.0 ml were transferred into 25 ml volumetric flask. Then 500 µl of internal standard solution was added and dilute to volume with mobile phase. Five dilutions were prepared as standard solution in the concentration range of 40-120 µg/ml.

Assay preparation:

An accurately weighed 0.100 g of microcapsules was placed into a 25 ml volumetric flask. The solution for nicotine content analysis was prepared by dissolving the coated granules with phosphate buffer pH 6.8 and 500 µl of internal standard solution was added. The solution was adjusted to volume with phosphate buffer pH 6.8. The samples were prepared in triplicate and analyzed. Content of nicotine in coated granules was calculated from the linear regression equation obtained calibration curve of standard solutions.

All solutions were filtered through 0.45 µm membrane filter before analysis and injected on column in triplicate.

Linearity:

The linearity of an analytical method is the ability to elicit test results that are directly proportional to the concentration of drugs in samples within a given range. Triplicate of each concentration of standard solutions in various concentrations range from 40 to 120 µg/ml were analyzed. The linear regression analysis of the peak area ratio versus the concentrations was calculated.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatly to multiple samplings of homogenous sample. The percentage of coefficient of variation (%CV) or relative standard deviation (%RSD) values of peak area of standard solutions both within run and between run less than 2.00% which indicates that HPLC methods can be used to determine the amount of nicotine over period of time studied.

Within run precision

The within run precision was determined by analyzing the standard solution at 100% of the test concentration (80 µg/ml). Repeatability was assessed using a minimum of six determinations. The percentage of relative standard deviation (%RSD) value of peak area of nicotine was determined.

Between run precision

The between run precision was determined by analyzing the standard solution at 100% of the test concentration which prepared and injected on different days. The percentage of relative standard deviation (%RSD) value of peak area of nicotine was determined.

Accuracy:

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. Three concentration levels of drug solution (80, 100 and 120% of assay concentration). Accuracy was calculated as the percentage of recovery of each drug solution. The mean percentage of recovery of 95-105% with percent of coefficient of variation (%RSD) < 2.00% indicates the high accuracy of the method.

Specificity:

The specificity of an analytical method is the ability to assess the peak of drug from the sample without interfered by other components, presented in the sample. To determine the specificity of the method, the contents consisting excipients without active ingredient present in final formulation was prepared in 25 ml of phosphate buffer pH 6.8. This solution was injected on column after filtration through 0.45 µm nylon filter and peak response was recorded. The chromatogram of excipients blend was compared with the chromatogram of the drug solution.

Actual nicotine content in mucoadhesive nicotine microcapsules was determined in triplicate by HPLC. The percentage of nicotine content in these microcapsules after coating process was calculated using the following equation:

$$\text{Nicotine content (\%)} = \frac{\text{Actual nicotine content}}{\text{Theoretical nicotine content}} \text{x } 100$$

3.4 Solid state characterization

The physical properties of mucoadhesive nicotine microcapsules after microencapsulation process were carried out by differential scanning calorimetry (DSC) technique, X-ray powder diffractometry and solid state NMR spectroscopy.

Part III. Preparation of buccal tablets containing mucoadhesive nicotine microcapsules

1. Tablets preparation

1.1 Tablet formulations

The tablets were designed as bilayer buccal tablets. From a preliminary study of tablet formulations, the compositions of bilayer buccal tablets which present the pleasant taste and good physical characteristic are shown in Table 11 and 12, respectively.

Table 11 Compositions of the placebo layer

Ingredients	Amount (mg/tablet)	Functions
Mannitol	480	Diluent
Microcrystalline cellulose (Avicel® PH102)	50	Disintegrant
Magnesium stearate	2	Lubricant
Talcum	8	Antiadherent, glidant
Peppermint oil in EtOH	q.s.	Flavoring agent

Mannitol and avicel were mixed until homogeneous. Then the lubricating agent and glidant were added and mixed for 5 minutes. Peppermint oil solution was sprayed at a last step.

Table 12 Compositions of the drug layer containing mucoadhesive nicotine microcapsules.

Ingredients	Amount (mg/tablet)	Functions
Mucoadhesive nicotine microcapsules	140*	Active ingredient
D-granules (mannitol, xylitol, avicel 102)	510	Diluent
Sucralose	1	Artificial sweetening agent
Menthol	12	Flavoring agent
Magnesium stearate	3.4	Lubricant
Talcum	13.6	Antiadherent, glidant
Peppermint oil in EtOH	q.s.	Flavoring agent

* Amount equivalent to nicotine 2 mg as determined by HPLC.

D-granules were prepared by wet granulation method. Mannitol, xylitol, and avicel PH102 (amounts were shown in appendix D) were mixed homogeneously by trituration in a planetary mixer. Then liquid binder, 5% HPMC E15 solution, was added and the damp mass was screen into granules using high speed granulator. Granules were dried at 50 ºC. The dried granules were passed through screen No.40 mesh and classified by sieve analysis, ranging from sieves No. 30, 50, and 80 mesh and a collecting pan.

Active ingredient, diluents and flavoring agents were mixed until homogeneous. The lubricating agent and glidant were added and mixed for 5 minutes and peppermint oil was sprayed at the end.

1.2 The process of compression

Buccal bilayer tablets were prepared by direct compression procedure involving two steps. First, 540 mg of mixture of the placebo layer was added and lightly compressed by manual single punch tabletting machine with 16 mm diameter die. The upper punch was raised then 680 mg of the drug mixture which composed of

mucoadhesive nicotine granules was added to the precompressed placebo layer. Then the two layers were finally compressed to form a bilayer buccal tablet. Total weight of each tablet was approximately 1,220 mg. A diagram of a bilayer buccal tablet is shown in Figure 21.

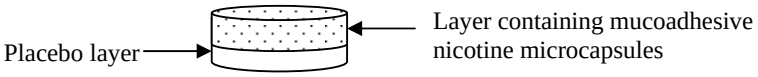


Figure 21 Diagram of bilayer buccal tablet containing mucoadhesive nicotine microcapsules

2. Evaluation of tablets

2.1 Determination of physical properties of tablets

2.1.1 Weight and weight variation of tablets

To perform the USP30 weight variation test, 20 tablets were randomly selected and individually weighed using an analytical balance. The average weight and the percent variation of each tablet were calculated.

2.1.2 Tablets thickness and hardness

Thickness and hardness of tablets were determined by evaluating 10 tablets using a Thermonik® instrument and the average value was calculated.

2.1.3 Friability

Friability was determined by first weighing 20 tablets (w_0) after dusting. The tablets were placed in a friability tester. The container was rotated for 4 minutes. After dusting off excess powder, the total remaining weight of the tablets (w) was recorded and the percent friability was calculated from the following equation:

$$\% \text{ Friability} = (1-w/w_0) \times 100$$

2.1.4 Disintegration

To perform the USP 30 disintegration test, 6 tablets were randomly selected and evaluated using a disintegration tester (Erweka, model ZT31). Disintegration time of each tablet was recorded and the average value was calculated.

2.2 Content uniformity of active pharmaceutical ingredient (API)

Accurately weighed tablet was triturated in a mortar and the contents were transferred quantitatively to 25 ml volumetric flask. The contents were dispersed in 20 ml of phosphate buffer pH 6.8 with the help of sonication for 10 minutes then 500 µl of internal standard was added and adjusted to volume with phosphate buffer. Allow the sample to stand for about 10 minutes until the sediments were separated. Clear solution of sample was filtered through Whatman#1 filter paper and the filtrate was collected. The filtrate was again filtered through 0.45 µm nylon filter before subjecting to HPLC analysis. The drug concentration was determined from previously obtained calibration curve. The experiment was repeated on another nine additional tablets.

2.3 In vitro nicotine release studies

The release of nicotine from tablets was studied using modified Franz diffusion cells. This diffusion cells consisted of two compartments, the donor compartment and the receiver compartment (Figure 22). The method used was modified from the method described by Ìkinci et al., 2004. The dissolution medium was 14 ml phosphate buffer pH 7.4 maintained at 37 ± 0.5 ºC by a circulating water jacket. Uniform mixing of the medium was carried out by magnetic stirring at 300±5 rpm. Each tablet was placed on top of the dialysis cellulose membrane with 2 ml simulated saliva fluid as the donor medium. Any air bubbles formed under the membrane were removed prior to the experiment. The experiment was done in triplicate.

One ml of sample was collected from the medium at various time intervals (i.e. 15, 30, 45, 60, 120, 180, 300, and 360 minutes) and replaced with the same amount of medium. The samples were filtered through 0.45 µm membrane filter and analyzed for nicotine concentration by HPLC method. The drug concentration was

determined from the calibration curve and the amount release was calculated by multiplying the drug concentration with the receiver volume. The release profiles were plotted as percent of cumulative drug released as opposed to time.

Commercial nicotine polacrilex lozenges, another buccal bioadhesive nicotine tablet were used as a reference product in this study. The difference factor (f_1) and similarity factor (f_2) were used to compare release profile between the two curves and calculated from the following equation (O'Hara et al., 1998):

$$f_1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\}*100$$

$$f_2 = 50*\log \{[1+ (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} *100\}$$

where n is the number of time points, R_t is the dissolution value of the reference (percentage) batch at time t, and T_t is the dissolution value of the test (percentage) batch at time t.

Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test and reference products.

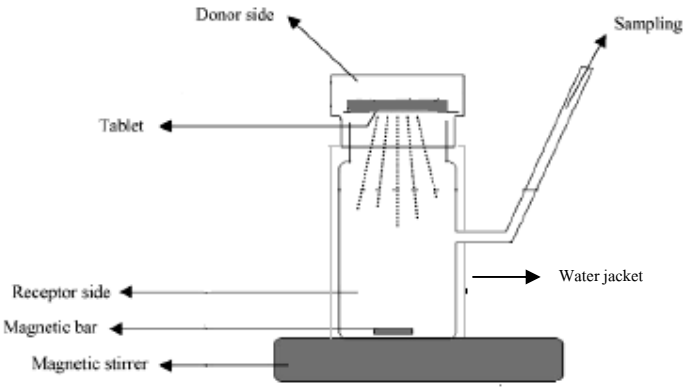


Figure 22 Schematic of representation of modified Franz diffusion cell used in the *in vitro* release study

2.4 Stability assessment of the product

Short term stability determination was performed under accelerated condition at 45 ºC, 75% relative humidity (RH) and room temperature of 30 ºC, 75% RH for a period of three months. Tablets were packaged in an aluminum foil-like material with light and moisture proof properties and sealed with poly sealer and placed at above specified condition in hot air oven. After each month tablet samples were evaluated for its physical characteristics and drug content.

CHAPTER IV
RESULTS AND DISCUSSION

Nicotine replacement therapy was the first successful pharmacological intervention for nicotine dependency and now widely manipulated (Mitrouska, Bouloukaki and Siafakas, 2007). Recently, Alberg et al. (2005) reported that the use of NRT was common in general population, particularly among heavy smokers. Numerous clinical trials have assessed the efficacy of nicotine medications for smoking cessation and concluded that these treatments were effective in very heavy and highly dependent smokers (Shiffman, Dresler and Rohay, 2004; Shiffman, Marino and Pilliteri, 2005).

NRT was available in several forms, such as, nicotine gum, patches and lozenge are found as an over the counter products (OTC) in many developed countries. Previous studies attempted to develop a buccal adhesive nicotine tablet by preparing the matrix-forming polymers (Park and Munday, 2002; Ìkinci, Senel, Wilson and Sumnu, 2004).

This study was aimed to formulate the buccal tablet containing mucoadhesive nicotine microcapsules using microencapsulation technique. The mucoadhesive nicotine microcapsules were characterized and evaluated before buccal tablets were produced.

The present study was devided into three parts which were characterization of nicotine hydrogen tartrate, preparation of mucoadhesive nicotine microcapsules and preparation of buccal tablets containing mucoadhesive nicotine microcapsules.

Part I. Characterization of nicotine hydrogen tartrate dihydrate (NHT) powder

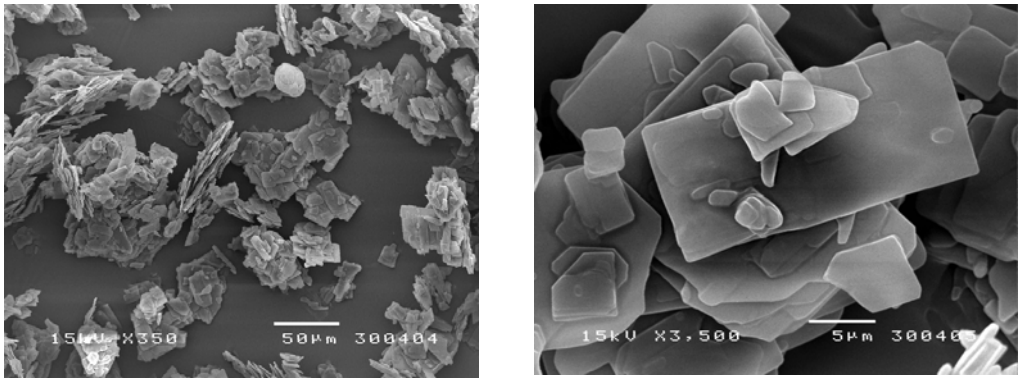
This section was focused on the identification of nicotine hydrogen tartrate dihydrate when used as an active pharmaceutical ingredient.

1. Solid state characterization of NHT powder

The solid-state behavior of nicotine hydrogen tartrate dihydrate powder was investigated using a variety of complementary techniques such as scanning electron microscopy, differential scanning calorimetry, thermogravimetry analysis and powder X-ray diffractometry.

