Formulation Development and *in vitro* Evaluation of Montelukast Nasal Sprays

Thunyaporn Jullaphant

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Pharmacy in Pharmaceutical Sciences

Prince of Songkla University

2017

Thesis Title	Formulation development and *in vitro* evaluation of montelukast nasal sprays
Author	Miss Thunyaporn Jullaphant
Major Program	Pharmaceutical Sciences

Major Advisor :

.....
(Assoc. Prof. Dr. Teerapol Srichana)

Examining Committee :

.....Chairperson
(Assoc. Prof. Dr. Suwipa Ungphaiboon)

.....Committee
(Assoc. Prof. Dr. Teerapol Srichana)

.....Committee
(Asst. Prof. Dr. Kwunchit Oungbho)

.....Committee
(Asst. Prof. Dr. Somchai Sawatdee)

The Graduate School, Prince of Songkla University, has approved this thesis as partial fulfillment of the requirements for the Master of Pharmacy Degree in Pharmaceutical Sciences.

.....
(Assoc. Prof. Dr. Teerapol Srichana)
Dean of Graduate School

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

……
(Assoc. Prof. Dr. Teerapol Srichana)
Major Advisor

……
(Miss Thunyaporn Jullaphant)
Candidate

I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

…….
(Miss Thunyaporn Jullaphant)
Candidate

ชื่อวิทยานิพนธ์ การพัฒนาและการประเมินสูตรตำรับสเปรย์พ่นจมูกมอนเทลูคาส

ผู้เขียน นางสาวธันยพร จุลพันธ์

สาขาวิชา เภสัชศาสตร์

ปีการศึกษา 2559

บทคัดย่อ

มอนเทลูคาสเป็นยากลุ่ม leukotriene receptor antagonist ใช้ในการป้องกันและรักษาการหดเกร็งของหลอดลม และอาการจมูกอักเสบจากภูมิแพ้ ปัจจุบันมีการบริหารยามอนเทลูคาสโดยการรับประทาน โดยยาจะอยู่ในรูปแบบเม็ด และแบบผงละลายน้ำ แม้ว่าการบริหารยาโดยการรับประทานจะมีความปลอดภัย แต่มีรายงานถึงผลข้างเคียงที่เกิดจากการรับประทานยาอย่างต่อเนื่อง ได้แก่อาการไอ มีไข้ หลอดลมอักเสบ มีผลต่อการทำงานของตับ เกิดภาวะกระสับกระส่าย และส่งผลให้เกิดอาการทางระบบประสาท ได้แก่ อาการประสาทหลอน ภาวะซึมเศร้า นอนไม่หลับ และอาจมีความคิดฆ่าตัวตายได้ วิธีการหนึ่งที่สามารถลดโอกาสการเกิดผลข้างเคียงจากการใช้ยาดังกล่าวคือการบริหารยาแบบเฉพาะที่ ดังนั้นการศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาสูตรตำรับยาพ่นจมูกมอนเทลูคาส โดยใช้พอลิเมอร์ hydroxypropyl cellulose (HPC) และ carbomer940 (C940) เป็นส่วนประกอบในตำรับ เพื่อช่วยในการยึดเกาะของตำรับยากับเยื่อบุภายในจมูก และลดการกำจัดยาโดยกลไก mucociliary clearance ภายในช่องจมูก ตำรับยาที่ได้เตรียมขึ้นมีทั้งหมด 11 สูตร ซึ่งแต่ละสูตรจะมีความแตกต่างในชนิดและความเข้มข้นของพอลิเมอร์ หลังจากนั้นนำตำรับยามาทำการประเมินตำรับทางด้านเคมีกายภาพ และทดลองกับเซลล์เยื่อบุจมูก (nasal epithelial cells) ในหลอดทดลอง เพื่อหาสูตรตำรับที่มีความเหมาะสมในการนำส่งยาทางจมูก ประเมินหาค่าความ

แตกต่างโดยใช้ student’s t-test โดยมีค่านัยสำคัญที่ p-value น้อยกว่า 0.05 จากการประเมินสูตรตำรับพบว่า ค่า pH ของสารละลายแต่ละตำรับอยู่ในช่วง 6.5 – 7.5 ขนาดอนุภาคของคอลลอยด์อยู่ในช่วง 70 – 300 ไมโครเมตร ศักย์ซีต้าของตำรับที่เตรียมด้วย C 940 อยู่ในช่วง -45 ถึง -30 มิลลิโวลต์ ส่วนตำรับที่เตรียมด้วย HPC อยู่ในช่วง -25 ถึง -3 มิลลิโวลต์ ความหนืดของทุกตำรับอยู่ในช่วง 6 – 320 เซนติพอยส์ โดยสมบัติทางเคมีกายภาพอื่นๆ เช่น มุมสัมผัส แรงตึงผิว ค่าในการยึดเกาะเซลล์เยื่อบุจมูก และขนาดของละอองสเปรย์ขึ้นกับความเข้มข้นของพอลิเมอร์ในตำรับ จากการประเมินความคงตัวของสูตรตำรับเป็นระยะเวลา 3 เดือนพบว่า สูตรตำรับมีความคงตัวดี ไม่เกิดการตกผลึกของยาคือ สูตรตำรับที่มีส่วนประกอบของ C940 0.01% w/v ซึ่งเมื่อทำการประเมินสูตรตำรับดังกล่าวด้วยเซลล์เยื่อบุจมูกพบว่า ไม่เป็นพิษต่อเซลล์เยื่อบุจมูก และไม่มีผลต่อค่าความต้านทานการผ่านไฟฟ้าของเซลล์โมโนเลเยอร์ (Cell monolayer) จึงสามารถสรุปได้โดยรวม สูตรตำรับดังกล่าวมีความเหมาะสมในการพัฒนายามอนเทลูคาสเพื่อนำส่งทางจมูก

Thesis Title	Formulation development and *in vitro* evaluation of montelukast nasal sprays
Author	Miss Thunyaporn Jullaphant
Major Program	Pharmaceutical Sciences
Academic Year	2016

ABSTRACT

Montelukast sodium is classified as a leukotriene receptor antagonist. It is used for prophylaxis and treatment of asthma and allergic rhinitis by oral administration. Although, montelukast safety profile is quite similar in adult and pediatric populations; there are some reports that montelukast has a number of the systemic side effects and adverse effects. The systemic side effects of montelukast include cough, fever, bronchitis and adverse effects along with liver dysfunction, agitation, hallucinations, depression, insomnia as well as suicidal ideation. Therefore, local administration of montelukast is expected to reduce the dose and possibly avoid the adverse effects. This study was aimed to develop montelukast nasal spray using either hydroxypropyl cellulose (HPC) or carbomer940 (C940) as mucoadhesive polymers and to characterize the physicochemical properties including *in vitro* evaluation of the prepared formulations. Eleven designed formulations comprising of one polymer with various concentrations were prepared. The statistical analysis were done using a Student's t-test with GraphPad Prism version 6.00 and significance level (*p-value*) of <0.05 was considered as statistically significant. The results indicated that the different polymer concentrations were the factors affecting the properties of nasal sprays. The pH of all

formulations were in the range of 6.5 - 7.5 and the hydrodynamic particle sizes were between 70 to 300 µm. The zeta potential of the formulations containing C940 ranged from -45 to -30 mV whereas for the formulations containing HPC were between -25 to -3 mV. The viscosity of all formulations were in the wide range of 6-320 cP. The contact angle, surface tension, adhesiveness and the droplet size of all formulations were dependent on the concentrations of the polymers. The stability data revealed that only 0.01% w/v C940 was stable when it was stored at 25^{o}C for 3 months. It showed no toxicity to human nasal epithelial cells as well as it did not decrease affect the transepithelial electrical resistance when it was observed before and after exposure to the spray formulation. We conclude that the MTS with 0.01% w/v C940 provided suitable nasal spray formulations.

ACKNOWLEDGEMENT

The completion of this study could not have been possible without the help of many other people. Therefore I would like to thank everyone who contributed to this thesis.

First and foremost, I would like to thank God for giving me the wisdom, the strength, peace of my mind and good health in order to finish this research.

I would like to high respectful gratitude and deep appreciation to my advisor, Assoc. Prof. Dr. Teerapol Srichana for his competent guidance, valuable suggestion, encouragement and support throughout this study. Moreover, I would like to thank for giving me the opportunity to study M. Pharm. Program and join with his research group.

I also thank the scholarship grant from Faculty of Pharmaceutical sciences and Graduate school of Prince of Songkla University for supporting facilities and everything related to this project. The grant of this study included of research fund, salary and conference scholarship throughout my study.

I also appreciate Drug Delivery System Excellence Center and Pharmaceutical Laboratory Service Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University for supporting the facilities throughout of the research. I deeply thankful to all scientists and staff of DDSEC team (Ms. Titpawan Nakpheng, Mrs. Wilaiporn Buatong, Ms. Kittiya Tinpun, Mr. Ekawat Thawitong and Ms. Kornkamon Petyord) for their friendship, training the lab skill and suggestions during my research work.

I wish to give special thanks to my brothers and sister in Postdoctoral and Ph.D. programs, Mr. Janwit Dechraksa, Mr. Faisal Usman, Mr. Charisopon Chuhachaichana,

Mr. Ruttapol Sritaradol and Ms. Sukanjana KamLungmak for their friendship, encouragement, helping and discussion during my study period.

I would especially like to thank Miss Panisara Boonsanong, education service officer of Faculty of Pharmaceutical Sciences, Prince of Songkla University, who co-ordinate and advice during academic process.

Lastly, I also thank my family, my close friend, Miss Naraporn Sornlertlumvanich, my brothers and sister in Christ who encouraged me and prayed for me throughout the time of my research.

Thunyaporn Jullaphant

CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOL

AR	Allergic rhinitis
BCS	Biopharmaceutics Classification system
°C	Degree Celsius
C940	Carbomer940
C18	Octadecyl carbon chain
cells/mL	Cells per milliliter
cm	Centimeter
cm^2	Square Centimeter
cP	Centipoise
CysLT1	Cysteinyl leukotriene receptor 1
Da	Dalton
EMEM	Eagle's minimum essential medium
FBS	Fetal bovine serum
GI	Gastrointestinal
h	Hour
HPC	Hydroxypropyl cellulose
HPLC	High-performance liquid chromatography
IV	Intravenous
LTRA	Leukotriene Receptor Antagonist
M	Molar
MCC	Mucociliary clearance
mg	Milligram

LIST OF ABBREVIATIONS AND SYMBOL (continued)

mg/mL	Milligram (s) per milliliter
mg/dose	Milligram (s) per dose
mL	Milliliter
mL/min	Milliliter (s) per minute
mM	Millimolar
mm	Millimeter
mm/s	Millimeter (s) per second
mN/m	Millinewton (s) per meter
mOsm/kg	Milliosmolarity (s) per kilogram
MS	Mass spectrophotometry
MTS	Montelukast sodium
MTT	Methyl thiazol tetrazolium
nm	Nanometer
N	Newton
N/m^2	Newton per square meter
PDI	Polydispersity index
PBS	Polyphosphate buffer solution
rpm	Round per minute
Sec	Second (s)
s^{-1}	Per second
TEER	Transepithelial electrical resistance
TEA	Triethanolamine

LIST OF ABBRIEVIATIONS AND SYMBOL (continued)

USA	United states of America
UK	United Kingdom
V	Volt (s)
v/v	Volume/volume
w/v	Weight per volume
$\Omega.cm^2$	Ohm (s) square centimeter
µJ	Microjoule
µL	Microliter
µm	Micrometer
µg/puff	Microgram (s) per puff
µg/mL	Microgram (s) per milliliter

SUBMITTED PAPER AND PROCEEDINGS

This thesis is based on the following papers:

Paper Jullaphant, T., Nakpeng, T. and Srichana, T. 2017. Formulation development and *in vitro* evaluation of montelukast nasal sprays. Submitted to Drug Development and Industrial Pharmacy. Impact factor (2016): 2.295

Proceedings Jullaphant, T. and Srichana, T. Physico-chemical properties of montelukast sodium nasal spray formulation. The 40th National Graduate Research Conference**,** 20-21 Oct 2016, Prince of Songkla University, Hatyai, Thailand.