1.1 Scanning electron microscopy

The shape and surface topography of NHT was examined by scanning electron microscope (SEM). The morphology of NHT are presented in Figure 23. Particles of nicotine hydrogen tartrate were aggregated (Figure 23a) and showed rectangular shape crystalline (Figure 23b).



(a) (b)

Figure 23 Scanning electron photomicrographs of NHT at magnification of (a) x350 and (b) x3,500

1.2 X-ray powder diffractometry

The crystalline form of NHT was confirmed by XRPD as shown in Figure 24. The crystallinity of material is related to sharp well-defined peak intensity.

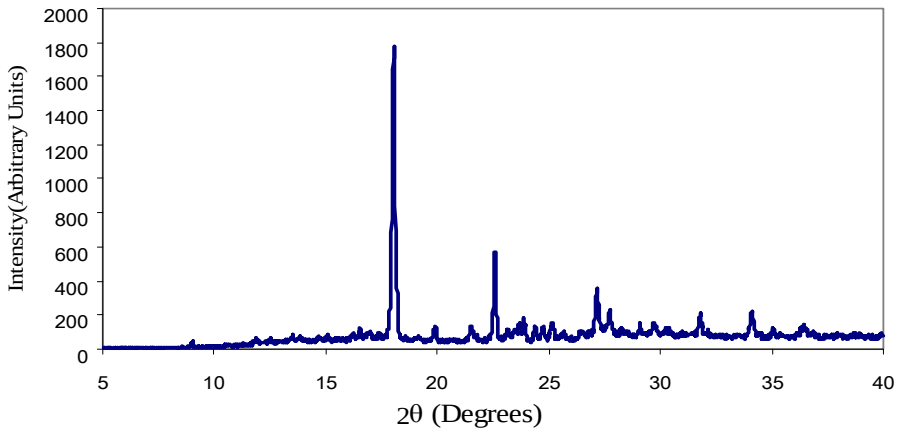


Figure 24 X-ray powder diffraction pattern of NHT

1.3 Differential scanning calorimetry and thermogravimetric analysis

Thermogram of NHT at a heating rate of 10°C/min, shows two endothermic peaks (Figure 25). The first endothermic peak was at 90 ºC and the second peak at 97 ºC. NHT underwent decomposition at 200 °C. The temperature at 80–90 °C where the first endothermic event occurred, mass loss was observed due to dehydration of dihydrate molecules. Meanwhile, weight of NHT was constant at the temperature where second endothermic peak occured.

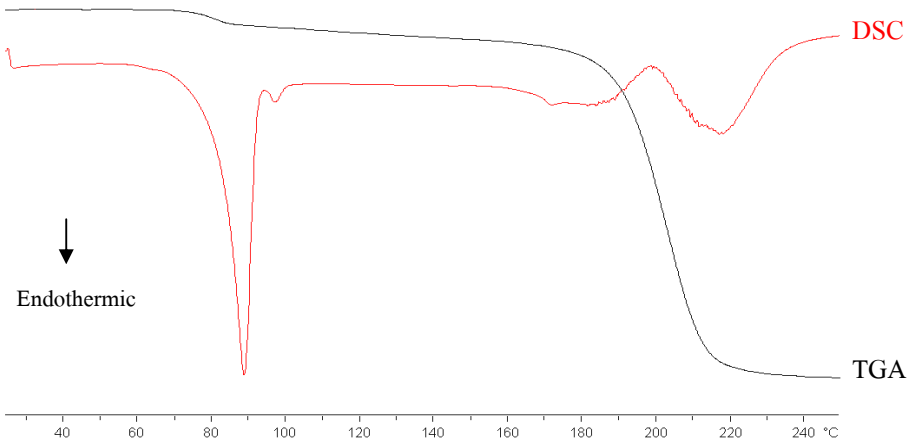


Figure 25 TGA and DSC thermograms of NHT in dynamic N_2 atmosphere and 10 °C/min heating rate from 25 – 250 ºC.

To increase the resolution of the second endothermic peak, repeated thermal analysis was carried out using a heating rate of 5 °C/min at 25 °C to 120 °C. The negligible shift of the first endotherm is shown in Figure 26 and the sharp endothermic peak at 97 °C represents the melting peak of NHT as anhydrous form. Hence, microencapsulation process, the drying temperature used in coating process should not exceed 80 °C to prevent degradation of drug.

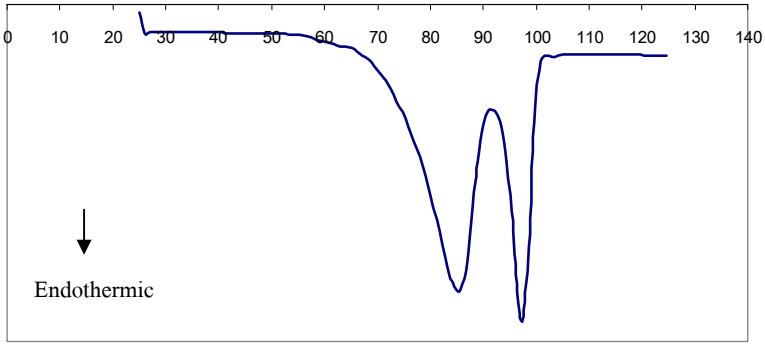


Figure 26 DSC thermogram of NHT at 5 °C /min heating rate from 25 - 120 ºC.

1.4 Solid state nuclear magnetic resonance spectrometry

When the sample is placed in a magnetic field, NMR active nuclei (^{13}C) absorb at a frequency characteristic of the isotope. The resonance frequency, energy of the absorption and the intensity of the signal are proportional to the strength of the magnetic field (Bernstein, 1994). Figure 27 illustartes chemical shift values (ppm) of NHT which determined by the local electronic environment of a carbon atom in the molecule.

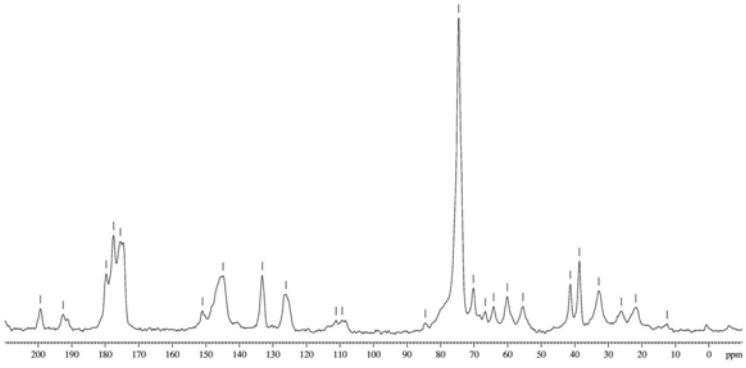


Figure 27 Solid state NMR ^{13}C spectra of NHT

2. **Particle size and size distribution of NHT powder**

The particle size and size distribution (Figure 28) of NHT powder were determined by laser light scattering technique and the results are shown in Table 13.

Table 13 The particle size and size distribution of NHT [mean (SD)]

Physical properties	Nicotine hydrogen tartrate dihydrate (NHT)
d (v,0.1), µm	12.69 (0.04)
d (v,0.5), µm	35.97 (0.24)
d (v,0.9), µm	73.29 (1.06)
D[4,3], µm	39.93 (0.40)
Span	1.68 (0.02)
Uniformity	0.52 (0.00)

Note: - d (v,0.5) is the size at which 50% of the sample is smaller and 50% is larger (mass median diameter).
- d (v,0.1) and d (v,0.9) are the size of particle below which 10% and 90% of the sample lies respectively.
- D[4,3] is the volume mean diameter.
- The span is the measurement of the width of the distribution, defined as the differences between the diameter at the 90 and the 10 percentage points relative to the median diameter.
- The uniformity is a measure of the absolute deviation from the median.

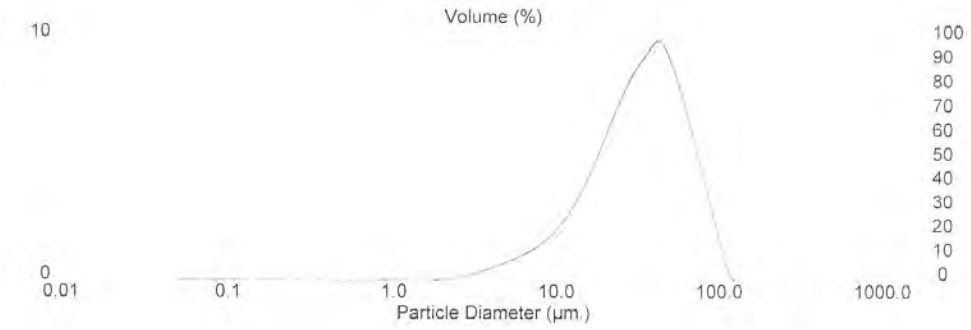


Figure 28 Particle size distribution of NHT powder

3. **Compatibility studies**

Compatibility study between active ingredient (NHT) and other major excipients such as mannitol and HPMC E15 was determined by DSC. Mannitol was selected as main filler in this experiment due to the fact that mannitol is an inert material, nonhygroscopic and gives cooling sensation when taken orally. HPMC E15 was used as binding agent and mucoadhesive coating material. Moreover, HPMC

polymer is a biocompatible material that do not damage tissue thus it is suitable to apply in the oral cavity.

Thermograms of NHT and mannitol show the endothermic melting peak at 97ºC and 168ºC, respectively. Meanwhile, each mixture (NHT:excipient, 1:1 and 1:1:1) gave negligible shift in NHT and mannitol melting temperatures (Figure 29). It could be concluded that no interactions between the NHT and other excipients were observed.

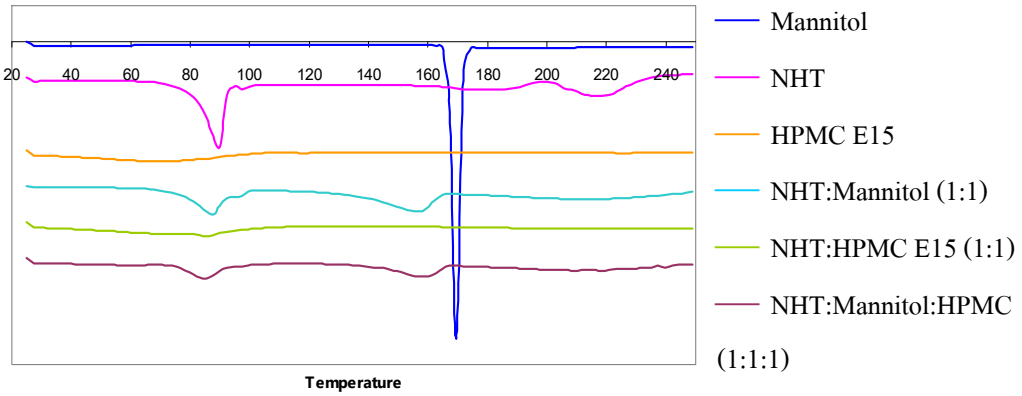


Figure 29 DSC thermograms of NHT, excipients and various ratios of its physical mixtures.

Part II. Preparation of mucoadhesive nicotine microcapsules

This section focused on the mucoadhesive performance and characterization of mucoadhesive nicotine microcapsules.

1. **Preliminary study on the coating process and mucoadhesion time of mucoadhesive microcapsules**

1.1 Preliminary study on top and bottom-spray fluidized bed coating

Factor affecting fluidized-bed coating such as atomizing air pressure, drying temperature and liquid spray rate were evaluated. The coating conditions and compositions of coating solution were described in appendix A. To select the appropriate coating conditions for subsequent studies, appearances and flowability in term of compressibility index of coated particles were evaluated.

The main objective of particle coating of this study is to encapsulate each particle or granule with sufficient coating polymer to improve the taste of nicotine and develop mucoadhesive microcapsules. From preliminary study, bottom-spray coating (B1 and B3) was operated with the same parameters used in formulation T3 and T6. The microcapsules which produced by top-spray coating technique exhibited bulky character, meanwhile, the microcapsules produced by bottom-spray coating technique exhibited excellent flow property which presented in terms of % compressibility less than 10% and angle of repose less than 30 degrees (Table 14). In addition, large particle aggregation could occur with top spray compared to bottom spray coating. Jones (2008) indicated that the high quality of films occurred by applying bottom spray fluidized-bed coater due to the concurrent spray and high drying efficiency of this process. As a result, bottom spray coating was used to produce mucoadhesive nicotine microcapsules with varying amount of HPMC E15 in the coating solution.

Table 14 Physical properties of the produced microcapsules [mean (SD)]

Parameters	**Formulations**					
	T3	**T5**	**T6**	**B1**	**B2**	**B3**
Moisture content (%LOD)	1.73 (0.03)	0.64 (0.12)	0.59 (0.04)	0.94 (0.03)	0.86 (0.04)	0.73 (0.05)
Bulk density (g/cm^3)	0.31 (0.00)	0.39 (0.00)	0.36 (0.00)	0.48 (0.00)	0.46 (0.00)	0.47 (0.00)
Tapped density (g/cm^3)	0.38 (0.002)	0.46 (0.00)	0.41 (0.002)	0.53 (0.004)	0.51 (0.004)	0.51 (0.004)
% Compressibility	18.49 (0.45)	13.44 (0.49)	11.21 (0.53)	9.92 (0.69)	9.69 (0.67)	8.91 (0.67)
Flow rate (g/s)	1.67 (0.01)	2.10 (0.01)	1.92 (0.003)	2.72 (0.01)	2.73 (0.02)	3.29 (0.03)
Angle of repose (deg)	35.4 (0.49)	32.67 (0.59)	31.53 (0.98)	26.77 (0.65)	25.52 (0.73)	27.99 (0.70)

T; top-spray coating, B; bottom-spray coating

Polyethylene glycols (PEG) are the suitable plasticizer for HPMC polymer, especially a high molecular weight type such as PEG6000 (Porter et al., 1993; Obara and Kokubo, 2008). Coating solution contained PEG6000 in order to reduce glass-transition temperature (Tg) of HPMC E15 and increase film flexibility (Carstensen, 2001). The used of coating solution without PEG6000 caused a marked

increase in aggregation of the granules. Tartrazine was added in the coating solution as a visual indicator for complete coating.

1.2 Preliminary study on the mucoadhesive performances of mucoadhesive nicotine microcapsules

Mucoadhesive nicotine microcapsules were produced by bottom-spray fluidized-bed coater with varying amounts of HPMC E15 in coating solution. The size of produced microcapsules was characterized by standard sieve analysis (Figure 30). Although the microcapsule size below 180 µm are shown in highest amount, this range was rejected due to its powdery character and was insufficiently coated. The size of microcapsules 300-600 µm was chosen for the future evaluation.

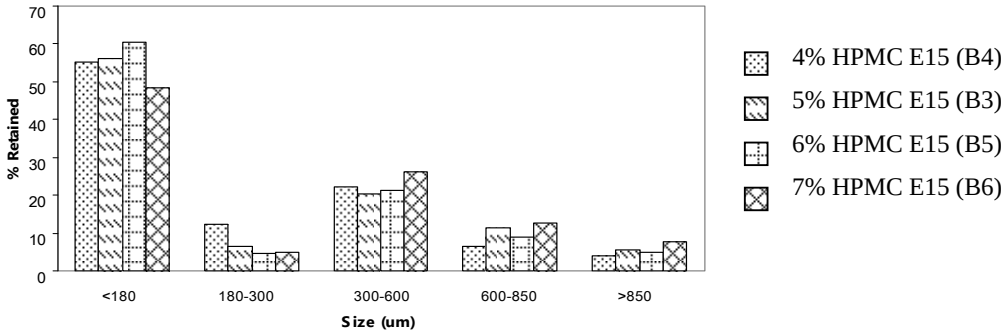


Figure 30 Particle size distribution of mucoadhesive nicotine microcapsules coated with 4, 5, 6 and 7% weight gain of HPMC E15 determined by standard analytical sieves.

The amounts of HPMC E15 were classified in groups with 4, 5, 6 and 7% weight gain using 6% w/w coating solutions and these groups were selected to evaluate mucoadhesion. The results (Figure 31 and Table 14) reveal that the coating solution which contained at least 5% weight gain of HPMC E15 gave optimal mucoadhesion time of 1.29±0.11 minutes. Although the amounts of the polymer were increased for more 5%, the mucoadhesion time was not significantly extended ($P > 0.05$, one-way ANOVA). The studies of Park and Robinson (1984) exhibited that increased concentration of mucoadhesive polymer would result in increased binding potential. However, for each polymer, there is a critical optimal concentration (Miller,

Chittchang and Johnston, 2005). Therefore, higher concentrations of polymer do not necessarily always improve the mucoadhesive properties.

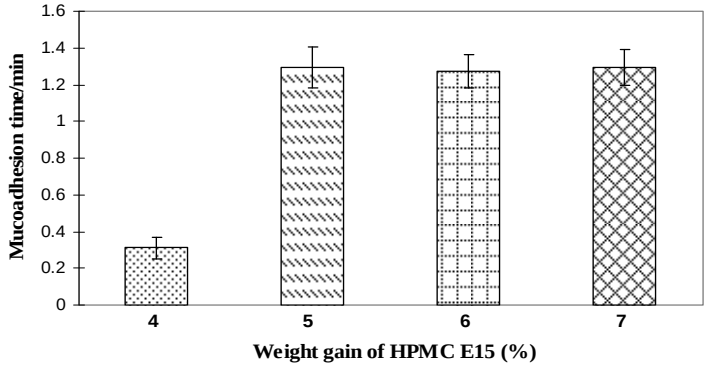


Figure 31 Mucoadhesion time of mucoadhesive nicotine microcapsules with varying amounts of HPMC E15 as 4, 5, 6 and 7% weight gain (mean±SD, n=9)

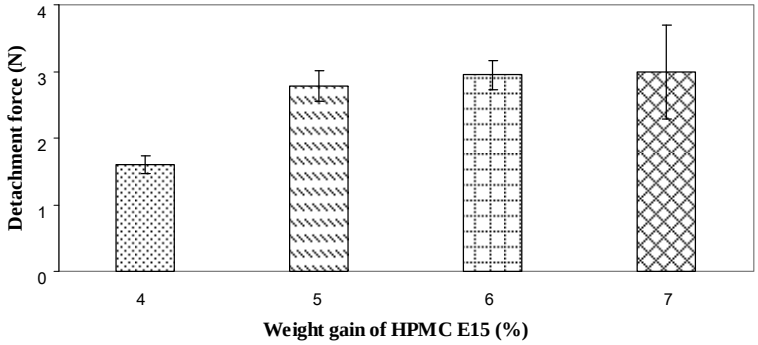


Figure 32 Comparative detachment force of mucoadhesive nicotine microcapsules with varying amounts of HPMC E15 as 4, 5, 6 and 7% weight gain (mean±SD, n=3)

Figure 32 represents the detachment force of the prepared microcapsules with varying amounts of HPMC E15. Mucoadhesive nicotine microcapsules contained 4% weight gain of HPMC E15 shows the lowest force of adhesion (lowest detachment force) that is significantly lower ($P < 0.05$, one-way ANOVA) than the other formulations. No significant difference ($P > 0.05$) was found between the detachment forces of the mucoadhesive nicotine microcapsules obtained from 5, 6 and 7% weight gain of HPMC E15 (Appendix G). The result illustrates that higher amount of HPMC E15 of more than 5% weight gain do not significantly increase the detachment force. This result was in correlation with the result of mucoadhesion time

(Table 15) therefore, the amount of 5% weight gain of HPMC E15 was selected as the coating solution for producing microcapsules throughout this study.

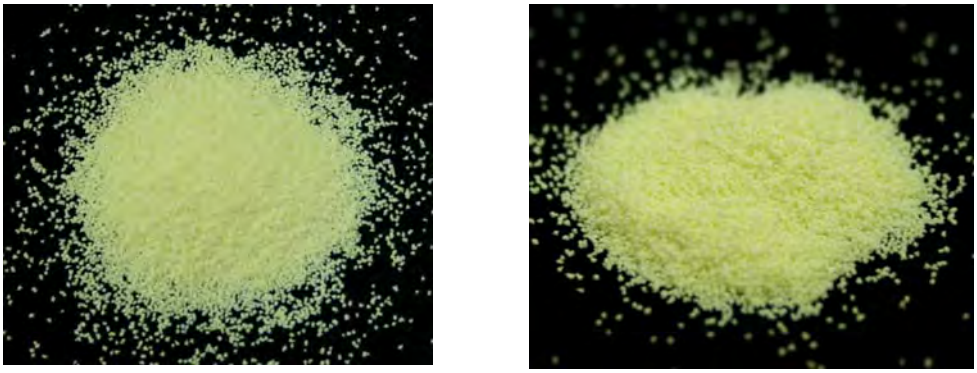
Table 15 Mucoadhesion performances of mucoadhesive microcapsules with varying amount of HPMC E15 (mean±SD)

Weight gain of HPMC E15 (%)	**4**	**5**	**6**	**7**
Mucoadhesion time (min)	0.31±0.06	1.29±0.01	1.27±0.09	1.29±0.10
Mucoadhesive force (N)	1.59±0.13	2.78±0.23	2.94±0.22	2.99±0.71

2. Preparation of mucoadhesive nicotine microcapsules

NHT and mannitol were first prepared as granules. Approximately 40 g of liquid binder (HPMC E15, 5% w/w) was added into 250 g of powder mixture. The prepared granules were coated with 5% weight gain of HPMC E15 in coating solution to form microcapsules. The coating process was performed in triplicate (M1-A, M1-B and M2) using bottom spray fluidized-bed coater with the most appropriate condition obtained from preliminary study. The ratio of NHT and mannitol of M1-A and M1-B were 1:50, unlike M2 formulation that the ratio of NHT and mannitol was 1:20.

The particle flow pattern in a bottom-spray fluidized bed coater was established with the aid of a cylindrical coating partition and an air distribution plate, which controlled the air flow. Most of the warm incoming air together with the atomizing air from the spray nozzle, caused the granules to circulate similar to spouting water fountain. Granules passing through the coating partition received a layer of coating material, dried in the expansion chamber and fell back onto the top of the bed outside the coating partition. The air in this down bed acts to cushion and dry the partially coated granules to continue the cycle through the coating partition (Mathur, 1992). The produced mucoadhesive nicotine microcapsules were further characterized. Figure 33 exhibits the bulk appearance of mucoadhesive nicotine microcapsules product of M1-A, M1-B and M2 formulations.



(a) (b)

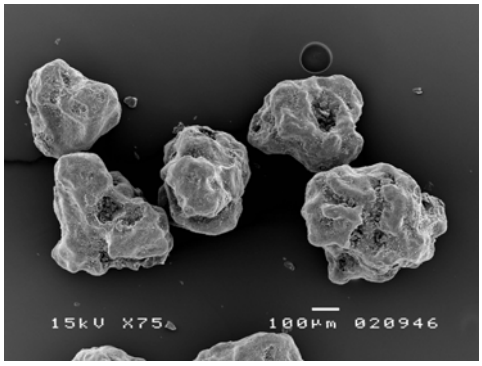
(c)

Figure 33 The appearance of mucoadhesive nicotine microcapsules bulk product of (a) M1-A, (b) M1-B and (c) M2

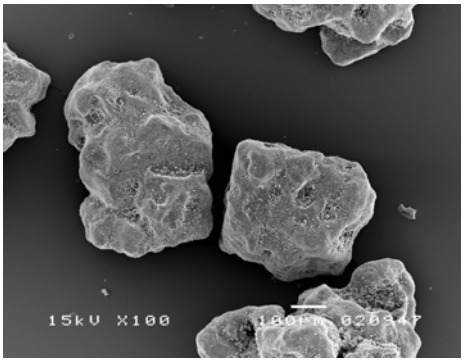
3. Physical characterization of mucoadhesive nicotine microcapsules

3.1 Morphology

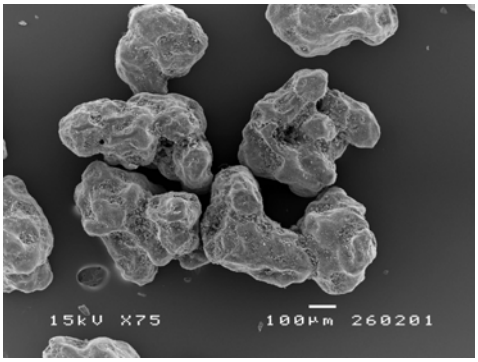
The shape and surface topography of mucoadhesive nicotine microcapsules were examined by scanning electron microscope (SEM). The microcapsules showed irregular shape, rough surface and observed to be covered with HPMC E15. The morphology of every formulations are presented in Figure 34.



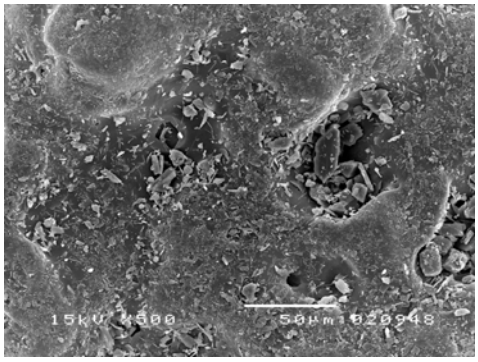
(a)



(b)



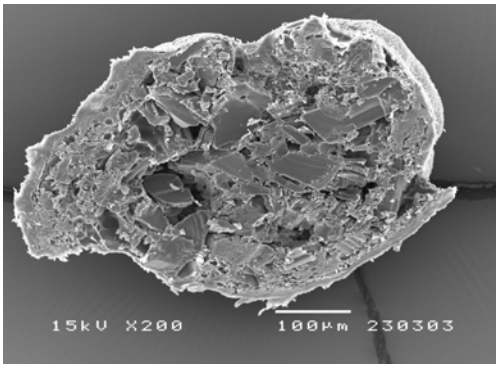
(c)



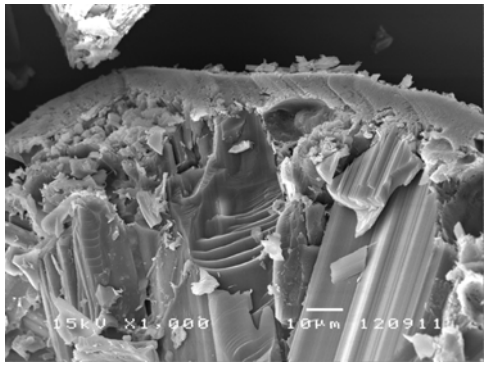
(d)

Figure 34 Scanning electron photomicrographs of mucoadhesive nicotine microcapsules (a) M1-A, (b) M1-B, (c) M2 at magnification of x75 and (d) outer surface of microcapsule at magnification of x500

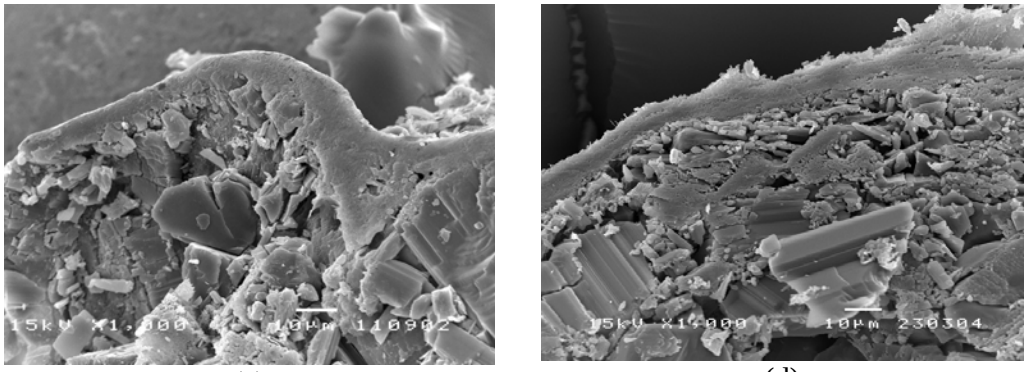
The cross section of the mucoadhesive nicotine microcapsules (Figure 35) illustrates that mucoadhesive nicotine microcapsules were coated with HPMC and the film layer was visible with approximately 10 µm thickness.