Certificate from attended to The 40th National Graduate Research Conference (NGRC) Prince of Songkla University, Hatyai, Thailand

บัณฑิตวิทยาลัย มหาวิทยาลัยสงขลานครินทร์
ขอมอบเกียรติบัตรนี้เพื่อแสดงว่า

นางสาวธันยพร จุลพันธ์

ได้เข้าร่วมนำเสนอผลงานประเภท Poster Presentation
เรื่อง "Physico-Chemical Properties of Montelukast Sodium Nasal Spray Formulation"
ในการประชุมวิชาการเสนอผลงานวิจัยระดับบัณฑิตศึกษาแห่งชาติ ครั้งที่ 40
ณ ศูนย์ประชุมนานาชาติฉลองสิริราชสมบัติครบ 60 ปี
ให้ไว้ ณ วันที่ 20 ตุลาคม 2559

(รองศาสตราจารย์ ดร.ธีระพล ศรีชนะ)
คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยสงขลานครินทร์

INTRODUCTION

Allergic rhinitis (AR) is the upper respiratory tract disease which is characterized by watery rhinorrhea, nasal itching, nasal obstruction and sneezing. The pathology of AR begins with nasal mucous membranes exposure to allergens such as plant pollens, dust mites, polluted air and weather change which induce immune response through IgE-mediated hypersensitivity. This causes penetration of mast cells, T-cells, eosinophils and basophils resulting in release of various chemokines and cytokines (Pawankar et al. 2011).

Cysteinyl leukotrienes (CysLTs) are one of the cytokines synthesized from arachidonic acid and released by various inflammatory cells. CysLTs play a major role in AR patients due to the presence of CysLTs receptors in intranasal tissues. On attachment of CysLTs to the receptors, the vascular permeability is increased which causes prolonged congestion and results in edema which may affect nasal cavity obstruction. Moreover, CyLTs also increase a number of nasal secretions in a dose-dependent manner that causes rhinorrhea. Those symptoms reduce the quality of life and impact the performance of work productivity, school learning, sleep disturbance and increased medical costs (Peters-Golden et al. 2006, Min 2010).

For AR treatment, guidelines suggest the avoidance of exposure to allergens. Moreover, pharmacological treatment of AR depends on the severity and duration of symptoms (Min, 2010). Leukotriene receptor antagonists (LTRAs) can be used alone or in combination with other drugs to treat severe cases of AR to produce better outcomes.

Montelukast sodium is a potent, selective cysteinyl leukotriene 1 (CysLT1) receptor antagonist, designed chemically as 1-[[[(1R)-1-[3-(1E)-2-(7-chloro-

2quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]-methyl]-cyclopropaneacetic acid, monosodium salt. It is prescribed to treatment allergic rhinitis and asthma attack (Peters-Golden et al. 2006, Min 2010, Pawankar et al. 2011). Montelukast is a hygroscopic, optically active, white to off-white powder. The empirical formula is $C_{35}H_{36}ClNO_3S$ and molecular weight is 586.18. It is an acidic lipophilic drug with estimated pKa of 2.7 and 5.8 and log P of 8.79. It is freely soluble in ethanol and methanol, practically insoluble in acetonitrile. The solubility of montelukast is reported between 0.2 – 0.5 μg/mL in water at 25°C. The solubility is low at the acidic condition but increases with the increase of pH. Montelukast possesses high permeability but low solubility therefore it is categorized in the Biopharmaceutics Classification Class (BCS Class) II (Okumu et al. 2008). The solubility of montelukast increases in the salt form of montelukast sodium (MTS). The empirical formula of MTS is $C_{35}H_{35}ClNNaO_3S$ and molecular weight of 608.18. The chemical structures of montelukast sodium are given in Figure 1. To determine the MTS dielectric constant, the MTS powder was transformed to tablet by using direct compression method. The dielectric constant of the MTS was measured by using dielectric constant apparatus (solid & liquid; AE 454) (Advanced Technocracy Inc Grain Market, Ambala, India). From this study, the dielectric constant of MTS is 13. On the other hand, the dielectric constant of water is 80 (Mohsen-Nia et al. 2010). Although, MTS was dissolve better than montelukast, MTS could not be dissolved clearly by water. Due to the dielectric constant between MTS and water are very different. Therefore, the solvents which are safe for nasal epithelial cells and have a similar dielectric constant as montelukast sodium were used to dissolve MTS. The main focus of cosolvent was ethanol, which has dielectric constant 24.5 and propylene

glycol has dielectric constant 38 which was the popular used as cosolvent in the pharmaceutical nasal administration (Bitter et al. 2011).

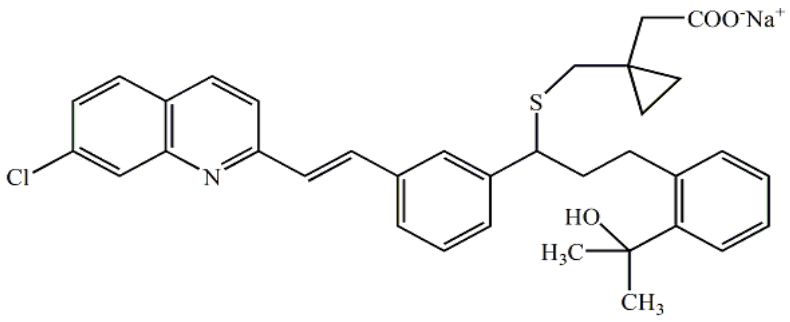


Figure 1. Structure of chemical formula of montelukast sodium

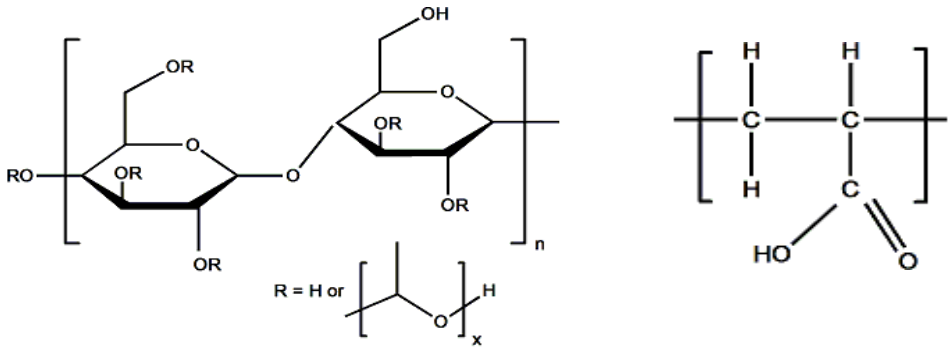
The stability of MTS depends on the exposure to light and pH of the solution. In the solid state, after exposure to the light the major degradation product is formation of montelukast sulfoxide. For the tablet dosage form after incubation at 40^{o}C and 75% RH for 6 months, it was also detected montelukast sulfoxide product. In solution state, after exposure to light, the photodegradation of MTS in solution leads to the formation of its cis-isomer as the major product. The thermal stress testing of MTS in solutions at 65^{o}C showed that it is degraded rapidly in both acidic and H_2O_2 solutions. The major degradation product in the latter solvent was montelukast sulfoxide (impurity product) (Al Omari et al., 2007). Therefore MTS should be kept in the closed container which can protect from light and avoidance of the acidic condition.

Nowadays MTS is available in the market as Singulair®. It is the commercial product imported into Thailand by MSD (Thailand) Ltd. The Singulair® has various dosage forms such as tablets, chewable tablets as well as oral granules. However, oral administration of montelukast sodium has many systemic side effects including

cough, fever, bronchitis and adverse effects agitation, aggression, anxiousness, hallucinations, depression, insomnia, restlessness, suicidal, ideation and liver dysfunction (Benninger and Waters 2009). Patil-Gadhe et al. (2014) developed the MTS-loaded nanostructured lipid carrier dry powder for the pulmonary route to increase bioavailability of montelukast sodium that could avoid the possible adverse effects (Patil-Gadhe et al. 2014, Patil-Gadhe and Pokharkar 2014). However, there is no reported work to deliver MTS as a nasal spray. Therefore, this study developed the MTS nasal spray for allergic rhinitis that is expected to reduce the dose and avoid systemic adverse effects.

The nasal drug delivery is widely used to treat of systemic and local diseases of the upper respiratory tract. The advantages of nasal spray include self-administration, non-invasive, suitable for children and adult, patient convenience and improve compliance. Moreover, nasal administration gives rapid onset of therapeutic effect with the rate of absorption comparable to IV medication, avoidance of first pass effect and destruction in GI tract. The use of low dose can reduce the adverse effects (Appasaheb et al. 2013). The important aspects of nasal sprays that affect nasal absorption were carefully considered consist of three factors include the anatomy and physiology of nasal cavity, physicochemical properties of drug and the formulation parameters (Illum 2002). The first factor of nasal absorption is the anatomy and physiology of human nasal cavity. The limitation from nasal physiology is the nasal mucus secretion and mucociliary clearance (MCC) (Ugwoke et al. 2005). The MCC mechanism protects the respiratory system against inhaled bacteria, irritants and foreign particles. It efficiently removes the product from the site of application, which reduces contact time in the potential absorption area. The strategy to improve nasal

absorption is using a mucoadhesive polymer which is focused on overcoming the MCC barrier effect. The mucoadhesive polymers act as adhesives material on the mucous membrane. Formation of an adhesive force between the formulation and nasal mucosa, increases the retention time of the drug inside the nasal cavity that minimizes the mucociliary clearance. The mucoadhesive polymers which were used in previous studies include hydroxypropyl cellulose (HPC) and carbomer940 (C940) (Chaturvedi *et al.*, 2011). Figure 2 showed the chemical structure of HPC and C940. Both polymers are used as thickening and bioadhesive and controlled-release agents. The physicochemical properties of HPC are a nonionic cellulose, white to slightly yellow-colored and hygroscopic powder. The molecular weight is about 1,150 kDa. It is soluble in water below 38°C, methanol, ethanol, propylene glycol and polar organic solvents. HPC is insoluble and precipitated as a highly swollen floc in hot water at a temperature between 40 to 45^{o}C. As it is a nonionic cellulose, it does not disturb other ionic materials and has thickening and mucoadhesive properties, thus it is preferable to use in local spray formulations.