(a)



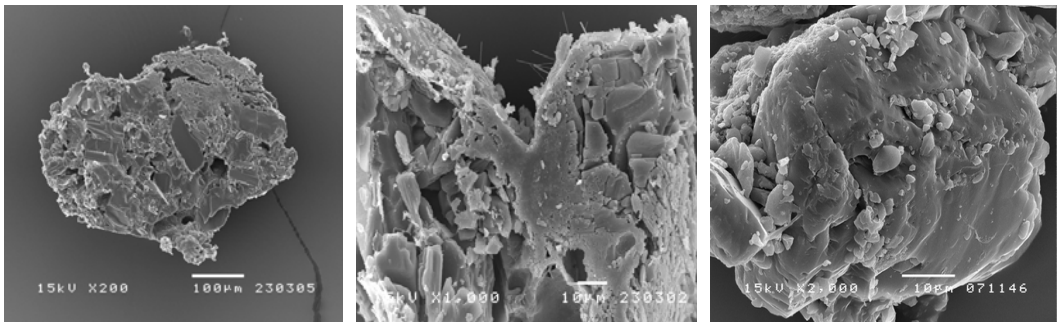
(b)



(c) (d)

Figure 35 Scanning electron photomicrographs of a cross-section of mucoadhesive nicotine microcapsule (a) HPMC E15 layer was observed to coat the microcapsule at magnification of x200, film layer of (b) M1-A, (c) M1-B and (d) M2 at magnification of x1,000

Figure 36 reveals the morphology of the granules before coating. The surface of the uncoated granules composed of small aggregates and the layer of polymer was not seen.



(a) (b) (c)

Figure 36 Scanning electron photomicrographs of uncoated granule before underwent fluidized-bed coating process at magnifications of (a) x200 of cross-sectioned, (b) x1,000 of cross-sectioned and (c) x2,000 of outer surface of the uncoated granule.

3.2 Particle size and size distribution

The particle size of mucoadhesive nicotine microcapsules was characterized by sieve analysis. Particle size distribution is displayed in Figure 37. The results reveal a good reproducibility of selected coating process. Agreed with the result obtained from the preliminary study, the microcapsule size below 180 μm was

rejected due to its powdery character and was incompletely coated. The size of microcapsules 300-600 µm was chosen for future evaluation.

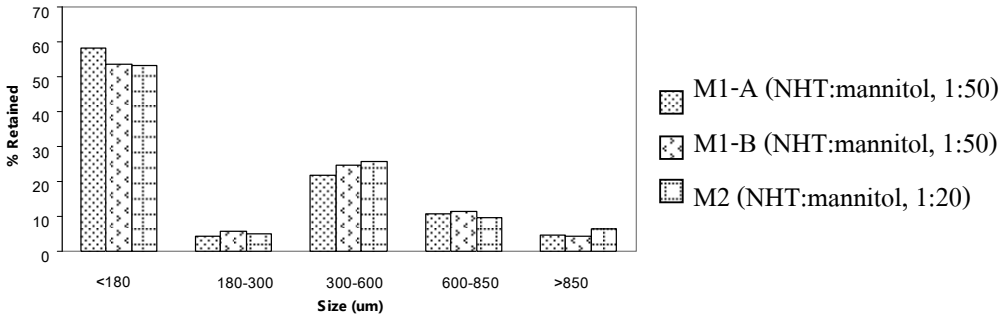


Figure 37 Particle size distribution of mucoadhesive nicotine microcapsules coated with 5% weight gain of 6% w/w HPMC E15 solution.

3.3 Moisture content

The moisture balance was used to measure the amount of residual moisture in the microcapsule after coating process. Moisture content of mucoadhesive nicotine microcapsules is presented in terms of % loss on drying (%LOD) as shown in Table 16. The values are in the range of 0.63 – 0.78%. Moisture content values were slightly different eventhough the amount of water used in each formulation was equivalent. Beside the amount of water used in the coating solution, the moisture content of microcapsules variability may depend on the different capability of water evaporation during the drying stage and the moisture content in the core granules.

3.4 Bulk, tapped densities and compressibility index

Bulk and tapped densities were determined and evaluated for compressibility index. Bulk density is used to determine the space required for the storage microcapsules, while, tapped density is used to investigate the packing property of the microcapsules. From the data shown in Table 16, it was found that the value of bulk density, tapped density and compressibility index of M1-A, M1-B and M2 microcapsules were only slightly different. The small difference of bulk and tapped density, resulting in low compressibility index value (<10%), indicating very free flowing behavior and good packing of microcapsules. This result may be due to its uniform shape and optimal size which was selected after sieving in 3.2.

3.5 Flowability and angle of repose

The flow rate was evaluated by monitoring the time taken for microcapsules to flow through an orifice of the glass funnel. Flow rate and angle of repose of microcapsules are reported in Table 16. Rapid flow rate and repose angle of < 30 deg indicated that microcapsules had excellent flowability in agreement with the results obtained from % compressibility interpretation.

Table 16 Physical properties of mucoadhesive nicotine microcapsules [mean (SD)]

Physical properties	**M1-A**	**M1-B**	**M2**
Moisture content (%LOD)	0.65 (0.03)	0.72 (0.02)	0.76 (0.03)
Bulk density (g/cm^3)	0.45 (0.01)	0.47 (0.01)	0.47 (0.01)
Tapped density (g/cm^3)	0.50 (0.004)	0.52 (0.01)	0.52 (0.01)
% Compressibility	9.39 (0.53)	9.38 (0.13)	9.05 (0.63)
Flow rate (g/s)	3.66 (0.03)	3.63 (0.02)	3.62 (0.02)
Angle of repose (deg)	25.30 (0.63)	25.10 (0.73)	27.85 (0.64)

The two different batches, M1-A and M1-B, of mucoadhesive nicotine microcapsules were prepared by bottom-spray fluidized bed coater. Physical properties showed no significant variation between the two batches. Although M2 formulation composed of concentrated NHT, the physical properties results are consistent with M1-A and M1-B. It could be concluded that the instrument and process parameters are appropriate to produce mucoadhesive nicotine microcapsules between these two concentration ranges.

4. Determination of mucoadhesive performances

Three major categories of polymers have been applied with some achievement as bioadhesive such as hydroxyl-containing, carboxyl-containing and others polymers mostly with charged types (Peppas and Buri, 1985). HPMC is an uncharged polymer which widely used in oral pharmaceutical formulations as a film coating polymer and used in an extended release tablet matrix. Hydration is required for mucoadhesive

polymer to permit a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or electrostatic interaction between the polymer and mucus network (Miller, Chittchang and Johnston, 2005). As addressed above, prehydration of HPMC occurred by moistening of saliva fluid in the oral cavity, and its molecular property containing hydroxyl groups allowed the polymer to come in close contact with the mucus membrane. In addition, derivatives of cellulose and poly(acrylic acid) with high molecular weight have been shown to possess the hydrogel-forming properties, which are necessary for mucoadhesion.

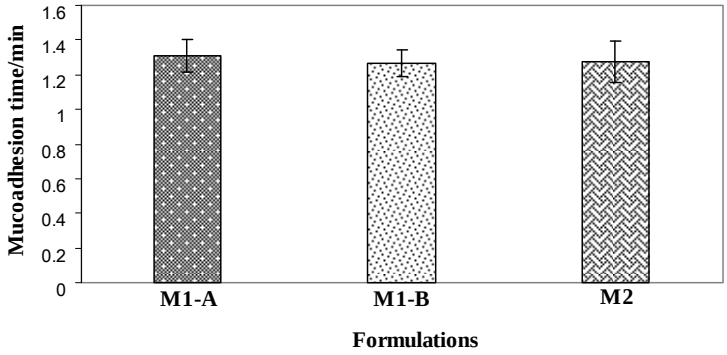


Figure 38 Mucoadhesion time of mucoadhesive nicotine microcapsules of M1-A, M1-B and M2 (mean±SD, n=9).

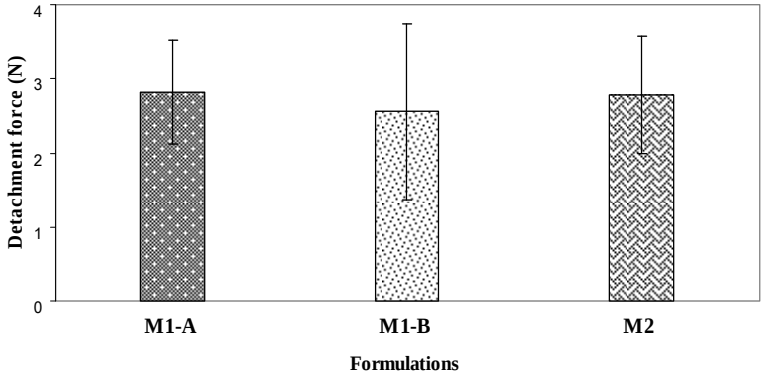


Figure 39 Mucoadhesive force of mucoadhesive nicotine microcapsules of M1-A, M1-B and M2 (mean±SD, n=3).

Mucoadhesion performances of produced mucoadhesive nicotine microcapsules (M1-A, M1-B and M2) were evaluated and the results are shown in Figures 38 and 39. Table 17 shows the mucoadhesion time and mucoadhesive force values which was found to be related to the results of 5% HPMC E15 coated microcapsules prepared in previous study.

Table 17 Mucoadhesion performances of produced mucoadhesive nicotine microcapsules of M1-A, M1-B and M2 (mean±SD).

Formulations	**M1-A**	**M1-B**	**M2**
Mucoadhesion time (min)	1.31±0.09	1.27±0.08	1.28±0.12
Mucoadhesive force (N)	2.82±0.71	2.56±1.19	2.78±0.79

5. Content of nicotine in mucoadhesive nicotine microcapsules

Nicotine content in mucoadhesive nicotine microcapsules was analyzed by high performance liquid chromatography and the results are shown in the Table 18. Nicotine content in 0.100 g microcapsules of M1-A and M1-B was lower than nicotine content of M2 due to amount of NHT in M1-A and M1-B formulation was lower than M2. The minute drug content variation of each formulation with a standard deviation of 0.01, 0.001 and 0.03 for M1-A, M1-B and M2, respectively indicated that the drug distribution was relatively uniform. The percentage of nicotine content of M1-A, M1-B and M2 formulations are also displayed in Table 19. Mucoadhesive nicotine microcapsules obtained from M2 were chosen for future buccal tablet production. The reason lies in the proper weight of microcapsules of 140 mg as equivalent to 2 mg of nicotine for tablet production.

Table 18 Content of nicotine in mucoadhesive nicotine microcapsules of M1-A, M1-B and M2 formulation (n=3)

	NHT : Mannitol (1:50)						**NHT : Mannitol (1:20)**		
Formulations	**M1-A**			**M1-B**			**M2**		
	n=1	**n=2**	**n=3**	**n=1**	**n=2**	**n=3**	**n=1**	**n=2**	**n=3**
Nicotine content in 0.100 g of microcapsule (mg)	0.542	0.562	0.565	0.565	0.563	0.566	1.448	1.439	1.392
Mean (SD)	**0.557 (0.01)**			**0.565 (0.001)**			**1.426 (0.03)**		
Weight of microcapsules (g), equivalent to 2 mg of nicotine	0.369	0.356	0.354	0.354	0.355	0.353	0.138	0.139	0.144
Mean (SD)	**0.359 (0.008)**			**0.354 (0.001)**			**0.140 (0.003)**		

Table 19 The percentage of nicotine content of M1-A, M1-B and M2 formulations

	NHT : Mannitol (1:50)		**NHT : Mannitol (1:20)**
Formulations	**M1-A**	**M1-B**	**M2**
Theoretical nicotine content in 0.100 g of uncoated granules (mg)	0.601	0.608	1.452
Analytical nicotine content in 0.100 g of mucoadhesive nicotine microcapsules (mg)	0.557	0.565	1.426
% Nicotine content	92.63	92.99	98.21

6. Solid state characterization of NHT in mucoadhesive nicotine microcapsules

6.1 Differential scanning calorimetry (DSC) and X-ray powder diffractometry (XRPD)

Transformation of excipients by incorporation of small amount of NHT during granulation and after fluidized-bed coating process was evaluated by XRPD and DSC. Figure 40 reveals that no unusual peaks were evident when comparing the X-ray diffraction patterns of the mucoadhesive nicotine microcapsules with HPMC E15 and mannitol. Similarly, thermograms of the mucoadhesive nicotine microcapsule and the physical mixture of NHT and each excipient (Figure 41) were not different. It could be concluded that the addition of minute amount of nicotine during granulation and the fluidized bed coating process did not affect the solid state modification of additive component within the mucoadhesive nicotine microcapsules. In addition, identification peaks of endotherm of NHT in mucoadhesive nicotine microcapsules were not visible possibly due to NHT was incorporated at a concentration below its XRPD and DSC detection limit.

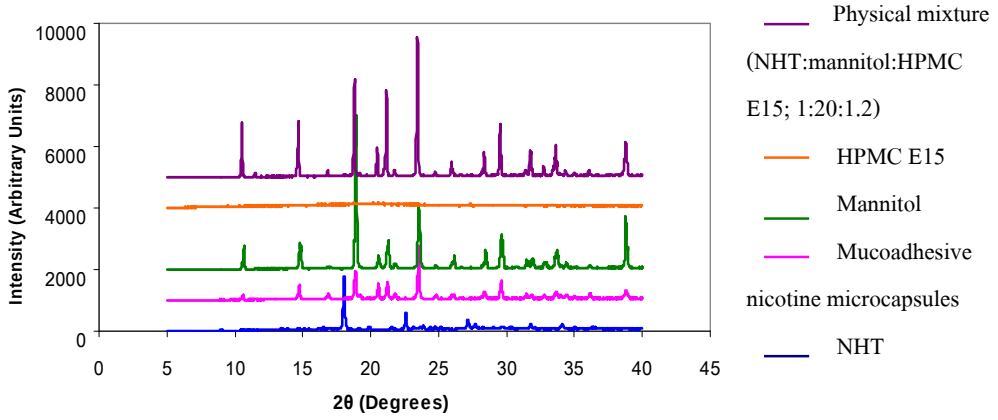


Figure 40 X-ray diffraction patterns of mucoadhesive nicotine microcapsules, excipients and its physical mixture.

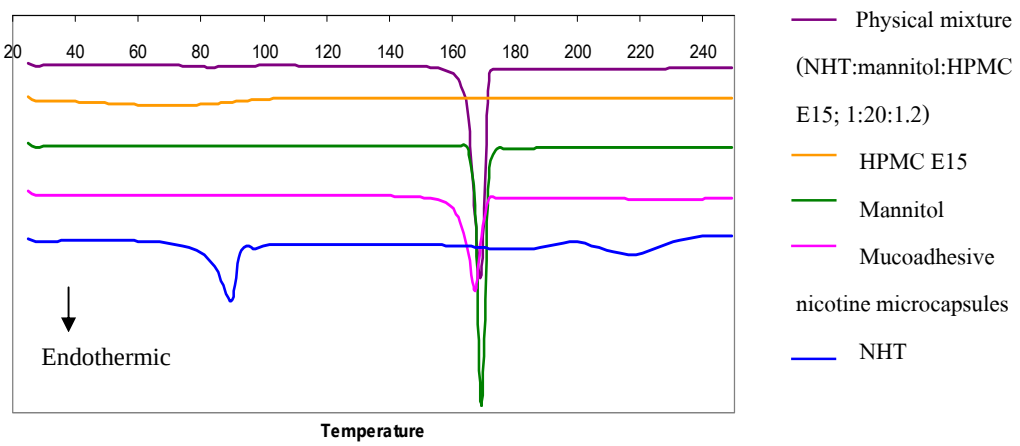


Figure 41 DSC thermograms of mucoadhesive nicotine microcapsules, excipients and its physical mixture.

6.2 Solid state nuclear magnetic resonance spectrometry

Structure determination is the main objective of NMR spectrometry to identify a molecule or for confirming the presence of a known molecule.

^{13}C NMR spectrum of each sample was obtained by solid-state NMR spectrometry. Chemical shift values of mucoadhesive nicotine microcapsules (Figure 42b) were detected at the same positions (21, 38, 74, 84, 103, 126, 144, 175 and 177 ppm) as chemical shift values of pure NHT (Figure 42a). Chemical shift values of placebo mucoadhesive microcapsules without NHT (Figure 42c) did not show any NHT signature values. The results indicated that NHT remained stable after granulation and coating process and the chemical interaction did not occur among the components during manufacturing. Thus, the fluidized bed coating process did not affect the solid state modification of NHT within the mucoadhesive nicotine microcapsules produced.

Nicotine hydrogen tartrate dihydrate is an active ingredient for mucoadhesive nicotine microcapsule production. The results exhibited NMR spectrum of NHT as dihydrate form. Previous solid-state NMR work was done with carbon nuclei of NHT in anhydrous form and found to be very different, from this finding which was due to different molecular arrangements. Single entity or

molecular adduct such as water (hydrates) within a compound structure affected molecular arrangement and eventually caused dissimilar NMR pattern.

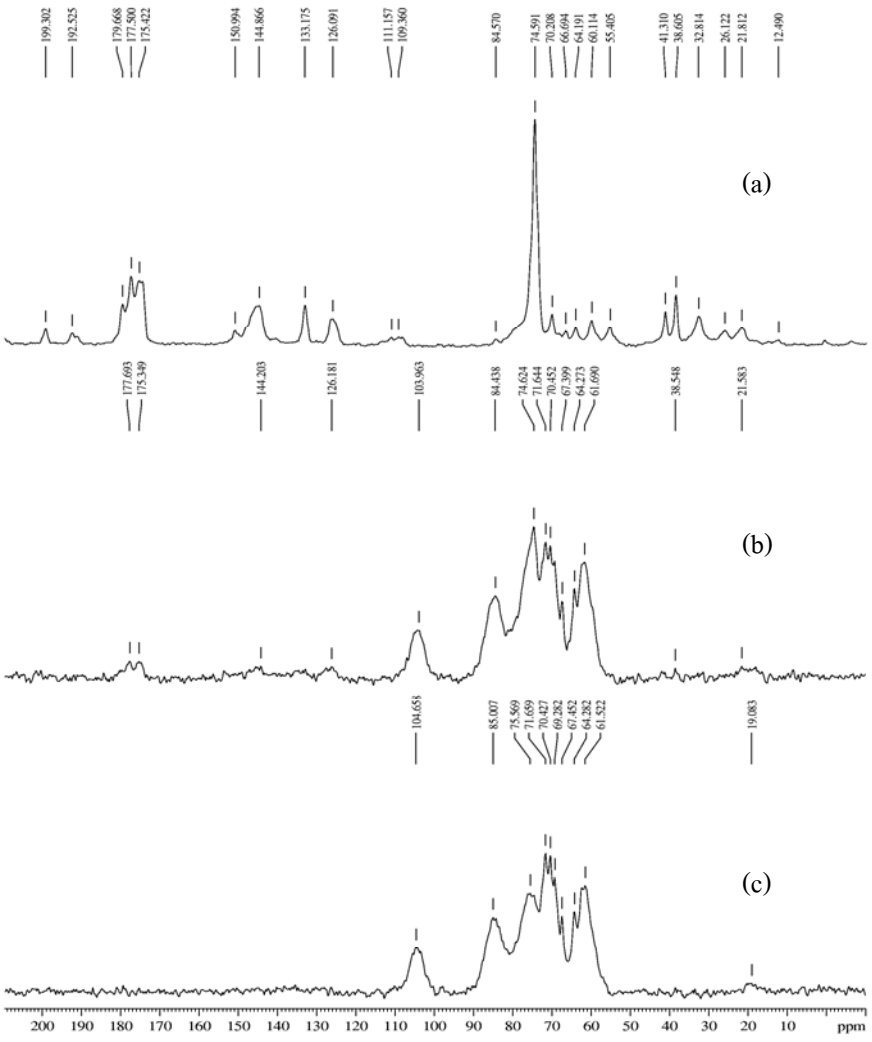


Figure 42 Solid state NMR ^{13}C spectra of (a) NHT, (b) mucoadhesive nicotine microcapsules and (c) mucoadhesive microcapsules without NHT

Part III. Preparation of buccal tablets containing mucoadhesive nicotine microcapsules

The tablets were designed as bilayer buccal tablets due to release the drug in a unidirectional way towards the buccal mucosa. Total weight of bilayer buccal tablet

was approximately 1,220 mg. The convex round shape of the layer containing mucoadhesive nicotine microcapsules was desired to be attached to the buccal mucosa. The placebo layer was designed as a concave shape aimed as a contact marker when the tablet was moved from one side of the mouth to the other. Mannitol and xylitol were used as the main filler due to its sweetness and cool mouth feel when taken orally. The mucoadhesive nicotine microcapsules prepared using HPMC E15 as coating will show mucoadhesive property and mask the acrid taste of nicotine. The compositions of buccal tablet formulation and the concentration of ingredients (Appendix D) were optimized during the preliminary trial to find the optimum formulation of bilayer buccal tablets.

The optimum formulation consists of the mixture of mannitol, xylitol and MCC to form preliminary granules. The segregation of mixing between additives and mucoadhesive nicotine microcapsules could occur due to their different sizes. Thus, the size of these granules was classified prior to mixing to avoid the segregation problem. Sucralose was also used in the formulation as nonnutritive sweetener whose sweetness is higher than sucrose. Likewise, because sucralose is not metabolized as sugar, it may be useful for weight control and has value for use with people who must restrict their sugar intake. The general appearances of bilayer buccal tablet are shown in Figure 43.

(a) (b) (c)

Figure 43 The general appearances of bilayer buccal tablet with 16 mm diameter (a) the placebo layer, (b) the drug layer containing mucoadhesive nicotine microcapsules and (c) bilayer buccal tablets.

1. Evaluation of tablets

The selected formulation of bilayer buccal tablets were found to be satisfactory when evaluated for weight variation (0.08±0.56%), thickness (5.59±0.01mm), hardness (12.95±0.68 kg/cm^2) and friability (0.14%). The average weight of the tablets was found to be 1,220.31±1.27 mg and the percent variation was within the specified limit. Bilayer buccal tablets did not show lamination of the two divided layer when the hardness of tablet was evaluated. Furthermore, friability was less than 1% which was considered acceptable for conventional tablets. The result indicates that the tablets endure the mechanical collisions reasonably well during handling. The general appearance of the tablets is smooth surface and free of any kind of visual defects.

Disintegration time of bilayer buccal tablets was 20.12±1.15 minutes meanwhile disintegration time of placebo layer was only 6.56±0.58 minutes hence the placebo layer disappeared before the drug layer. The placebo layer consisted mannitol powder, avicel powder and glidants then compressed by direct compression and the thickness of this layer was approximately 1.5 mm which was thinner than the drug layer (3.97±0.05 mm) and resulted in shorter disintegration time. Therefore, the amounts and thickness of the placebo layer have to be future improved. Besides, granulation of mannitol and avicel with the retarded polymer would be beneficial to extend the disintegration time of the placebo layer.

Factors contributing directly to content uniformity problems in low-dose tablet formulation are nonuniform distribution of mixing or granulation process, segregation of the powder mixture or granulation during the manufacturing process and tablet weight variation. The precision and variation of the assay used in the content uniformity test is also a factor that leads as an error in the determination of content uniformity (Rosanske et al., 1962). To ensure uniform potency for tablets of low-dose drugs, a content uniformity test was applied. In this test, not less than 30 tablets were randomly selected and at least 10 tablets were assayed individually. Each tablet must contain nicotine not less than 85% or not more than 115% of the labeled amount (USP30/NF25). The percentage of content uniformity of each tablet was within the acceptable range (Appendix F). The result demonstrated that uniform

distribution of the drug substance throughout powder mixing, granulation and tabletting.

2. *In vitro* nicotine release studies

Data shown in Figure 44 were plotted to exhibit percent nicotine released versus time of developed buccal tablet containing mucoadhesive nicotine microcapsules compared to commercial nicotine polacrilex lozenges, another buccal bioadhesive nicotine tablet. Both formulations are equivalent to 2 mg of nicotine. The release was monitored over 6 hours and both release kinetics were found to follow near zero-order rate kinetics that nicotine showed greater release for extended period of time.

To assure similarity in product performance, the f_2 comparison has been the focus in guidance. The f_2 factor measures the closeness between the two profiles. Although, the difference factor (f_1) is 31.73 and should not be more than 15, f_2 is the principal consideration in practice. Figure 44 indicates that the release profile of commercial nicotine lozenge are similar to those obtained with the developed buccal tablets with a similarity factor (f_2) of 56.23 with developed formulation showed slightly higher release value ($P < 0.05$, independent student's t-test) at each time point.

Percent cumulative released of nicotine at 6 hour for the developed buccal tablets and commercial nicotine lozenges were 61.83±0.93% and 45.90±1.05%, respectively. It could be concluded that this developed formulation can enhance the release of drug for extended period of time. However, both buccal tablet formulations were completely dissolved within 20-30 minutes when placed in the mouth, unlike the tablets which were placed in the modified Franz cell diffusion. The limitation of apparatus caused a very slowly nicotine released from the dosage form, due to the lack of high mobility and high salivary flow rate that actually occurred in the oral cavity. Although, several apparatus and conditions have been used by different researchers (Fabregas and Garcia, 1995; Khanna, Agarwal and Ahuja, 1996; Park and Munday, 2002), no standard methods have been especially developed for the *in vitro* release assessment of buccal tablet dosage form.