(a) **(b)**

Figure 2. The chemical structure of hydroxypropyl cellulose (HPC) (a) and C940 (C940) (b).

Moreover, the advantages of HPC are prolonging the retaining time of drug in the nasal cavity thus sustaining the release of drug owing to high viscosity besides acting as absorption enhancer and effectively increasing intranasal bioavailability (Appasaheb et al. 2013).HPC solutions are non-irritant and less allergic to the skin (Khairnar and Sayyad 2010, Fini et al. 2011). It was used in various dosage form for intranasal drug administration such as leuprolide nasal powder (Suzuki and Makino 1999), metoclopramide in situ gel and dopamine nasal drop (Chaturvedi et al. 2011). The physicochemical properties of C940 are an anionic polymer, white color to off-white, acidic and hygroscopic powders. The molecular weight is about 1,044 kDa. C940 disperses in water to form acidic colloidal dispersions. After neutralization, C940 forms cross-linked polymers and swellable in water, glycerin and ethanol. The advantages of C940 include excellent mucoadhesive and gel formation ability as well as attachment and tightening potential between the formulation and membrane surface (Appasaheb et al. 2013). It was used in various dosage form for intranasal drug administration such as apomorphine, metoclopramide nasal powder and levonorgestrel nasal drop (Chaturvedi et al. 2011).

The second factor involves the physicochemical properties of the drug including size, molecular weight (should be less than 500 Da), solubility (should be more than 50 mg/mL), lipophilicity (should be less than 5), charge, pKa, polymorphism, chemical state, and physical state. The third factor includes the formulation parameters, for example, the pH of the formulation should be in the range of 4.5 - 7.5. The osmolarity of the formulation should be 200 to 500 mOsm/kg. The drug concentration should not exceed 5 mg/dose. The viscosity should be in the range

of 3 - 6 cP. Finally, the applied volume of solution should be 25 - 200 µL per nostril (Illum 2002, Jadhav et al. 2007, Bitter et al. 2011, Menaka and Pandey 2014).

The rationale of the dose specified for montelukast nasal sprays was calculated. Table 1 shows the data which is related to dose calculation.

Table 1. The amount of MTS in blood circulation and volume of targeted organ.

Oral bioavailability	**Average C_{max} (ng/mL)**	**Volume of nasal cavity (mL)**	**Lung fluid (mL)**
58% – 62%	375	3	10

Due to the oral bioavailability of montelukast sodium is about 58 - 62% (Zhao et al., 1997, Cheng et al., 1996), an alternative route such as nasal delivery could possibly improve and reduce the dose of MTS at the target site (the turbinates located at the lateral nasal wall are highly vascularized with a very high blood flow). The pharmacokinetic studies of montelukast sodium showed C_{max} of the males as 385 ng/mL whereas for females, C_{max} was found to be 350 ng/mL followed by oral administration of a 10 mg tablet of MTS (Cheng et al., 1996). From the anatomy of human nasal cavity, it is seen that the total surface area is approximately 200 cm^2 (Harkema et al., 2006) and the thickness of the low viscosity mucous layer is 5-10 µm and for the more viscous upper layer is about 0.5-5 µm (Wadell, 2002).

$$\text{Dose for nasal spray} = (C_{max} \times \text{volume of nasal cavity}) \times \frac{100\%}{50\%}$$

$$= 375 \text{ ng/mL} \times 3 \text{ mL} \times 2$$

$$= 2.25 \text{ µg/dose}$$

The dose of MTS was calculated from the C_{max} (average value from male and female data mentioned aforesaid) multiplied by the volume of nasal cavity (calculated

from the total surface area multiplied with the average of mucous thickness). Assuming that the dose can be delivered 50% to nasal cavity, the calculated dose is about 2.25 µg/dose.

$$\text{Dose for pulmonary route} = (C_{max} \times \text{lung fluid}) \times \frac{100\%}{25\%}$$

$$= 375 \text{ ng/mL} \times 10 \text{ mL} \times 4$$

$$= 15 \text{ µg/dose}$$

On the other hand, the calculated dose for pulmonary route is 15 µg/dose. The dose for pulmonary route was more than nasal route because the lung fluid is about 10 mL and the drug reach to target site only 20 – 25%. This dose estimation may depict variability based on the performance of the device, anatomical and personal variations and the stage of disease. As the calculated dose from nasal cavity was too low therefore the selected dose was as suitable for pulmonary route. However, the dose 15 µg/dose was low, hence the formulation should be in the form of nasal spray solution.

The containers of the nasal spray are important factor to consider. The Figure 3 shows the containers used in this study were plastic nasal spray bottle with pump sprayer mist nose refillable 10 mL. The estimation volume per spray was 50 µL, therefore the obtained delivered doses were 200 doses.

Figure 3. The plastic nasal spray bottle with pump sprayer mist nose **r**efillable 10 mL.

However, the spray volume dependent on the pump and the force applied at the finger rest. The accuracy of metered-dose pump spray is dependent on the viscosity and surface tension of the formulation.

To obtained the suitable nasal spray, the prepared formulations were evaluated for physicochemical properties including appearance, color, clarity, foreign particulates, pH, osmolality, drug content, spray content uniformity, stability, impurities and degradation products, preservative and stabilizing excipients assay, microbial limit, the particle hydrodynamic size, droplet size distribution, viscosity, zeta potential, contact angle, and surface tension. Furthermore, the formulations were assessed the drug release profile and *in vitro* evaluation. The Figure 4 shows the human nasal epithelial cell line (squamous carcinoma cell line) or RPMI 2650 that represented a valid *in vitro* model to evaluate the cytotoxicity as well as the opening of the tight junctions. It was expected that the formulations would not alter the tight junctions and transepithelial electrical resistance (TEER).

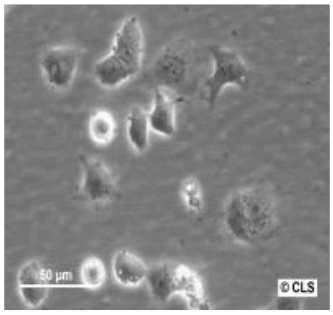


Figure 4. The human nasal epithelial cell line (RPMI2650) (Kreft et al. 2015).

OBJECTIVES

1. To design and develop montelukast nasal sprays
2. To characterize the physicochemical properties to obtain the suitable and stable formulation.
3. To *in vitro* evaluate the formulation to obtain the safety and not alter the tight junction of the nasal epithelial cells.

SIGNIFICANT RESULTS AND GENERAL DISCUSSION

Formulations and physicochemical properties of MTS nasal sprays

Preparation of the montelukast nasal spray

The formulation consists of montelukast sodium 0.0375% w/v as an active ingredient, carbomer940 (C940) or hydroxypropyl cellulose (HPC) as a mucoadhesive polymer. Due to the dielectric constant of MTS is 13 whereas the dielectric constant of water is 80. To dissolve MTS clearly, the cosolvent having the similar dielectric constant as MTS was used. Therefore, ethanol has dielectric constant 24.5 and propylene glycol has dielectric constant 38 were utilized as a cosolvent. The ratio of cosolvent in the formulation is ethanol 20% v/v and propylene glycol 30% v/v as a cosolvent and preservative. Table 2 shows the quantity of nasal spray ingredients in different formulations. The formulations containing C940 (0.005 – 0.15 %w/v) or HPC (0.005 – 0.5 %w/v) were clear solution.

Physicochemical properties evaluation

The polymers and its concentrations affected to the physicochemical properties of the formulations that are showed in the Table 1. The hydrodynamic particle size of the MTS colloid without the polymer was 53 nm with PDI of 0.13 indicating a monodisperse system. The hydrodynamic particle size of formulations with C940 or HPC were in the range of 70 – 300 nm. Perhaps the size of the colloid particle in MTS formulation increased by the polymers that covered the MTS particle. The size of colloid system remained constant. The zeta potential of the MTS without

polymer was -28.5 mV. The obtained zeta potential of HPC formulations were in the range of -25 to -3 mV. The less negative charge presented in the formulations containing a higher quantity of HPC. The zeta potential of the C940 formulations were in the range of -45 to -30 mV and it was more negative than -30 mV when C940 concentration was risen. The zeta potential of the formulations could be explained according to the Figure 5. As the HPC is a nonionic polymer, without charge when the MTS molecules were covered by the HPC resulting in a decrease in the zeta potential. On the other hand, the C940 is an anionic polymer. When the C940 covered the MTS, it promoted the negative charge of the system.

Table 2. The quantity of nasal spray ingredients in different formulations.

Formulation (mg)	**Polymer (%w/v)**	**MTS (%w/v)**	**Propylene glycol (%v/v)**	**Ethanol (%v/v)**	**Water q.s. to (%v/v)**
HPC 5	0.005	0.0375	30	20	100
HPC 10	0.01	0.0375	30	20	100
HPC 50	0.05	0.0375	30	20	100
HPC 100	0.10	0.0375	30	20	100
HPC 300	0.30	0.0375	30	20	100
HPC 500	0.50	0.0375	30	20	100
C940 5	0.005	0.0375	30	20	100
C940 10	0.01	0.0375	30	20	100
C940 50	0.05	0.0375	30	20	100
C940 100	0.10	0.0375	30	20	100
C940 150	0.15	0.0375	30	20	100

Table 3. The Physical and chemical properties of the MTS nasal sprays (mean ± SD, n=3).

Formulation	Particle size (nm)		Zeta potential (mV)*	Viscosity (cps)	Contact angle (degree)	Work of adhesion (μJ)
	Z-average	PDI				
No polymer	53.01	0.13	-28.50	5.7 ± 0.2	66.9 ± 1.3	0.5 ± 0.1
HPC 5	70.57	0.14	-24.63	6.2 ± 0.0	23.7 ± 3.7	0.9 ± 0.1†
HPC 10	78.48	0.17	-21.37	6.7 ± 0.0	24.3 ± 2.3	0.9 ± 0.1†
HPC 50	80.52	0.38	-9.68	8.0 ± 0.0	29.0 ± 4.4	1.1 ± 0.1†
HPC 100	93.21	0.27	-6.33	11.4 ± 0.1	36.8 ± 3.1	1.3 ± 0.4†
HPC 300	96.77	0.23	-5.09	46.8 ± 0.1	42.8 ± 3.5	1.8 ± 0.6†
HPC 500	100.09	0.38	-3.66	318.7 ± 1.3	43.5 ± 4.6	3.9 ± 0.7†
C940 5	84.25	0.15	-32.37	6.8 ± 0.0	17.6 ± 1.8	0.2 ± 0.1
C940 10	94.02	0.16	-37.80	7.3 ± 0.0	20.9 ± 1.4	1.0 ± 0.2†
C940 50	100.37	0.14	-44.30	18.2 ± 0.1	27.0 ± 2.9	3.6 ± 0.4†
C940 100	103.14	0.31	-43.97	115.1 ± 0.9	31.8 ± 0.9	8.1 ± 0.9†
C940 150	267.17	0.64	-43.30	NA	34.5 ± 0.3	27.1 ± 0.8†

* Indicates significant difference between HPC and C940 formulations $p<0.05$, †Indicates significant difference between the solution without polymers and the formulation with HPC or C940 $p<0.05$. NA is not applicable.