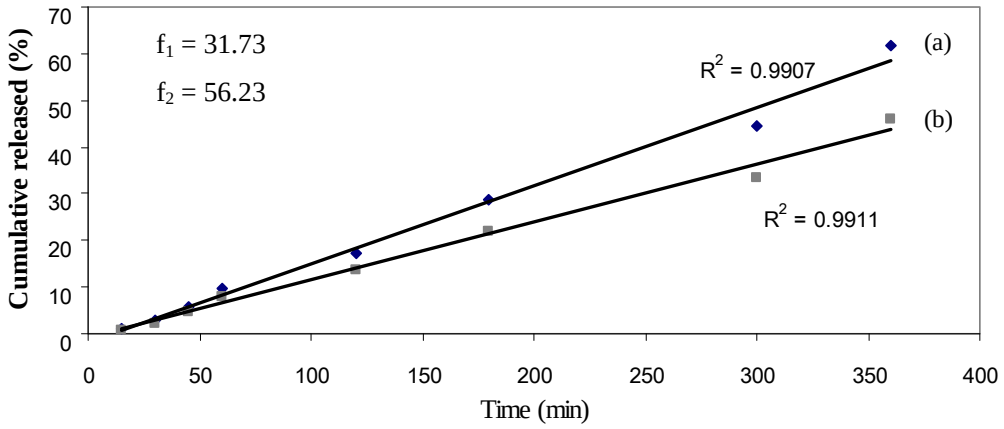


Figure 44 Release profile of nicotine from (a) developed bilayer buccal tablets and (b) commercial nicotine polacrilex lozenge in phosphate buffer pH 7.4 (mean, n=3)

3. Stability assessment of the product

Tablets of optimum formulation were selected for short term stability study. It was carried out at room temperature (30 ºC, 75% RH) and accelerated condition (45ºC, 75% RH) for three months. Physical characteristic and content uniformity of drug are shown in Tables 20 and 21. The results indicate that there are no significant changes in the physical characteristics and content uniformity is within acceptable range. Hence, it may be concluded that the tablets from selected formulation are stable for the period of three months at 30 ºC and 45 ºC.

Table 20 Physical characterization of optimum formulation at room temperature 30 ºC, 75% RH (mean±SD)

Parameters	Time (month)			
	0	1	2	3
Weight of tablets (mg)	1,220.31±1.27	1,219.96±1.81	1,219.16±1.03	1,219.52±1.32
Weight variation (%)	0.08±0.06	0.12±0.08	0.07±0.04	0.09±0.06
Thickness (mm)	5.59±0.01	5.65±0.02	5.65±0.02	5.63±0.02
Hardness (kg/cm^2)	12.95±0.68	12.64±0.50	12.87±0.51	12.71±0.42

Table 20 (Continue), Physical characterization of optimum formulation at room temperature 30 ºC, 75% RH (mean±SD)

Parameters	Time (month)			
	0	1	2	3
Friability (%)	0.14	0.07	0.09	0.09
Content uniformity of drug (%)	97.60±3.26	95.50±1.40	97.18±2.60	98.32±1.97

Table 21 Physical characterization of optimum formulation at accelerated condition; 45 ºC, 75% RH (mean±SD)

Parameters	Time (month)			
	0	1	2	3
Weight of tablets (mg)	1,220.31±1.27	1,221.55±1.37	1,219.75±0.99	1,219.41±1.34
Weight variation (%)	0.08±0.06	0.09±0.06	0.07±0.04	0.10±0.06
Thickness (mm)	5.59±0.01	5.61±0.02	5.63±0.02	5.63±0.03
Hardness (kg/cm^2)	12.95±0.68	12.92±0.51	12.86±0.43	12.78±0.36
Friability (%)	0.14	0.09	0.12	0.12
Content uniformity of drug (%)	97.60±3.26	95.41±2.26	96.39±1.64	97.99±1.07

CHAPTER V
CONCLUSIONS

The purpose of this study was to develop mucoadhesive microcapsules of nicotine hydrogen tartrate (NHT) by fluidized-bed coating technique and to formulate buccal tablet containing mucoadhesive NHT microcapsules. The mucoadhesive NHT microcapsules were prepared by using fluidized-bed coater. Hydroxypropyl methylcellulose E15 (HPMC E15) solution was used as mucoadhesive coating agent. The effects of type of fluidized bed coater, coating parameters, and amount of mucoadhesive material were investigated. The mucoadhesive nicotine microcapsules and buccal tablets containing these microcapsules which were selected from the optimum formulation were evaluated. It can be concluded from the study that:

Bottom spray fluidized bed coater provided the desirable characteristics of the developed microcapsules which exhibited excellent flow property. The satisfactory microencapsulation of nicotine was possible by using HPMC E15 as mucoadhesive coating material at least 5% weight gain through carefully controlled bottom spray fluidized-bed coating process.

Solid state NMR measurement indicated that the components and the fluidized bed coating process did not affect the solid state modification of NHT within the mucoadhesive nicotine microcapsules. These mucoadhesive nicotine microcapsules were subsequently processed to produce a novel suitable buccal drug delivery system.

Buccal tablet containing mucoadhesive NHT microcapsules was designed as a bilayer tablet and prepared by direct compression method. The formulation of drug layer consisted of mucoadhesive nicotine microapsules (140 mg), D-granules (510 mg), sucralose (1 mg), menthol (12 mg), magnesium stearate (0.5%) and talcum (2%) was selected as optimum formulation. The buccal tablets that were prepared from an optimum formulation provided pleasant taste and good physical appearance. Various physicochemical parameters were tested on this formulation and showed satisfactory results. The release rate kinetic of optimum formulation was found to follow near

zero-order rate kinetic. From the *in vitro* release study, it may be concluded that this novel formulation can enhance the release of drug for extended period of time and will provide a longer period of contact time of microcapsules reducing side effects and loss of drug through swallowing, which would in turn resulted in higher bioavailability. The results from short term stability study indicated that this formulation was stable for the period of three months at 30 ºC and 45 ºC.

Although these microcapsules showed good appearance and physical characteristics, variables should be further carefully evaluated in future development. In addition, the formulation of buccal tablets containing mucoadhesive nicotine microcapsules still needs adjustment for scale-up production.

The future plans should be as follows:

1. Incorporation of buffering agents into the buccal tablet formulation such as sodium carbonate, sodium bicarbonate, calcium carbonate and magnesium hydroxide may be necessary in order to increase local buccal absorption of nicotine.
2. Use of alternative techniques to measure the nicotine release from tablet containing mucoadhesive nicotine microcapsules that truly mimic with a real condition in oral cavity.
3. Assessment of local irritation of NHT, additives and tablets to human buccal mucosa is important.

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APPENDICES

APPENDIX A

The conditions used in fluidized-bed coating process and physical properties of produced microcapsules

Table 1A The conditions used in fluidized-bed coating process

Parameters	Formulations											
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Type of spray coating	top	top	top	top	top	top	bottom	bottom	bottom	bottom	bottom	bottom
Weight of granules (g)	250	250	250	250	250	250	250	250	250	250	250	250
Pre-heating time (min), temperature (ºC)	10, 65	10, 65	10, 65	10, 70	10, 70	10, 65	10, 65	10, 65	10, 65	10, 65	10, 65	10, 65
Inlet temperature (ºC)	65±2	65±2	65±2	70±2	70±2	65±2	65±2	65±2	65±2	65±2	65±2	65±2
Outlet temperature (ºC)	50±2	50±2	50±2	55±2	55±2	50±2	50±2	50±2	50±2	50±2	50±2	50±2
Fluidized air velocity (m/s)	6.5	6.5	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Atomizing pressure (kg/cm^2)	1.5	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Feed rate (ml/min)	2.2	2.2	2.2	2.2	2.0	2.0	2.2	2.0	2.0	2.0	2.0	2.0
Post-heating time (min), temperature(ºC)	10, 65	10, 65	10, 65	10, 70	10, 70	10, 65	10, 65	10, 65	10, 65	10, 65	10, 65	10, 65

Table 2A Composition of coating solution used in fluidized-bed process

Coating solution (g)	Formulations											
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
HPMC E15, 6% w/w (% weight gain)	12.5 (5%)	12.5 (5%)	12.5 (5%)	12.5 (5%)	12.5 (5%)	12.5 (5%)	12.5 (5%)	12.5 (5%)	12.5 (5%)	10 (4%)	15 (6%)	17.5 (7%)
PEG 6000	-	-	-	-	2.5	2.5	-	-	2.5	2	3	3.5
DI Water	208	208	208	208	208	208	208	208	208	167	250	292
Tartrazine	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.018	0.027	0.031

Table 3A Physical properties of produced microcapsules [mean (SD)], size range between 300 to 600 µm

Physical properties	Formulations								
	T3	**T5**	**T6**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**
Moisture content (%LOD)	1.73 (0.03)	0.64 (0.12)	0.59 (0.04)	0.94 (0.03)	0.86 (0.04)	0.73 (0.05)	0.39 (0.00)	0.67 (0.03)	1.05 (0.08)
Bulk density (g/cm^3)	0.31 (0.00)	0.39 (0.00)	0.36 (0.00)	0.48 (0.00)	0.46 (0.00)	0.47 (0.00)	0.46 (0.003)	0.43 (0.003)	0.46 (0.00)
Tapped density (g/cm^3)	0.38 (0.002)	0.46 (0.00)	0.41 (0.002)	0.53 (0.004)	0.51 (0.004)	0.51 (0.004)	0.50 (0.00)	0.48 (0.003)	0.52 (0.004)
% Compressibility	18.49 (0.45)	13.44 (0.49)	11.21 (0.53)	9.92 (0.69)	9.69 (0.67)	8.91 (0.67)	8.74 (0.60)	9.38 (0.60)	10.08 (0.67)
Flow rate (g/s)	1.67 (0.01)	2.10 (0.01)	1.92 (0.003)	2.72 (0.01)	2.73 (0.02)	3.29 (0.03)	3.26 (0.05)	3.05 (0.01)	3.19 (0.04)
Angle of repose (deg)	35.4 (0.49)	32.67 (0.59)	31.53 (0.98)	26.77 (0.65)	25.52 (0.73)	27.99 (0.70)	26.35 (0.38)	26.77 (1.53)	27.37 (0.33)

Coating conditions of formulation T6 gave the most satisfactory method to prepare mucoadhesive microcapsules with top-spray coating technique. On the other hand, bottom-spray coating (B1 and B3) was operated with the same parameters used in formulation T3 and T6. The microcapsules which produced by top-spray coating technique were bulky, meanwhile, the microcapsules produced by bottom-spray coating technique exhibited excellent flow property which presented in terms of % compressibility less than 10% and angle of repose less than 30 degrees. The aggregation problem did not seem to improve with an increase in the inlet temperature (T4). PEG6000 was a suitable plasticizer for HPMC coating solution. A reduction of aggregated particles was found in the case of the employing of coating solution with PEG6000. Moisture content of microcapsules obtained from formulations containing PEG6000 were lower than the formulations without PEG6000.

The coating condition used in formulation B3 were the most appropriate conditions for further mucoadhesive nicotine microcapsules production. Therefore, mucoadhesive microcapsules of formulation B4, B5 and B6 were coated with the same conditions applied in B3 production. Different amounts of HPMC E15 was used in these formulations then mucoadhesive property of the produced microcapsules was evaluated.

APPENDIX B

Data of mucoadhesion performances determination

Table 1B Mucoadhesion time (min) of mucoadhesive microcapsules with varying amounts of HPMC E15 and mucoadhesive nicotine microcapsules with 5% weight gain of HPMC E15

Number	Mucoadhesive nicotine microcapsules, varied amounts of HPMC E15 (% weight gain)				Mucoadhesive nicotine microcapsules with 5% weight gain of HPMC E15		
	4%	5%	6%	7%	M1	M2	M3
1	0.4	1.32	1.25	1.35	1.24	1.18	1.4
2	0.24	1.11	1.19	1.22	1.36	1.32	1.35
3	0.28	1.28	1.37	1.28	1.41	1.3	1.12
4	0.3	1.38	1.43	1.41	1.39	1.19	1.23
5	0.28	1.31	1.14	1.42	1.12	1.25	1.32
6	0.39	1.12	1.23	1.32	1.35	1.2	1.32
7	0.25	1.35	1.32	1.3	1.36	1.38	1.2
8	0.33	1.43	1.25	1.12	1.31	1.36	1.44
9	0.3	1.35	1.3	1.22	1.22	1.21	1.11
Mean	0.31	1.29	1.28	1.29	1.31	1.27	1.28
SD	0.06	0.11	0.09	0.10	0.09	0.08	0.12

Table 2B Mucoadhesive force (N) of mucoadhesive microcapsules with varying amounts of HPMC E15 and mucoadhesive nicotine microcapsules with 5% weight gain of HPMC E15

Number	Mucoadhesive nicotine microcapsules, varied amounts of HPMC E15 (% weight gain)				Mucoadhesive nicotine microcapsules with 5% weight gain of HPMC E15		
	4%	5%	6%	7%	M1	M2	M3
1	1.688	2.835	3.201	3.078	3.438	3.910	3.038
2	1.441	2.987	2.831	2.253	2.050	1.691	1.896
3	1.653	2.529	2.802	3.657	2.979	2.067	3.411
Mean	1.594	2.784	2.945	2.996	2.823	2.556	2.782
SD	0.13	0.23	0.22	0.71	0.71	1.19	0.79

APPENDIX C

Validation of HPLC method

The HPLC method was used to determine the nicotine content of mucoadhesive nicotine microcapsules and buccal tablets. The validation of HPLC methods used are presented as follows:

1.Specificity

Figure1C reveals that standard solution of nicotine was eluted at 4.6 min. Figures 2C and 3C shows the chromatogram in presence of the contents consisting excipients without active ingredient and nicotine with other excipients. It indicated that the other ingredients did not interfere the peaks of drugs.

2.Accuracy

Tables 1C shows the percentage of analytical recovery of nicotine. The mean percentage of analytical recovery complied to the range of 95-105 % with low % RSD (<2.00 %) indicated the high accuracy of this method.

3.Precision

Data of within run precision and between run precision of nicotine analyzed by HPLC method are shown in table 2C. The percentage of coefficient of variation (%CV) values of peak area both within run and between run were low (<2.00 %) which indicated that HPLC methods could determine the amount of the drugs over period of time studied.

4.Linearity

Figures 6C shows the relationship between peak area ratio and drug concentrations is linear with the correlation of determination value was 1.0. This result indicated that HPLC method was acceptable for quantitative analysis of nicotine drug in the range studied.

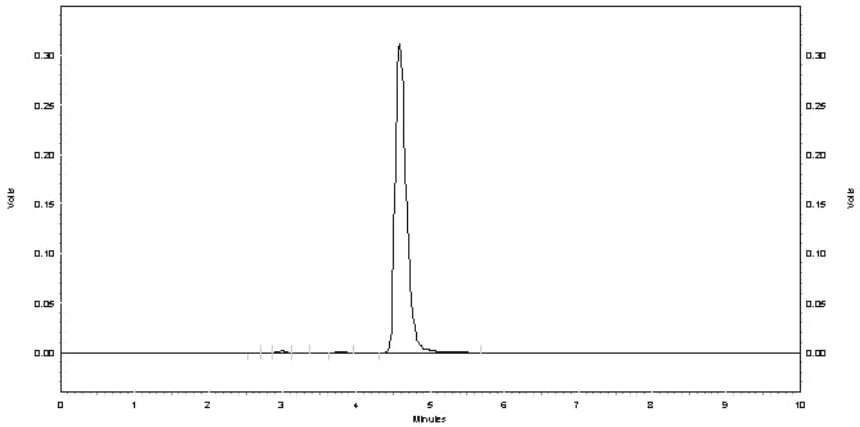

Figure 1C The chromatogram of standard of nicotine

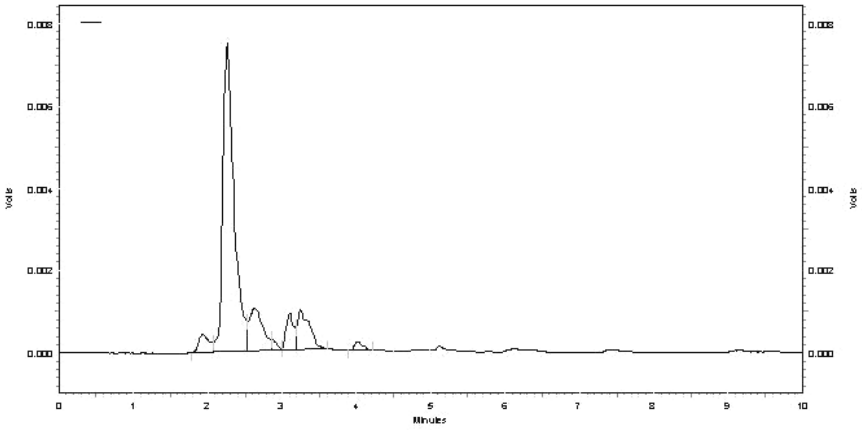

Figure 2C The chromatogram of excipients without nicotine

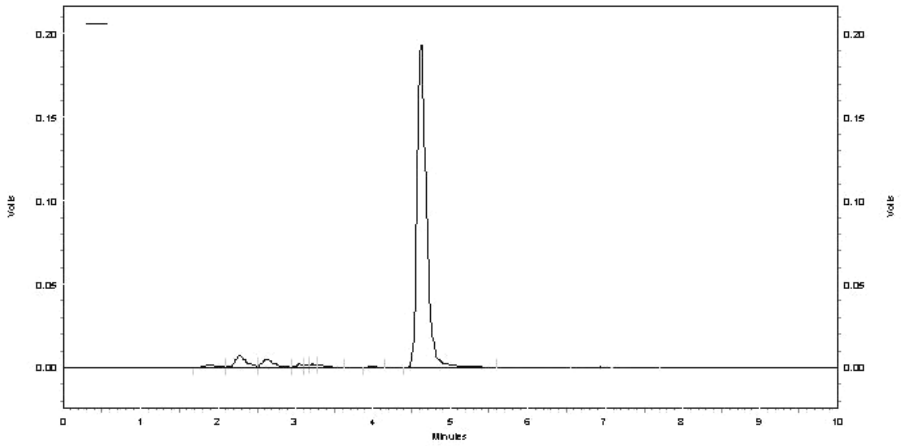

Figure 3C The chromatogram of specificity of nicotine

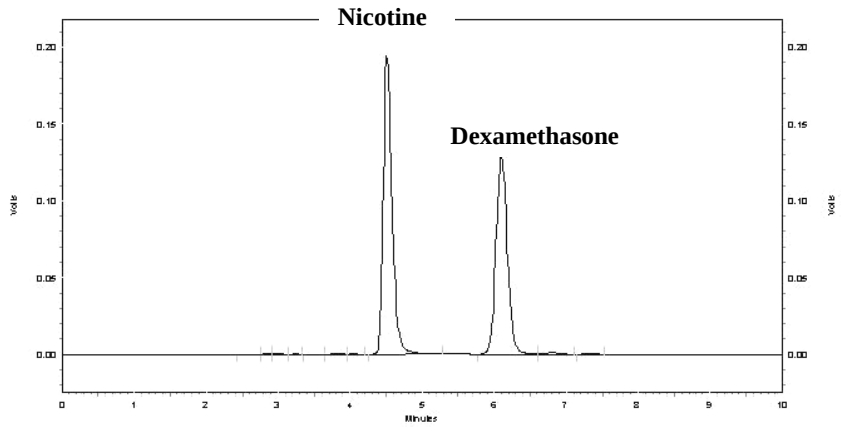

Figure 4C The chromatogram of resolution of nicotine

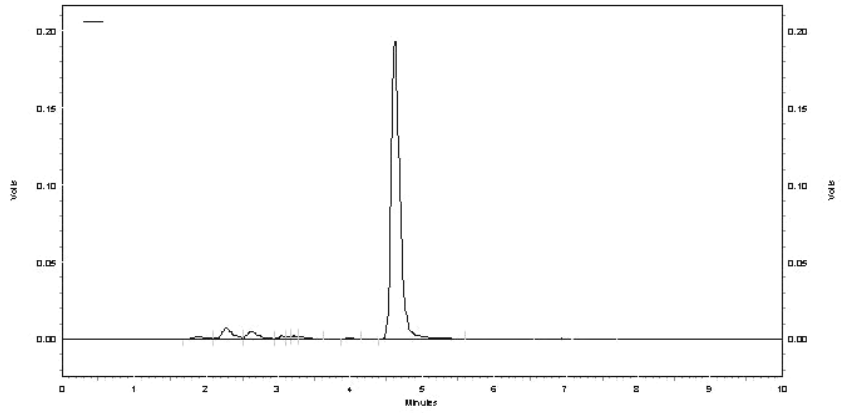

Figure 5C The chromatogram of nicotine of the bilayer buccal tablet

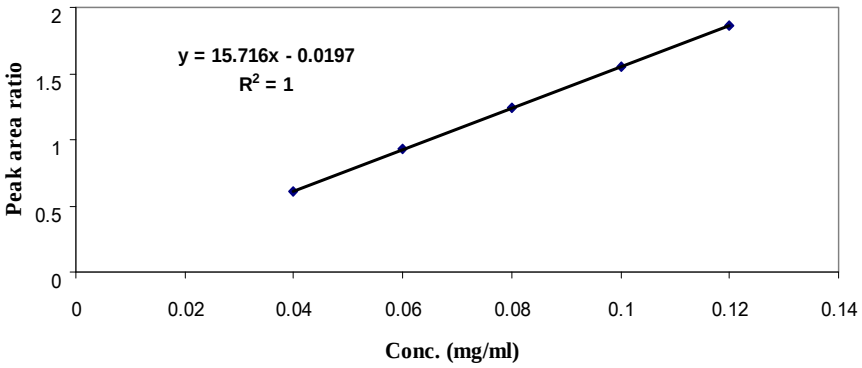

Figure 6C Calibration curve showing linearity between peak area ratio and nicotine concentrations analyzed by HPLC method.

Table 1C Accuracy data of percentage of analytical recovery of nicotine

Actual concentration (µg/ml)	Peak area ratio	Analytical concentration (µg/ml)	%Recovery	Average (SD)	%RSD
64.06 (N=1)	0.98166	63.72	99.46	99.39 (0.07)	0.07
	0.98066	63.65	99.36		
	0.98035	63.63	99.33		
64.06 (N=2)	0.99387	64.49	100.68	100.86 (0.12)	0.15
	0.99665	64.67	100.95		
	0.99657	64.66	100.94		
63.94 (N=3)	0.97731	63.44	99.22	99.19 (0.03)	0.03
	0.97678	63.41	99.17		
	0.97703	63.42	99.19		
80.31 (N=1)	1.22316	79.08	98.47	98.46 (0.01)	0.01
	1.22300	79.07	98.46		
	1.22285	79.06	98.45		
80.17 (N=2)	1.22444	79.16	98.75	98.74 (0.01)	0.01
	1.22445	79.16	98.75		
	1.22425	79.15	98.73		
80.17 (N=3)	1.23620	79.91	99.68	99.70 (0.03)	0.03
	1.23629	79.92	99.69		
	1.23702	79.96	99.74		
96.42 (N=1)	1.51023	97.35	100.96	100.93 (0.03)	0.03
	1.50970	97.31	100.93		
	1.50916	97.28	100.89		
96.29 (N=2)	1.51792	97.84	101.61	101.62 (0.02)	0.02
	1.51796	97.84	101.61		
	1.51847	97.87	101.64		
96.42 (N=3)	1.49683	96.50	100.21	100.20 (0.01)	0.01
	1.49640	96.47	100.19		
	1.49660	96.48	100.20		

Table 2C Precision data of nicotine/dexamethasone

Number	Peak Area at 259 nm		
	Day1	Day2	Day3
1	1700184/1370575	1690997/1376556	1744111/1369239
2	1700976/1372666	1692786/1378991	1773257/1394659
3	1701761/1373140	1690804/1377038	1773744/1394299
4	1703079/1376563	1693155/1379124	1758571/1380545
5	1703168/1376539	1692831/1379049	1784849/1399743
6	1705322/1378517	1684013/1371068	1788143/1396453
Average	1.2384	1.2279	1.2745
SD	0.0014	0.0003	0.0032
%CV	0.12	0.03	0.25

APPENDIX D

The compositions of buccal tablet formulations

Table 1D Compositions of the drug layer containing mucoadhesive nicotine microcapsules

Ingredients (mg/tab)	Formulations													
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**
Mucoadhesive nicotine microcapsules	140 (M1)	140 (M1)	140 (M1)	140 (M1)	140 (M1)	140 (M1)	140 (M2)	140 (M2)	140 (M2)	140 (M2)	140 (M2)	140 (M2)	140 (M3)	140 (M3)
Avicel 102	240	-	-	-	-	-	36	36	-	36	36	-	-	-
Mannitol	-	200	200	300	300	250	220	-	-	-	-	-	-	-
Xylitol	405	443	365	310	302.4	300	300	-	-	-	-	-	-	-
Mannitol granules	-	-	-	-	-	-	-	220	220	-	-	-	-	-
Xylitol granules	-	-	-	-	-	-	-	300	270	-	-	-	-	-
B-granules	-	-	-	-	-	-	-	-	-	520	-	-	-	-
C-granules	-	-	-	-	-	-	-	-	-	-	520	-	-	-
D-granules	-	-	-	-	-	-	-	-	-	-	-	550	540	510
Polyplasdone S630	40	40	40	40	-	-	-	-	-	-	-	-	-	-
Fructose	6	8	8	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	8	-	-	-	-	-	66	-	-	-	-	-

Ingredients (mg/tab)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
Sorbitol	-	-	-	-	-	63	-	-	-	-	-	-	-	-
Sucralose	-	-	-	-	2	2	2	2	2	2	2	1	1	1
Menthol	4	4	2	4	4	5	5	5	5	5	5	10	12	12
Magnesium stearate	5	5	5	4	4	6	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Talcum	-	-	-	-	7.6	14	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6
Peppermint oil in EtOH	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total weight (mg)	**840**	**840**	**840**	**800**	**760**	**780**	**720**	**720**	**720**	**720**	**720**	**718**	**710**	**680**

Table 2D Compositions of the placebo layer

Ingredients (mg/tab)	Formulations							
	N1	N2	N3	N4	N5	N6	N7	N8
Mannitol	400	200	200	200	300	400	440	480
Xylitol	-	200	200	-	-	-	-	-
Sorbitol	-	-	-	200	100	-	-	-
Avicel 102	-	-	-	-	-	50	50	50
Magnesium stearate	1	1	2	2	2	2	2	2
Talcum	4	4	8	8	8	8	8	8
Peppermint oil in EtOH	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total weight (mg)	**405**	**405**	**410**	**410**	**410**	**460**	**500**	**540**

Table 3D Compositions of B, C, and D-granules

Ingredients	**Amount (% w/w)**		
	B-granules	C-granules	D-granules
Mannitol	42	38.5	36.4
Xylitol	58	57.7	54.5
Sorbitol	-	3.8	-
Avicel PH102	-	-	9.1

APPENDIX E

In vitro nicotine release study

Table 1E The percentage of cumulative released of nicotine from developed buccal tablets containing mucoadhesive nicotine microcapsules

Time (min)	% Cumulative released of nicotine			
	n=1	n=2	n=3	Mean
15	1.026	1.328	1.106	1.153
30	2.857	2.933	3.072	2.954
45	5.439	5.811	6.038	5.763
60	9.393	9.703	10.004	9.7
120	16.524	17.673	17.738	17.312
180	27.643	28.951	29.180	28.591
300	43.567	44.269	45.240	44.356
360	61.154	61.461	62.894	61.836

Table 2E The percentage of cumulative released of nicotine from commercial nicotine polacrilex lozenges

Time (min)	% Cumulative released of nicotine			
	n=1	n=2	n=3	Mean
15	0.834	0.721	0.811	0.789
30	2.215	2.258	2.337	2.270
45	4.495	4.793	4.697	4.662
60	7.486	8.241	7.924	7.884
120	13.358	14.218	13.767	13.781
180	21.722	22.088	21.602	21.804
300	33.674	33.393	32.611	33.226
360	46.901	45.999	44.799	45.9