Therefore, the colloid particle with C940 had more repulsive force between each particle than that of the HPC formulations. In brief, the MTS colloid solution containing C940 was found to be more stable with a lower chance of precipitation than the formulations containing HPC according to their zeta potential.

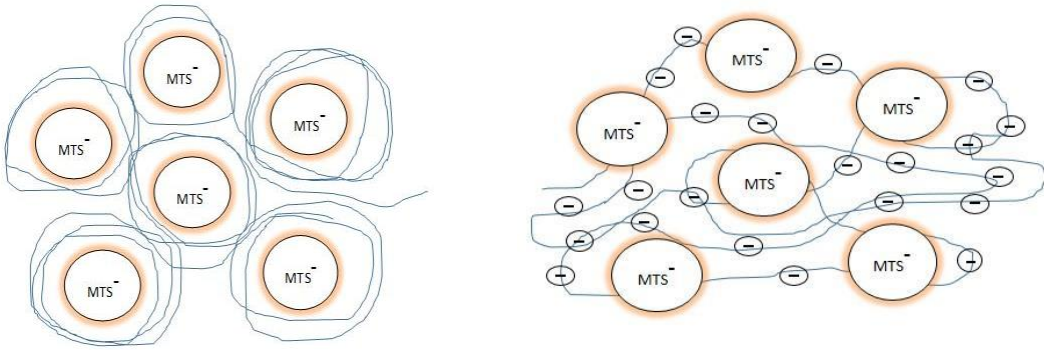


Figure 5. The illustration of HPC-coated (a) and C940-coated (b) the MTS in nasal spray formulation.

The contact angle of the colloid solution without the polymers was 67°, whereas the solutions containing the 0.005 – 0.05% HPC and C940 had a contact angle in the range of 10 – 30°. Therefore, containing the C940 or HPC had low contact angle with good spreadability and increase contact time in the nasal cavity to enhance drug absorption in the nasal epithelial cell (Zheng 2010). The formulations with HPC and C940 had low surface tension in the range of 30 – 36 and 30 – 38 mN/m, respectively.

The viscosity of HPC and C940 formulations were dependent on the concentration of polymer. The viscosity of the formulations will prolong a residence time of the drug in the nasal cavity for sustained release manner. A greater viscosity of formulation impacts the droplet size distribution which may altered deposition in the nasal cavity. The droplet volume mean diameter of the formulations with HPC and C940 were in the range of 100 – 600 µm and 70 – 120 µm, respectively. The droplet volume mean diameter obtained from the formulations with C940 were smaller than HPC formulations significantly. The parameters related to droplet volume mean diameter are the viscosity and surface tension of the liquid. When the viscosity and

surface tension rose, the droplet size will be increased. The adhesive force of the solution without the polymers was 0.5 µJ. The formulations with HPC were in the range of 0.9 – 4.0 µJ and the formulations with C940 were in the range of 0.2 – 28.0 µJ. The work of adhesion of the formulations with mucoadhesive polymers had higher adhesions than the solution without polymer. However, there were no significant differences between each formulation on the work of adhesion with different concentrations of polymer solution.

The stability and drug content in the montelukast nasal sprays

All formulations were kept in clear bottles and stored at 25ºC. After one month, the solutions precipitated as yellow crystals. The precipitation occurred in all samples of the solution containing HPC. Similar circumstances also occurred in the samples containing C940 and even the zeta potential was less than -30 mV. However, no precipitation took place in the formulation containing 0.01% w/v C940. The formulation containing 0.01% w/v C940 had an average drug content of 95.5%. Therefore, a lower polymer concentrated formulation could produce an appropriate zeta potential for the hydrodynamic size in the proper viscous media. In conclusion, only the formulation of C940 10 demonstrated good stability when stored at 25ºC for three months. In addition, the yellow crystals were further analyzed by MS-MS spectrometry.

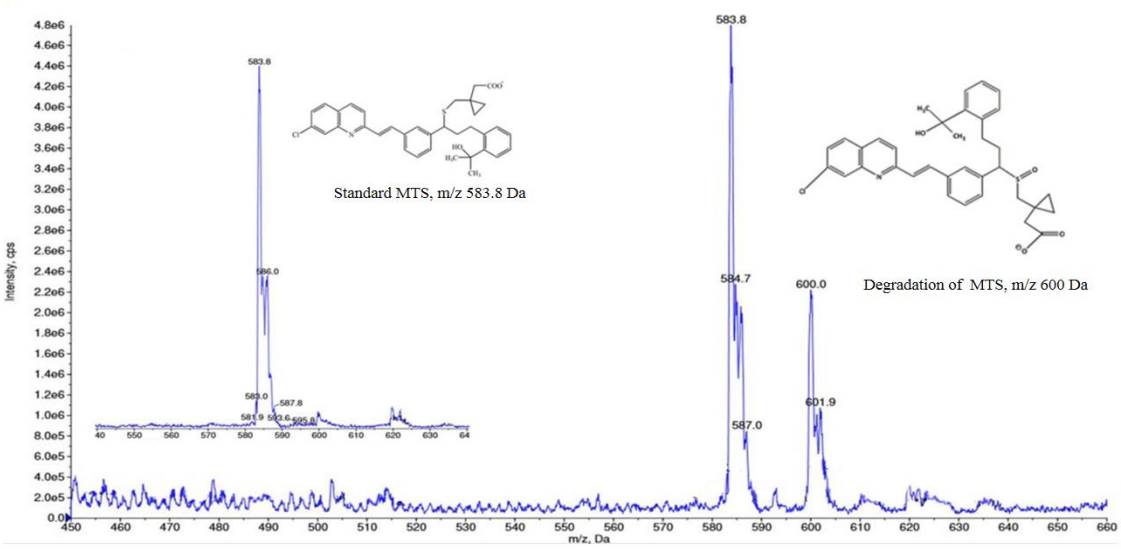


Figure 6. Degradation of MTS, montelukast sulfoxide m/z 600

The Figure 6 shows the MS-MS of MTS spectra, the molecular weight of montelukast sodium was about 584 Da. The peaks of yellow crystal consist of the peak at 584 Da and some other peaks were degradation products of MTS, such as the molecular weight of 600 Da which was montelukast sulfoxide (Al Omari et al. 2007, Saravanan et al. 2008). The crystal of montelukast nasal spray was montelukast with degradation product. MTS is sensitive to the light and pH of the solution. The major photodegradation product is montelukast formation of its cis-isomer (impurity) whereas the major degradation product from the acidic solution is montelukast sulfoxide (impurity). Therefore, MTS should be kept in the closed container that can protect from light and avoidance of the acidic condition. From overall physicochemical properties evaluation, the formulation C940 10 had excellent physicochemical properties and was also stable. The formulation was chosen for further investigation including drug release profile.

The release of the montelukast nasal sprays

The control formulation (MTS solution) and formulations with 0.01% w/v HPC and 0.01% w/v C940 were stored at 37°C for 24 h in 0.5% sodium dodecyl sulfate (Okumu et al. 2008). The release rate of the solution without the polymers and the solution with 0.01% w/v HPC gradually increased until it reached the maximum at 24 h, while the formulation with 0.01% w/v C940 released continuously until the maximum concentration was achieved at 3 h. The % cumulative drug release was then obtained as 44.6%, 41.8% and 50.1%, respectively.

The effect of MTS nasal spray formulation on the nasal epithelial cells

In this study, the human nasal epithelial cell (RPMI 2650) line was used as a model of the progressive formation of the cellular toxicity, the effect of formulation on the tight junction and drug permeation. As with the cytotoxicity test, C940 alone, the formulation with 0.01% w/v C940, and the different concentrations of MTS did not induce cell toxicity and was safe for nasal epithelial cells. The Figure 7 shows the results of the cellular viability of the samples was similar to the control (95-109%).

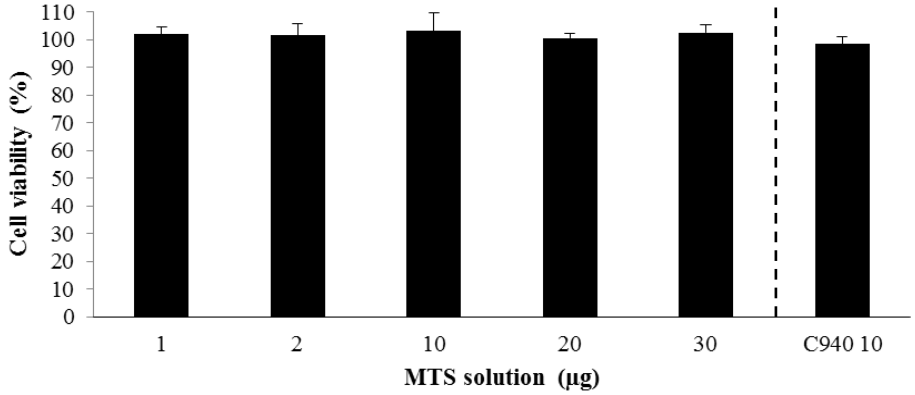


Figure 7. Nasal epithelium cell viability incubated with the dose of 1 – 30 ug MTS and the formulation C940 10 on RPMI 2650 cells, (mean ± SD, n=3**)**.

Figure 8 shows the % transepithelial electrical resistance (% TEER) of the RPMI 2650 cell monolayer. Two days before the experiment was performed, the TEER value was constant around 515 $\Omega.cm^2$ therefore this TEER value was assumed as 100% TEER. Thereafter, the RPMI 2650 cell monolayer incubated with MTS formulation as a function of time from 0 to 24 h which was significantly higher than the control ($p<0.05$) indicating no interference of the formulation with the tight junction. Figure 9 shows that only a small amount of MTS permeated to the basolateral compartment. The apparent permeability (P_{app}) of the MTS solution at 3 h was 3.73 x 10^{-6} cm/s but after 3 and 6 h later, the P_{app} decreased to 0.43 x 10^{-6} cm/s and 0.49 x 10^{-6} cm/s, respectively. After medium replacement at 12 h, P_{app} of the MTS solution was raised to 2.65 x 10^{-6} cm/s. For the formulation with 0.01% w/v C940, the P_{app} at 3 h was 1.79 x 10^{-6} cm/s and was decreased to 0.74 x 10^{-6} cm/s and 0.73 x 10^{-6} cm/s at 6 and 9 h respectively. The P_{app} value was raised again to 1.79 x 10^{-6} cm/s at 12 h which was the time to replace the medium. When the P_{app} value was less than 2 x 10^{-6} cm/s, it was indication of the low permeability but if the P_{app} was between 2 x 10^{-6} cm/s and 20 x 10^{-6} cm/s, it was indication for medium permeability but if it was more than 20 x 10^{-6} cm/s, it indicated high permeability. However, the P_{app} of formulation with 0.01% w/v C940 from 3 to 12 h was less than 2 x 10^{-6} cm/s. Therefore, using the 0.01% w/v C940 in the formulation showed less drug permeation into the basolateral compartment compared to MTS without the C940. It was assumed that using the C940 would retain the drug at the nasal epithelial cell longer than without the C940.

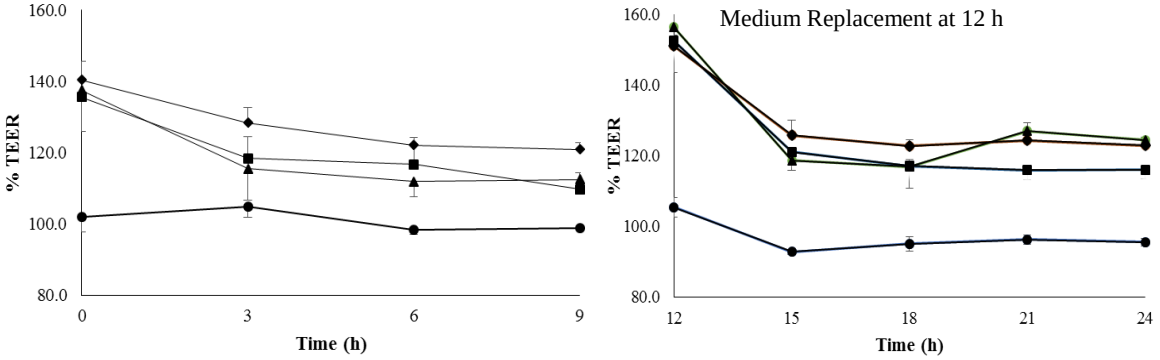


Figure 8. % Transepithelial electrical resistance (TEER) of RPMI 2650 cells cultured in the medium with the formulation containing 0.01% w/v C940 (◆), MTS solution (■), the polymeric solution without MTS (▲) and control (●) using LLI conditions 12 h, (mean ± SD; n = 3).

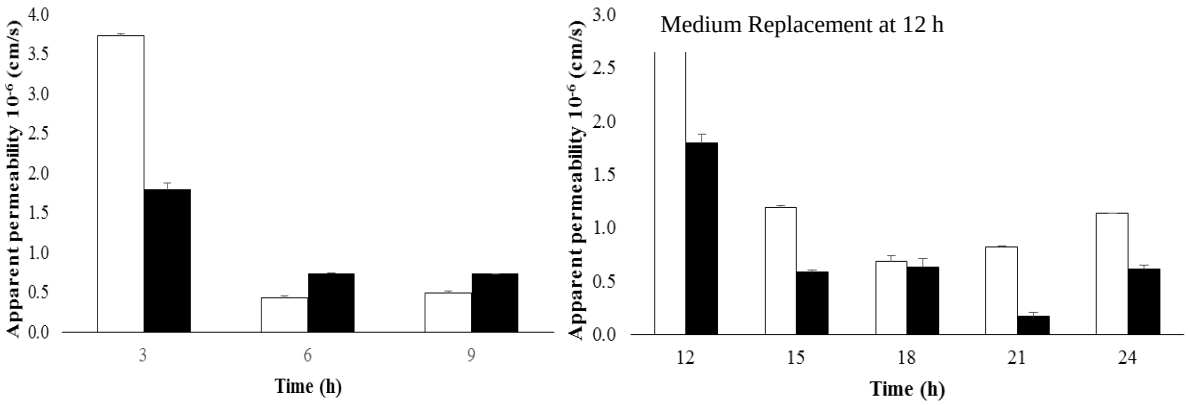


Figure 9. Comparison between permeability of MTS solution and the formulation containing 0.01% w/v C940 in a nasal epithelial cell monolayer. Apparent permeability (P_{app}) of MTS solution (□) and the formulation containing 0.01% w/v C940 (■) from apical to basolateral (AP-BL), (mean ± SD; n=3).

CONCLUSIONS

In this study, montelukast sodium was developed as the nasal spray formulations. The eleven designed formulations were prepared to investigated the physicochemical properties for the suitable nasal sprays, then the suitable formulation was chose to study related drug releasing and *in vitro* evaluation further. It was demonstrated that the formulation with 0.01% w/v C940 had been selected according to the good physicochemical properties and adhesiveness as well as good stability. Moreover, no toxicity was observed from all ingredients in the formulation. The TEER value of nasal epithelial cells after exposure with the formulation was not decreased but it was rose from control. Therefore, the formulation with 0.01% w/v C940 could not alter the opening of the nasal epithelial tight junction. It can be concluded that this montelukast spray can be an effective candidate for a nasal administration.

BIBLIOGRAPHY

Al Omari, M. M., Zoubi, R. M., Hasan, E. I., Khader, T. Z. and Badwan, A. A. (2007). Effect of light and heat on the stability of montelukast in solution and in its solid state. J Pharmaceut Biomed 45(3): 465-471.

Appasaheb, P. S., Manohar, S. D., Bhanudas, S. R. and Anjaneri, N. (2013). A review on intranasal drug delivery system. JAPER 3(4): 333-346

Chaturvedi, M., Kumar, M. and Pathak, K. (2011). A review on mucoadhesive polymer used in nasal drug delivery system. J Adv Pharm Technol Res 2(4): 215-222

Bitter, C., Suter-Zimmermann, K. and Surber, C. (2011). Nasal drug delivery in humans. Topical Applications and the Mucosa, Karger Publishers. 40: 20-35.

Fini, A., Bergamante, V. and Ceschel, G. C. (2011). Mucoadhesive gels designed for the controlled release of chlorhexidine in the oral cavity. Pharmaceutics 3(4): 665-679.

Illum, L. (2002). Nasal drug delivery: new developments and strategies. Drug Discov Today. 7(23): 1184-1189.

Jadhav, K. R. Gambhire, M. N. Shaikh, I. M. Kadam, V. J. and Pisal, S. S. (2007). Nasal drug delivery system-factors affecting and applications. Curr Drug ther. 2(1): 27-38.

Khairnar, G. and Sayyad, F. (2010). Development of buccal drug delivery system based on mucoadhesive polymers. Int J PharmTech Res. 2(1): 719-735.

Kreft, M. E., Jerman, U. D., Lasič, E., Rižner, T. L., Hevir-Kene, N., Peternel, L. and Kristan, K. (2015). The characterization of the human nasal epithelial cell line

RPMI 2650 under different culture conditions and their optimization for an appropriate in vitro nasal model. Pharm. Res. 32(2): 665-679.

Menaka, M., Pandey, V. P. (2014). Formulation development and evaluation of cinnarizine nasal spray. JPHS. 2(4): 339-346.

Min, Y. G. (2010). The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy Asthma Immunol Res 2(2): 65-76.

Mohsen-Nia, M., Amiri, H. and Jazi, B. (2010). Dielectric constants of water,methanol, ethanol, butanol and acetone: measurement and computational study. J Solution Chem 39(5): 701-708.

Okumu, A., DiMaso, M. and Löbenberg, R. (2008). Dynamic dissolution testing to establish *in vitro/in vivo* correlations for montelukast sodium, a poorly soluble drug. Pharmaceut Res 25(12): 2778-2785.

Patil, V. B., Kalkotwar, R. S., Patel, A., Tathe, S. and Jadhav, V. B. (2012). Evaluation and quality control of nasal spray. JDDT. 1(2): 1-4.

Patil-Gadhe, A., Kyadarkunte, A., Patole, M. and Pokharkar, V. (2014). Montelukast-loaded nanostructured lipid carriers: part II pulmonary drug delivery and *in vitro – in vivo* aerosol performance. Eur J Pharm Biopharm. 88(1): 169-177.

Patil-Gadhe, A. and Pokharkar, V. (2014) Montelukast-loaded nanostructured lipid carriers: part I oral bioavailability improvement. Eur J Pharm Biopharm. 88(1): 160-168.

Pawankar, R., Mori, S., Ozu, C. and Kimura, S. (2011). Overview on the pathomechanisms of allergic rhinitis. Asia Pacific Allergy 1(3): 157-167.

Peters-Golden, M., Gleason, M. and Togias, A. (2006). Cysteinyl leukotrienes:

multi-functional mediators in allergic rhinitis. Clinical & Experimental Allergy 36(6): 689-703.

Rowe, R. C., Sheskey, P. J. and Weller, P. J. (2006). Handbook of pharmaceutical excipients.

Saravanan, M., Reddy, P. P., Naidu, M. N., Babu, J. M., Srivastava, A. K., Kumar, T. L., Sekhar, B. V. V. N. C. and Satyanarayana, B. (2008). Identification, synthesis, isolation and spectral characterization of potential impurities of montelukast sodium. J Pharmaceut Biomed 48(3): 708-715.

Suzuki, Y. and Makino, Y. (1999). Mucosal drug delivery using cellulose derivatives as a functional polymer. J Control Release 62(1): 101-107.

Ugwoke, M. I., Agu, R. U., Verbeke, N. and Kinget, R. (2005). Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives. Adv Drug Deliver Rev. 57(11): 1640-1665.

Washington, N., Steele, R. J. C., Jackson, S. J., Bush, D., Mason, J., Gill, D. A., Pitt, K. and Rawlins, D. A. (2000). Determination of baseline human nasal pH and the effect of intranasally administered buffers. Int J Pharmaceut 198(2): 139-146.

Zheng, W. (2010). Surface wetting characteristics of rubbed polyimide thin films. Polymer Thin Films, InTech.

APPENDIX

Submitted paper and proceedings

PAPER

Formulation development and *in vitro* evaluation of

montelukast nasal sprays

(Manuscript was submitted to *Drug Development and Industrial Pharmacy*)

Drug Development and Industrial Pharmacy

Formulation development and *in vitro* evaluation of montelukast nasal sprays

Journal:	*Drug Development and Industrial Pharmacy*
Manuscript ID	LDDI-2017-0312
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	08-Jun-2017
Complete List of Authors:	Jullaphant, Thunyaporn; Faculty of Pharmaceutical Sciences, Prince of Songkla University, Drug Delivery System Excellence Center Nakpheng, Titpawan; Faculty of Pharmaceutical Sciences, Prince of Songkla University, Drug Delivery System Excellence Center Srichana, Teerapol; Faculty of Pharmaceutical Sciences, Prince of Songkla University, Department of Pharmaceutical Technology; Faculty of Pharmaceutical Sciences, Prince of Songkla University, Drug Delivery System Excellence Center
Keywords:	montelukast, allergic rhinitis, nasal sprays, Carbomer 940, hydroxypropyl cellulose

Formulation development and *in vitro* evaluation of montelukast nasal sprays

Thunyaporn Jullaphant[a], Titpawan Nakpeng[b], Teerapol Srichana[a,b*]

[a] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.*

[b] *Nanotec-PSU Excellence Center on Drug Delivery System, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.*

*Corresponding author; Dr. Teerapol Srichana, Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand. E-mail address: teerapol.s@psu.ac.th;

Tel: +66 74288979; Fax: +6674288979.