Appendix F

Stability study of optimum formulation

Table1F Tablet weight of optimum formulation at room temperature 30 ºC, 75% RH

	Weight (mg)			
Number	Month 0	Month 1	Month 2	Month 3
1	1219.2	1222.5	1220.4	1218.4
2	1219.2	1223.9	1218.5	1218.0
3	1222.0	1219.6	1218.2	1219.9
4	1219.1	1217.6	1218.0	1217.6
5	1222.1	1219.0	1220.6	1219.3
6	1221.4	1219.1	1218.8	1218.0
7	1220.1	1221.5	1220.9	1220.5
8	1218.3	1220.5	1218.5	1218.0
9	1221.6	1218.9	1218.0	1218.3
10	1220.1	1218.6	1218.0	1218.8
11	1218.6	1217.6	1219.6	1218.6
12	1220.9	1219.0	1218.1	1220.4
13	1219.8	1220.1	1218.1	1220.9
14	1219.5	1218.8	1218.5	1220.4
15	1220.2	1223.2	1220.3	1219.6
16	1220.2	1218.4	1219.8	1222.2
17	1222.4	1219.0	1219.0	1221.2
18	1222.1	1222.0	1219.2	1221.4
19	1220.3	1219.1	1220.6	1219.2
20	1219.0	1220.8	1220.1	1219.6
Mean	1220.31	1219.96	1219.16	1219.52
SD	1.27	1.81	1.03	1.32

Table 2F Thickness and hardness of optimum formulation at room temperature

Months	Thickness (mm)				Hardness (kg/cm^2)			
	0	1	2	3	0	1	2	3
1	5.56	5.67	5.67	5.63	12.96	12.7	13.12	12.35
2	5.6	5.66	5.65	5.64	12.09	12.36	13.35	13.23
3	5.6	5.68	5.64	5.61	12.07	12.37	13.34	12.96
4	5.6	5.67	5.64	5.6	13.72	13.51	12.8	12.75
5	5.58	5.67	5.61	5.64	13.84	12.6	13.6	12.6
6	5.58	5.65	5.65	5.62	12.73	12.35	12.7	13.19
7	5.6	5.66	5.64	5.65	12.4	12.54	12.03	13.18
8	5.59	5.62	5.63	5.59	12.96	12.03	12.19	12.42
9	5.59	5.61	5.65	5.66	13.9	13.54	12.55	12.04
10	5.57	5.63	5.67	5.64	12.8	12.43	13.02	12.36
Mean	5.59	5.65	5.65	5.63	12.95	12.64	12.87	12.71
SD	0.01	0.02	0.02	0.02	0.68	0.50	0.51	0.42

Table 3F Drug content of optimum formulation at room temperature

Months	Content of nicotine (mg)				Content uniformity of drug (%)			
	0	1	2	3	0	1	2	3
1	2.05	1.91	1.96	1.96	102.60	95.65	98.04	98.05
2	2.06	1.94	1.99	1.94	102.79	97.02	99.59	97.12
3	1.95	1.95	2.03	1.95	97.32	97.63	101.28	97.44
4	1.91	1.92	1.85	1.98	95.68	96.15	92.26	98.76
5	1.92	1.91	1.94	1.99	96.05	95.74	96.94	99.29
6	1.98	1.92	1.91	2.02	99.22	96.07	95.58	100.93
7	1.87	1.87	1.90	1.90	93.30	93.48	95.24	94.76
8	1.98	1.90	1.96	2.02	98.82	95.25	98.02	100.88
9	1.88	1.86	1.98	1.99	93.96	93.19	99.17	99.62
10	1.92	1.90	1.91	1.93	96.23	94.79	95.73	96.37
Mean	1.95	1.91	1.94	1.97	97.60	95.50	97.18	98.32
SD	0.07	0.03	0.05	0.04	3.26	1.40	2.60	1.97

Table 4F Weight of optimum formulation at accelerated condition; 45 ºC, 75% RH

Number	Weight (mg)			
	Month 0	Month 1	Month 2	Month 3
1	1219.2	1222.5	1218.6	1218.3
2	1219.2	1223.6	1220.5	1218.2
3	1222.0	1222.5	1219.2	1217.8
4	1219.1	1221.1	1219.2	1220.7
5	1222.1	1222.4	1219.4	1218.1
6	1221.4	1220	1220.1	1218.5
7	1220.1	1221.4	1220.2	1219.6
8	1218.3	1222.4	1218.9	1220.7
9	1221.6	1223.4	1219.4	1222.4
10	1220.1	1222.2	1218.9	1218.6
11	1218.6	1222.5	1220.2	1218.9
12	1220.9	1220.8	1220.8	1220.9
13	1219.8	1221.6	1221.5	1217.3
14	1219.5	1219.4	1218.7	1221.6
15	1220.2	1221	1219.1	1218
16	1220.2	1223.5	1220.1	1219.5
17	1222.4	1221.5	1222	1218.6
18	1222.1	1220.8	1219	1220.4
19	1220.3	1218.9	1218.5	1220.4
20	1219.0	1219.6	1220.8	1219.6
Mean	1220.31	1221.56	1219.76	1219.41
SD	1.27	1.37	0.99	1.40

Table 5F Thickness and hardness of optimum formulation at accelerated condition

Months	Thickness (mm)				Hardness (kg/cm^2)			
	0	1	2	3	0	1	2	3
1	5.56	5.59	5.65	5.59	12.96	12.55	12.58	13.05
2	5.6	5.61	5.64	5.61	12.09	13.56	13.21	12.48
3	5.6	5.65	5.65	5.62	12.07	13.65	12.63	12.52
4	5.6	5.6	5.62	5.66	13.72	12.61	12.63	12.52
5	5.58	5.59	5.64	5.65	13.84	13.18	13.62	13.34
6	5.58	5.62	5.61	5.65	12.73	12.57	12.15	12.56
7	5.6	5.62	5.62	5.62	12.4	13.42	12.92	12.51
8	5.59	5.59	5.6	5.64	12.96	12.11	13.14	13.07
9	5.59	5.62	5.59	5.58	13.9	12.65	12.53	12.5
10	5.57	5.66	5.64	5.65	12.8	12.92	13.15	13.28
Mean	5.59	5.62	5.63	5.63	12.95	12.92	12.86	12.78
SD	0.01	0.02	0.02	0.03	0.68	0.51	0.43	0.36

Table 6F Drug content of optimum formulation at accelerated condition

Months	Content of nicotine (mg)				Content uniformity of drug (%)			
	0	1	2	3	0	1	2	3
1	2.05	1.91	1.90	1.94	102.60	95.30	95.08	96.85
2	2.06	1.98	1.98	1.98	102.79	99.02	98.79	99.04
3	1.95	1.93	1.94	1.97	97.32	96.47	96.84	98.43
4	1.91	1.95	1.92	1.94	95.68	97.39	96.04	96.80
5	1.92	1.94	1.86	1.96	96.05	97.07	92.86	97.78
6	1.98	1.92	1.92	1.98	99.22	96.15	96.00	99.14
7	1.87	1.85	1.92	1.98	93.30	92.57	96.09	98.99
8	1.98	1.90	1.96	1.98	98.82	94.98	97.97	99.16
9	1.88	1.84	1.94	1.94	93.96	92.20	96.90	96.93
10	1.92	1.86	1.95	1.94	96.23	92.92	97.31	96.81
Mean	1.95	1.91	1.93	1.96	97.60	95.41	96.39	97.99
SD	0.07	0.05	0.03	0.02	3.26	2.26	1.64	1.07

APPENDIX G

Statistical analysis

Table 1G One-way ANOVA analysis of mucoadhesion time of the developed mucoadhesive microcapsules with varying the amounts of HPMC E15.

Test of Homogeneity of Variances

time

Levene Statistic	df1	df2	Sig.
.932	3	32	.437

ANOVA

time

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.485	3	2.162	264.211	.000
Within Groups	.262	32	.008		
Total	6.747	35			

Multiple Comparisons

Dependent Variable: time

Scheffe

(I) Conc	(J) Conc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4	5	-.98667*	.04264	.000	-1.1125	-.8609
	6	-.96778*	.04264	.000	-1.0936	-.8420
	7	-.98556*	.04264	.000	-1.1113	-.8598
5	4	.98667*	.04264	.000	.8609	1.1125
	6	.01889	.04264	.978	-.1069	.1447
	7	.00111	.04264	1.000	-.1247	.1269
6	4	.96778*	.04264	.000	.8420	1.0936
	5	-.01889	.04264	.978	-.1447	.1069
	7	-.01778	.04264	.981	-.1436	.1080
7	4	.98556*	.04264	.000	.8598	1.1113
	5	-.00111	.04264	1.000	-.1269	.1247
	6	.01778	.04264	.981	-.1080	.1436

*. The mean difference is significant at the .05 level.

Table 2G One-way ANOVA analysis of mucoadhesive force of the developed mucoadhesive microcapsules with varying the amounts of HPMC E15.

Test of Homogeneity of Variances

Force

Levene Statistic	df1	df2	Sig.
2.520	3	8	.132

ANOVA

Force

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.959	3	1.320	8.516	.007
Within Groups	1.240	8	.155		
Total	5.199	11			

Multiple Comparisons

Dependent Variable: Force

Scheffe

(I) Conc	(J) Conc	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4	5	-1.18966*	.32144	.038	-2.3123	-.0670
	6	-1.35068*	.32144	.020	-2.4734	-.2280
	7	-1.40208*	.32144	.016	-2.5248	-.2794
5	4	1.18966*	.32144	.038	.0670	2.3123
	6	-.16103	.32144	.967	-1.2837	.9616
	7	-.21243	.32144	.930	-1.3351	.9102
6	4	1.35068*	.32144	.020	.2280	2.4734
	5	.16103	.32144	.967	-.9616	1.2837
	7	-.05140	.32144	.999	-1.1741	1.0713
7	4	1.40208*	.32144	.016	.2794	2.5248
	5	.21243	.32144	.930	-.9102	1.3351
	6	.05140	.32144	.999	-1.0713	1.1741

*. The mean difference is significant at the .05 level.

Table 3G Independent t-test of nicotine released at each time interval between the developed buccal tablets and commercial nicotine polacrilex lozenges

At 15 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	3.244	.146	3.770	4	.020	.36446	.09668	.09603	.63290
	Equal variances not assumed			3.770	2.574	.042	.36446	.09668	.02587	.70306

At 30 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	.996	.375	9.418	4	.001	.68393	.07262	.48231	.88555
	Equal variances not assumed			9.418	3.170	.002	.68393	.07262	.45969	.90816

At 45 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	1.253	.326	5.633	4	.005	1.10122	.19551	.55840	1.64403
	Equal variances not assumed			5.633	2.953	.012	1.10122	.19551	.47338	1.72905

At 60 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	.159	.710	6.454	4	.003	1.81611	.28138	1.03486	2.59735
	Equal variances not assumed			6.454	3.827	.003	1.81611	.28138	1.02075	2.61146

At 120 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	1.481	.290	7.575	4	.002	3.53042	.46605	2.23646	4.82438
	Equal variances not assumed			7.575	3.372	.003	3.53042	.46605	2.13571	4.92513

At 180 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	5.987	.071	13.558	4	.000	6.78716	.50059	5.39729	8.17703
	Equal variances not assumed			13.558	2.369	.003	6.78716	.50059	4.92498	8.64934

At 300 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	.398	.562	19.192	4	.000	11.13258	.58008	9.52203	12.74313
	Equal variances not assumed			19.192	3.449	.000	11.13258	.58008	9.41532	12.84984

At 360 minutes

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
relesed	Equal variances assumed	.006	.944	19.645	4	.000	15.93644	.81124	13.68409	18.18879
	Equal variances not assumed			19.645	3.938	.000	15.93644	.81124	13.66995	18.20293

Appendix H

Calculation of the percentage of nicotine content

Model formulation is M3 (NHT:Mannitol, 1:20)

1) Theoretical nicotine content in 0.100 g of granules

Weight of NHT = 12.32 g (equivalent to 4.002 g of nicotine)
Weight of mannitol = 246.24 g
Weight of HPMC E15 (dry powder, binder) = 2.03 g
Weight of HPMC E15 (dry powder, coating material) = 12.5 g
Weight of PEG6000 (plasticizer) = 2.5 g
Total weight = 12.32 + 246.24 + 2.03 + 12.5 + 2.5 = 275.59 g

Theoretical nicotine content:
Granules 275.59 g equivalent to nicotine 4.002 g
Granules 0.1 g equivalent to nicotine **1.452** mg

2) Actual nicotine content in 0.100 g of mucoadhesive nicotine microcapsules determined by HPLC

Coated granules 0.1 g equivalent to nicotine **1.426** mg

3) Percentage of nicotine content in mucoadhesive nicotine microcapsules after coating process

$$= \frac{\text{Actual nicotine content}}{\text{Theoretical nicotine content}} \text{x } 100$$

= (1.426 mg/1.452 mg) x100 = **98.21 %**

BIOGRAPHY

Miss Chonwipa Yarangsee was born on January 27th, 1983. She received her bachelor of Pharmacy degree (First Class Honors) in 2005 from Faculty of Pharmacy, Chiangmai University, Chiangmai, Thailand. After graduation, she intended to study the Master's degree program in industrial pharmacy at Chulalongkorn University.

During her study at Chulalongkorn University, she had a presentation and publication on the topic of "Development of mucoadhesive nicotine microcapsules by fluidized-bed coating technique for buccal nicotine delivery" at The 35th Congress on Science and Technology of Thailand, Burapha University on October 15th, 2009.