Keywords: Montelukast, Allergic rhinitis, Nasal sprays, Carbomer940, Hydroxypropyl cellulose

Abstract:

Objectives: The aim of this study is to formulate and characterize montelukast sodium (MTS) nasal spray for the treatment of allergic rhinitis to attain a local effect.

Materials and methods: The formulations were prepared using various concentrations of hydroxypropyl cellulose (HPC) or Carbomer940 (C940). The prepared formulations were evaluated for their physicochemical properties including clarity, pH, hydrodynamic particle size, zeta potential, viscosity, contact angle, surface tension, droplet size and distribution, muco-adhesiveness, drug release profile, and stability. The suitable formulations were also assessed for their effects on nasal epithelial cells (RPMI 2650).

Results and discussion: At room temperature (25ºC), the formulation containing 0.01% w/v C940 exhibited good physicochemical properties and showed no sign of precipitation during 3 month storage. The works of adhesion of the formulations containing HPC or C940 were in the range of 0.8–4.0 and 0.2–27 µJ, respectively. In addition, the formulation containing 0.01% w/v C940 and the 0.5–15 µg/50 µL concentrations of MTS showed no signs of cytotoxicity.

Conclusion: The formulation containing 0.01% w/v C940 had no effect on the tight junction. In conclusion, the formulated MTS nasal spray is ideal for nasal administration for the treatment of allergic rhinitis.

1. Introduction

Montelukast sodium (MTS) is a leukotriene receptor antagonist (LTRA) that binds with high affinity and selectivity to the CysLT1 receptor. It is widely prescribed for upper and lower respiratory diseases as well as for the relief of symptoms of allergic rhinitis, the treatment and prophylaxis of asthma attacks. MTS is available in various oral dosage forms such as tablets, chewable tablets, and oral granules. In the management of allergic or perennial rhinitis, it is suggested as 10 mg oral administration once daily (Knorr et al. 2000, Lagos and Marshall 2007, Philip et al. 2009). Although MTS is accepted with a safety profile that is similar in the adult and pediatric populations, Benninger et al. (2009) reported that MTS has a number of systemic side effects. The side effects of MTS include cough, fever, bronchitis, and adverse effects like liver dysfunction, agitation, aggression, anxiousness, hallucinations, depression, insomnia, restlessness, and suicidal ideation (Benninger and Waters 2009). Some studies reported to have reduced the systemic side effects of MTS and improved bioavailability by using MTS-loaded nanostructured lipid carriers for oral administration which focused directly on the target such as MTS-loaded nanostructured lipid carrier dry powder for the pulmonary route (Patil-Gadhe et al. 2014, Patil-Gadhe and Pokharkar 2014). However, no study has confirmed the local effects of MTS for allergic rhinitis on nasal epithelial cells. Therefore, a nasal formulation for local delivery in the form of a nasal spray was developed.

The factors that affect nasal absorption were carefully considered. The first factor is the anatomy and physiology of the nasal cavity which is related to membrane transport, deposition, enzymatic degradation, and the mucociliary clearance

mechanism. The second factor involves the physicochemical properties of the drug including size, molecular weight, solubility, lipophilicity (log P), charge, pKa, polymorphism, chemical state, and physical state. The third factor includes the formulation parameters, for example, the pH of the formulation should be in the range of 4.5 – 6.5. The osmolarity of the formulation should be 200 to 500 mOsm/kg. The drug concentration should not exceed 5 mg/dose. The viscosity should be in the range of 3-6 cP. Finally, the applied volume of solution should be 25 – 200 μL per nostril (Illum 2002, Jadhav et al. 2007, Bitter et al. 2011, Menaka and Pandey 2014). The advantages of nasal spray include rapid onset, low dose, low systemic side effects, and avoidance of first-pass metabolism (Illum 2002, Ugwoke et al. 2005, Jadhav et al. 2007). Even though intranasal drug delivery offers many advantages, one of the limitation is the mucociliary clearance mechanism (MCC) (Illum 2002, Ugwoke et al. 2005, Jadhav et al. 2007, Touitou and Illum 2013, Gizurarson 2015). The formulation must prolong the residence time within the nasal cavity to enhance the bioavailability of the drug. To achieve this goal, the use of mucoadhesive polymer-based systems must demonstrate a prolonged contact time with the mucosa. In this work, the mucoadhesive polymers employed were hydroxypropyl cellulose (HPC) and carbomer940 (C940).

The objective of the study was to prepare MTS nasal sprays using HPC and C940 as the mucoadhesive agents. The formulations were characterized for their physicochemical properties and *in vitro* cytotoxicity on a nasal epithelial cell line as well as the opening of the tight junctions. It was expected that the formulations would not alter the tight junctions and transepithelial electrical resistance (TEER).

2.Materials and Methods

2.1 Chemicals and materials

Montelukast sodium was purchased from Sigma-Aldrich (Singapore). Hydroxypropyl cellulose (HPC) (Klucel® HF pharm grade, MW 1,150 kDa) was obtained from Bronson and Jacobs International Co., Ltd. (Bangkok, Thailand). Carbomer940 (C940, MW 1,044 kDa) was obtained from Sigma - Aldrich (Shanghai, China). Propylene glycol was purchased from S. Tong Chemicals Co., Ltd. (Bangkok, Thailand). Methanol and ethanol were from RCI Labscan Ltd., (Bangkok, Thailand). Disodium hydrogen orthophosphate dihydrate and triethanolamine (TEA) were obtained from Ajax Finechem Pty Ltd., (Bangkok, Thailand). Orthophosphoric acid was purchased from Merck KGaA (Darmstadt, Germany). Mucin from the porcine stomach type III, bound sialic acid 0.5 – 1.5% was purchased from Sigma-Aldrich (St. Louis, USA). Eagle's minimum essential medium (EMEM), phosphate buffered saline (PBS) solution, penicillin/streptomycin, and trypsin - EDTA solution were purchased from ATCC® (Virginia, USA). Fetal bovine serum (FBS) was purchased from Gibco® Thermo Fisher Scientific Inc. (New York, USA). Dialysis membrane (Cellu-Sep® H1 molecular cut-off 1 kDa) was obtained from Membrane Filtration Products, Inc. (Texas, USA). Nupore nylon-66 membrane disc filter - type HNN pore size 0.45 µm was from Nupore Filtration Systems Pvt. Ltd. (Ghaziabad, India). Distilled water was immediately filtered through a 0.22 µm cellulose acetate membrane filter before use. All chemicals and reagents were of analytical grad

2.2 Preparation of the mucoadhesive polymers

One gram of hydroxypropyl cellulose (HPC) was dissolved in 100 mL of distilled water and stirred continuously overnight at room temperature. Thereafter, the HPC solution was diluted to make different formulations (HPC: 0.005, 0.01, 0.05, 0.10, 0.30, and 0.50 %w/v). Carbomer940 (C940) solution was prepared by dispersing 0.5 g into 100 mL of distilled water to obtain a colloidal dispersion. The C940 colloidal solution was diluted to obtain various concentrations (C940: 0.005, 0.01, 0.05, 0.10, and 0.15 %w/v). After that, they were neutralized with 20µl/mL of TEA to obtain pH values of 6.0 to 6.5.

2.3 Preparation of the MTS nasal sprays

MTS (3.75 mg) was dissolved in 5 mL of the mixed ethanol-propylene glycol (2:3 volume ratio) to obtain a clear solution of MTS (Final concentration is 0.0375 %w/v). Afterwards, the MTS solution (5 mL) was slowly added to the mucoadhesive polymer solution (5 mL of either HPC or C940). The mixture was mixed continuously until the clear solution was obtained.

2.4 Evaluation of physicochemical properties

2.4.1 Clarity Test

The prepared formulations were visually examined for any foreign particles and were discarded if any foreign particles were present (Worthey 1985).

2.4.2 Determination of pH

The pH values of the MTS nasal spray formulations were measured using a pH meter (BP3001, Trans Instruments Ltd., Singapore).

2.4.3 Determination of hydrodynamic particle size and zeta potential

The hydrodynamic particle size and zeta potential of the formulations were measured using the Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK). The hydrodynamic particle size was measured based on the dynamic light scattering technique, while the zeta potential was calculated by determining the electrophoretic mobility. The measurements were performed at 25^{o}C. Before measurements of the hydrodynamic particle size and zeta potential of the samples were made, all solution samples were diluted 1:10 by volume with distilled water. The samples obtained were then filtered using a nylon membrane filter with a 0.45µm pore size.

2.4.4 Determination of viscosity

The rheological properties and the viscosity of the formulations were assessed using a Brookfield viscometer (Model DV-III, Brookfield Engineering Laboratories, Massachusetts, USA). The viscosities of the formulations were examined at a rotational speed of 250 rpm at 25°C, while the assessment of the flow behavior was performed at 25^{o}C with a shear rate of 17-85 s^{-1}.

2.4.5 Determination of surface tension and contact angle

The surface tension and contact angle were measured by drop shape analysis using a contact angle meter (OCA 15EC, DataPhysics Instruments GmbH, Filderstadt, Germany) to determine the spreadability of the formulations.

2.4.6 Texture analysis of the nasal spray formula

The method of Pardeshi et al. (2016) was modified to measure the mucoadhesion of the formulation systems (Pardeshi and Belgamwar 2016). The mucoadhesive property was measured using a texture analyzer (TA.XT plus Texture Analyser, Stable Micro Systems, Surrey, England). The load cell was set up with a capacity equivalent to 5 kg. The cylindrical probe (P/0.5R) was utilized in this experiment. The dialysis membrane, which was previously hydrated overnight with 0.5%w/v mucin, was attached to the upper probe of the instrument. The sample solution was kept below the probe. The upper probe was then lowered at a speed of 0.5 mm/s to touch the surface of the solution. A force of 6.0 N was applied for 20 sec, and the probe was lifted upwards at a similar rate (0.5 mm/s). The surface area of the exposed dialysis membrane was 1.13 cm^2. The mucoadhesive force (detachment stress, N) required to detach each polymer compact from membrane was considered as an index of adhesiveness. The area under the curve was calculated by multiplying force and distance resulting in work of adhesion. The greater value in the work of adhesion, the greater was the mucoadhesive property.

2.4.7 Droplet size and distribution analysis

To determine the potential *in vivo* deposition characteristics of the proposed nasal formulations, a multi-dose pump spray delivery system was used to characterize the formulation droplet size distributions using a laser diffraction technique. The Spraytec® (Malvern Instrument, Worcestershire, UK) system was used with a 300 mm lens (measuring range 0.1-900 µm). The droplet sizes of the MTS formulations were analyzed at room temperature. The nasal pump was vertically mounted 3 cm away

from the laser path, and a vacuum source was mounted anterior to the pump system. Before measurement, 5 doses were discharged to waste. Then 5 puffs were sprayed at two seconds per puff. Data were reported as volume diameters at 10%, 50%, and 90% of the cumulative undersized volumes. Span was calculated using the equation: Span = [(Dv90-Dv10)/Dv50].

2.5 In vitro release studies of the MTS nasal sprays

The in *vitro* drug release profile of the formulations was carried out in the Franz diffusion cell (Variomag Telesystem, Thermo Fisher Scientific Inc., Waltham, MA, USA). The MTS formulation was sprayed 3 puffs onto a nylon membrane (1.77 cm^2) and placed the membrane interfacing with the dissolution media (12 mL) in the lower chamber of the Franz diffusion cell. The donor compartments were placed on top of the receptor compartments and were clamped tightly. It is expected that the MTS will therefore release into the dissolution medium while the media is continuously stirred with magnetic bar (5 x 2 mm) at the speed of 400 rpm. The dissolution media was adapted from the US Pharmacopeia using 0.5% sodium dodecyl sulfate in water (US Pharmacopeia 2016). Franz cells were fixed on a preheated water bath (PolyScience, Niles, IL, USA) at 37°C. Syringes were rinsed three times before sampling to ensure homogeneity and to acquire an equal volume of preheated 0.5% sodium dodecyl sulfate solution for replacement after each sampling. One milliliter samples were withdrawn at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h from the receiver compartment and analyzed by high-performance liquid chromatography

(HPLC) (Thermo Fisher Scientific, San Jose, CA, USA). The receptor compartment was replaced with fresh medium after each sampling to allow sink condition.

2.6 Stability Assessment

All formulations were kept at room temperature for three months [12]. The stability determinations were made once a month until three months. The formulations that did not meet the specified criteria were excluded from further studies.

2.6.1 Physical stability evaluation

Physical stability of the MTS nasal spray formulations was observed for three months. Parameters included color change, drug, and other excipient precipitation, formulation miscibility, container and suitable spray performance. The formulations not meeting the specified criteria were excluded from further studies.

2.6.2 Chemical stability

2.6.2.1 High-performance liquid chromatography

The chemical stability of the formulations was analyzed using HPLC. The separation was done using an octadecylsilane column (C18). The mobile phase consisted of methanol: 10 mM disodium hydrogen orthophosphoric acid 85% (pH 6.5 adjusted with orthophosphoric acid) in 75:25 (v/v) with a flow rate of 1 mL/min at

ambient temperature. The UV detector was operated at 285 nm, and the injection volume was 20 μL (Ethiraj et al. 2011).

2.6.2.2 Mass spectrometry

A sample of each formulation was dissolved in acetonitrile and 0.05 M aqueous ammonium acetate (adjusted pH to 3.7 with acetic acid) in the ratio of 75:25.

The electrospray ionization and MS/MS studies were performed on an LC/MS/MS spectrometer (AB Sciex Instruments, Model API 3200, Ontario, Canada). The negative electrospray MS data were obtained at -4500 V. The MS–MS data were generated with the collision energy ramping from 30 to 60 V in a nitrogen atmosphere (Saravanan et al. 2008).

2.7 Evaluation of toxicity of nasal epithelium cells

2.7.1 Culture of human nasal epithelial cells

The human nasal epithelial cell line (RPMI 2650; ATCC, Virginia, USA) was used for toxicity evaluation. The cells were cultured in complete growth medium (EMEM: Eagle's Minimum Essential Medium) supplemented with 10% fetal bovine serum (FBS), 50 units/mL of penicillin, and 50 units/mL of streptomycin (100 U/mL penicillin/streptomycin). The RPMI 2650 was incubated at 37ºC in a fully humidified atmosphere of 5% CO_2 in the air. The medium was changed every day, and the cells were cultured for one week. When the cells reached 70-80% confluence, they were rinsed with phosphate buffered saline (PBS) solution. The PBS was aspirated, and the cells were detached from the plate by 0.25% trypsin - EDTA solution followed by the addition of fresh culture medium to create a new single cell suspension for further incubation. Detached cells were centrifuged, resuspended, and transferred into a new culture flask (Kreft et al. 2015, Pozzoli et al. 2016).

2.7.2 Determination of cytotoxicity

Cell number and viability were determined by MTT assay. The cell suspension was diluted to $1x10^5$ cells/well and added to a 96-well plate (Corning® Costar,

Corning, New York, USA), 100 µL per well or 10,000 cell per well. The medium was prepared by spraying 20 doses of the MTS solution onto the blank well and waited for 60 min until the solvent completely evaporated. The residue on the well was adjusted with 1 mL of the medium to obtain final concentration at 300 µg/mL. Thereafter, the MTS in the medium was diluted to a concentration of MTS 10 to 200 µg/mL. Other samples were C940 10 and MTS formulation. All samples (100 µL) were replaced the culture medium in the 96-well plate containing RPMI 10,000 cell per well. Cells without a sample served as a negative control. After incubation for 24 h, methyl thiazol tetrazolium (MTT) assay was performed to evaluate cell viability. Briefly, the cells were treated with 100 µL of fresh media along with 50 µL of MTT solution and incubated at 37^{o}C under 5% CO_2 for 4 h. Thereafter, media containing MTT was removed, and 100 µL of dimethyl sulfoxide was added. The quantity of formazan (directly proportional to the number of viable cells) was measured by recording the changes in absorbance at 570 nm using a microplate reader (Biohit 830, Biohit Healthcare Ltd, Helsinki, Finland). Viable cells with an active metabolism would convert MTT into a purple colored formazan product which has an absorbance maximum at 570 nm (Riss et al. 2016). The percentage of cell proliferation was calculated and compared to a negative control.

2.8 TEER measurement and permeability studies across nasal epithelial cells

2.8.1 TEER measurement

The human nasal epithelial cells (RPMI 2650) were seeded into a 6 transwell plate (Corning Costar, Corning, New York, USA) with polycarbonate translucent

membrane. The membrane pore size was 0.4 µm, membrane thickness 10 µm, cell growth area is 4.67 cm^2 and nominal pore density is $1x10^8$ pores per cm^2. The cells were seeded at a density of $1x10^6$ cells/well and cultured in EMEM. The cells were incubated at 5% CO_2 at 37^oC for 1 week with the culture medium changed daily. Transepithelial electrical resistance (TEER) of the cell monolayer was measured using the epithelial voltohmmeter (Merck KGaA, Darmstadt, Germany) every day for 1 week (Kreft et al. 2015). When the TEER value was constant for 2 days, the experiment was performed by adding 2.5 mL of the medium to the basolateral compartment. 2 mL of either medium with MTS solution or suitable formulation to the apical compartment. The cells were then incubated, and the TEER measurement was performed every 3 h until 24 h in a laminar airflow hood. Sample solution (500 µL) from the basolateral compartment was taken and replaced with 500 µL of cell culture medium every 3 h followed by incubation. The sampling solutions was analyzed to determine the MTS by the HPLC.

2.8.2 Sample preparation for HPLC analysis

The sample solution from section 2.8.1 was subjected to liquid-liquid extraction. MTS was extracted the sample (500 µL) with ethyl acetate 1 mL and the solution was vortexed for 10 sec. After that the clear solution was pipetted. Nitrogen gas was used to evaporate the ethyl acetate and final volume was adjusted to 2 mL with mobile phase. The quantity of MTS was analyzed by HPLC

2.9. Statistical analysis

All experiments were performed in triplicate and the data were represented as mean ± SD. Statistical analysis was done using a Student's t-test with GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, www.graphpad.com) and a significance level (*p-value*) of *<0.05* was considered as statistically significant.

3. Results and Discussion

3.1 Improvement of MTS solubility

MTS is an acidic drug and categorized as BCS class II (Okumu et al. 2008). To dissolve MTS clearly, a co-solvent, which has a similar dielectric constant to MTS, can be employed. Therefore, ethanol and propylene glycol were used as cosolvents, where the dielectric constant of MTS is 13. Moreover, the advantage of propylene glycol is that it acts as a preservative in concentrations of 15-30% (Rowe et al. 2006). All formulations of the prepared nasal sprays were inspected visually with black and white backgrounds. The formulations were clear and without foreign particles.

3.2 pH of the MTS nasal sprays

The pH values of the HPC and C940 formulations are shown in Table 2. The pH values of all formulations were kept in the range of 6.5-7.5 since the ideal suitable range is 4.5-7.5. The pH values of the C940 formulations were significantly lower than the HPC formulations ($p<0.05$) because the C940 formulations were neutralized

using the proper amount of TEA to obtain a greater viscosity of the carbomer (Rowe et al. 2006). Nevertheless, the pH of the formulations with C940 could be controlled in a suitable range for the nasal spray. The viscosity of the formulation with HPC depended only on the concentration of the polymer.

3.3 Viscosity of the MTS nasal sprays

The flow behavior of the formulations with 0.05% HPC and 0.05% C940 depicted a linear relation between shear stress (N/m^2) and shear rate (s^{-1}) and categorized as "Newtonian" flow (Figure 1). At a particular temperature, the samples of both polymers had a constant viscosity for both shear rates.

The viscosities of the HPC and C940 formulations were in the range of 6 – 320 and 6 – 120 cP, respectively (Table 1). The acceptable limit is 3 – 6 cP (Menaka and Pandey 2014). Therefore, the formulations with acceptable viscosities were HPC 5, HPC 10, HPC 50, C940 5, and C940 10.

3.4 Particle hydrodynamic size and zeta potential of the MTS nasal sprays

The hydrodynamic size of the drug without the polymer was 53 nm with a polydispersity index of 0.126 indicating a monodisperse system. The polymer formulations gave larger hydrodynamic sizes compared with the pure drug particle. The HPC formulations had a slightly smaller particle size than the C940, but at the same concentrations, they were not significantly different (Table 1).

The zeta potential of the MTS without polymer was -28.5 mV. The zeta potentials of the HPC formulations were in the range of -25 to -3 mV. The more negative zeta potentials were found in the formulations which contained lower

quantities of HPC. Moreover, the zeta potentials of the samples of HPC formulations were different from the C940 formulations at the same concentrations (Table 1). The zeta potentials of the C940 formulations ranged from -45 to -30 mV and they seemed more negative than -30 mV in the higher C940 concentrations.

3.5 Surface tension and contact angle of the MTS nasal sprays

For nasal spray formulation development, a cellulose membrane was used to mimic nasal epithelial cells in contact angle measurement (Tas et al. 2003). The spreadability of the polymer solutions was observed as it could increase the contact area in the nasal cavity. The contact angle of the solution without the polymers was 67^{o}, whereas the solutions containing the 0.005-0.05% HPC or C940 had a contact angle in a range of 0-30^{o} and they were categorized as having good spreadability (Yuan and Lee 2013). The other solutions containing concentrations of 0.15%, 0.30% and 0.50% polymers gave contact angles in the range of 31-90^{o}. Therefore, adding polymer in the formulations gave a better spreadability than the solution without the polymers. The degree of contact angle was slightly different between the samples of HPC formulations and C940 formulations at the same concentrations (Table 1). The surface tension value of the solution without the polymers was 35.48 $mN{\cdot}m^{-1}$. Table 1 shows the surface tension of all formulations. The cohesive forces of the formulations were dependent on the polymer concentration. A greater surface tension value resulted in a greater contact angle but less spreadability.

3.6 Droplet size and distribution of the MTS nasal sprays

The droplet size of the nasal spray gradually increased with the polymer concentration. The droplet volume mean diameter obtained from the formulations with C940 were significantly smaller than the HPC formulations ($p<0.05$) when compared with the same concentration. To ensure retention of the drug within the nasal passages, the cut-off points for the droplet sizes are greater than 10 µm but less than 100 µm (Aurora 2002, Ghori et al. 2015). The formulations with 0.005% and 0.01% w/v HPC and the formulations with 0.005%, 0.01%, 0.05% and 0.10% w/v C940 demonstrated diameters in the range of the criteria (Table 2). It was observed that the size of the droplets relied on the contact angle of the formulation. The higher polymer concentrations provided greater cohesive forces which gave larger droplet sizes.

3.7 In vitro mucoadhesion

Table 3 shows the adhesive forces between the formulations and mucin as work of adhesion. Overall, the results showed that the work of adhesion of the formulations with mucoadhesive polymers gave dramatically higher adhesions than the solution without polymer. However, there were no significant differences between each formulation on the work of adhesion.

3.8 Stability of the MTS nasal sprays

Optimized formulations were assessed by batches that were prepared and kept in clear bottles. It was observed that HPC precipitated at temperatures above 40^{o}C.

Therefore, the stability test of all formulations was carried out at room temperature for three months. All formulations were stored at 25^{o}C. After one month, the solutions precipitated as yellow crystals. The precipitation occurred in all samples of the solution containing HPC. Similar circumstances also occurred in the samples containing C940 and even the zeta potential was less than -30 mV. Surprisingly, no precipitation occurred in the formulation containing 0.01% w/v C940. Table 4 shows the % drug content in all of the formulations. The formulation containing 0.01% w/v C940 had a drug content of 95.50% on average. Therefore, a lower concentrated formulation could produce an appropriate zeta potential for the hydrodynamic size in the proper viscous media. In conclusion, only the formulation of 0.01% w/v C940 demonstrated good stability when stored at 25^{o}C for three months. In addition, the yellow crystals were further analyzed by MS-MS spectrometry using 1 µg/mL of MTS as the standard. Figure 2 shows the MTS spectra. The molecular weight was about 584 Da and the highest peak was the yellow crystal peak which was 584 Da but some other peaks were degradation products of MTS, such as the molecular weight of 600 Da which was montelukast sulfoxide (Al Omari et al. 2007, Saravanan et al. 2008). From overall physicochemical properties evaluation, the formulation with 0.01% w/v C940 had good physicochemical properties and was also stable. Therefore, this formulation was chosen for further investigation including MTS releasing profile and *in vitro* evaluation.

3.9 In vitro drug release of the MTS nasal sprays

Figure 3 shows the release profiles of the solution without the polymers. The nasal spray formulations with 0.01% w/v HPC and 0.01% w/v C940 were stored at 37°C for 24 h in 0.5% sodium dodecyl sulfate (Okumu et al. 2008). The release rate of the solution without the polymers and the solution with 0.01% w/v HPC gradually increased until it reached the maximum at 24 h, while a small dose of the MTS formulation with 0.01% w/v C940 released continuously until the maximum concentration was achieved at 3 h. The % cumulative drug release was then obtained as 44.6, 41.8 and 50.1, respectively.

3.10 Effect of MTS nasal spray formulation on the nasal epithelial cells

In this study, the human nasal epithelial cell (RPMI 2650) line was used as a model of the progressive formation of the cellular toxicity, the effect of formulation on the tight junction and drug permeation. As with the cytotoxicity test, Figure 4 shows the safety of the different concentrations of MTS on the nasal epithelial cells and the formulation with 0.01% w/v C940. The results showed that the cellular viability of the samples was comparable to the control (95-109%). Therefore, the MTS 15 µg and the formulation with 0.01% w/v C940 were chosen to studied the alternation of transepithelial electrical resistance (TEER) of the nasal epithelial cells. Figure 5 provides the % transepithelial electrical resistance (%TEER) of the RPMI 2650 cell monolayers incubated with different samples including the control, the polymeric solution, MTS solution, and the formulation as a function of time. The %TEER value of all samples from 0 h to 24 h were significantly higher than the

control ($p<0.05$) which indicated no interference of the formulation with the tight junction. Only a small amount of MTS permeation could be detected in the basolateral compartment (Figure 6). The addition of C940 in the formulation showed significantly slower drug permeation into the basolateral compartment compared to MTS without the C940 ($p<0.05$). It was assumed that using the C940 would retain the drug at the nasal epithelial cell longer than without the C940.

4. Discussion

The evaluation of nasal sprays consists of many formulation parameters. The suitable pH for nasal sprays is 4 – 7.5 because the pH range of the human nasal mucosa is 5.5 – 6.5 which is slightly acidic (England et al. 1999, Washington et al. 2000). The adjustment of the pH is to avoid nasal irritation and obtain drug absorption. Because drugs are absorbed well in unionized forms and to prevent the growth of pathogenic bacteria in the nasal cavity, the lysozymes found in nasal secretions are destroyed by certain bacteria at an acidic pH, whereas in the alkaline conditions lysozymes are not activated, and the nasal tissue is susceptible to microbial infection (Behl et al. 1998, Patil et al. 2012). In this study, the physicochemical properties of the formulations were dependent on the polymer concentrations. The sizes of the particles in the formulation solutions were in the range of nanometers. Since the drug could dissolve in the co-solvent, the detected particles were colloids of polymer which covered the drug in the formulation. The zeta potential measurement was used to predict the dispersion stability. The particles showed stable dispersion and stable system with less chance of aggregation or precipitation when the particles

carried more negative charges (-30 mV) or positive charges (+30 mV) (Salopek et al. 1992). MTS is an acidic drug, which has a negative charge after dissolution. Moreover, ethanol and propylene glycol also show negative charges as well. The zeta potentials of the HPC formulations tended to be higher as the concentrations of the polymer increased. This indicated that since HPC is a nonionic polymer, the drug molecules were covered by the polymer leading to a decrease in the zeta potential compared with the solution without polymer (Figure 7a) (Rowe et al. 2006). On the other hand, the C940 is an anionic polymer. The polymer had covered the drug and promoted the negative charge of the system. Its zeta potential was lower than -30 mV when the concentration of C940 was higher (Figure 7b) (Rowe et al. 2006). Therefore, the formulations with C940 had more impulsive interaction between each particle than the HPC formulations. In brief, the solution containing C940 was found to be more stable with a lower chance of precipitation than the HPC formulations according to their zeta potential. The surface tensions of the formulations were low suggesting good spreadability when applied to the nasal epithelial cells (Zheng 2010). Moreover, easy spraying and providing fine droplets could be obtained in the formulation that had less surface tension and cohesive force. The formulation which had a low polymer concentration obtained good spreadability and increased surface area for drug absorption on the nasal epithelial cell. The viscosity of the formulations was raised when the polymer concentrations increased. The advantages from the viscosity of the polymer include a prolonged residence time of the drug in the nasal cavity for sustained release of the drug. A greater viscosity impacts the droplet size distribution which result in an altered deposition in the nasal cavity. Mucoadhesion is defined as a

state, where one biological material adheres to another material to prolong the contact time. The work of adhesion rose gradually as the concentration of polymers in solution increased. Since the HPC and C940 have hydroxyl and carboxyl groups, respectively, these chemical structures adhered to the tissue by using the net result of the forces including hydrogen bonding, hydrophobic bonding and van der Waal's forces between two surfaces. Mucoadhesion may be affected by a number of factors, including hydrophilicity, molecular weight, cross-linking, swelling, pH, and the concentration of the polymer (Rahamatullah et al. 2011). MTS is sensitive to light and the pH of the solution. The major photodegradation product of MTS is the formation of its cis-isomer, whereas the major degradation product from an acidic solution is montelukast sulfoxide (Al Omari et al. 2007). Therefore, MTS formulations should be kept in closed containers and protected from light to avoid an acidic condition. The TEER can be used as an indicator of the alternation of tight junctions. The TEER steadily increased with the density of the cells until day 7 when the TEER value was 122 x 4.2 cm^2 or 515 $\Omega.cm^2$ (1 x 10^6 cells/well seeding). This trend is similar to previously published data (Pozzoli et al. 2016). The formulation with 0.01% w/v C940 was chosen in this study because it passed the stability exam with the suitable physicochemical properties required for the nasal spray. When the TEER decreases it means the tight junction opened, but if the TEER value is the same as or higher than the control group, it indicates the tight junction did not open. However, some drugs could pass from the apical to the basolateral chamber of nasal epithelial cells.

5. Conclusion

In general, the route of localized nasal drug delivery can reduce the amount of drug entering the human body and also avoids systemic adverse effects of the drug. According to this work, the formulation with 0.01% w/v C940 was found to be an optimal montelukast nasal spray comprised of MTS 0.0375% w/v as an active ingredient, 0.01% w/v C940 as a mucoadhesive polymer, propylene glycol as preservative and co-solvent, ethanol as a co-solubilizer, and water. In addition, the selected formulation demonstrated good physicochemical properties as well as good adhesiveness and a good drug release profile. In other words, it was safe to nasal epithelial cells and did not alter the opening of the tight junction. It can be concluded that this montelukast spray can be an effective candidate for nasal administration to attain local drug delivery with reduced adverse effects and better patient compliance.

Acknowledgement

We would like to thank the Drug Delivery System Excellence Center and Nanotech-PSU Excellence Center on Drug Delivery System for the use of their facilities. This work was financially supported by a scholarship grant from the Discipline of Excellence in Pharmacy, phase 2, Faculty of Pharmaceutical Sciences and Graduate School of Prince of Songkla University.

Conflict of interest

The authors report no declarations of interest.

References

Al Omari, M. M., Zoubi, R. M., Hasan, E. I., Khader, T. Z. and Badwan, A. A. (2007). Effect of light and heat on the stability of montelukast in solution and in its solid state. J Pharmaceut Biomed 45(3): 465-471.

Appasaheb, P. S., Manohar, S. D., Bhanudas, S. R. and Anjaneri, N. (2013). A review on intranasal drug delivery system. Journal of Advanced Pharmacy Education & Research Oct Dec 3(4): 333 - 346.

Aurora, J. (2002). Development of nasal delivery systems: a review. Drug Deliv Technol 2(7): 1-8.

Behl, C. R., Pimplaskar, H. K., Sileno, A. P. and Romeo, V. D. (1998). Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv Drug Deliver Rev 29(1): 89-116.

Benninger, M. S. and Waters, H. (2009). Montelukast: pharmacology, safety, tolerability and efficacy. Clin Med Insights Ther 1: 1253-1261.

Bitter, C., Suter-Zimmermann, K. and Surber, C. (2011). Nasal drug delivery in humans. Topical Applications and the Mucosa, Karger Publishers. 40: 20-35.

Chaturvedi, M., Kumar, M. and Pathak, K. (2011). A review on mucoadhesive polymer used in nasal drug delivery system. Journal of advanced pharmaceutical technology &research 2(4): 215.

England, R. J. A., Homer, J. J., Knight, L. C. and Ell, S. R. (1999). Nasal pH measurement: a reliable and repeatable parameter. Clin Otolaryngol 24(1): 67-68.

Ethiraj, T., Revathi, R., Thenmozhi, P., Saravanan, V. S. and Ganesan, V. (2011).

High performance liquid chromatographic method development for simultaneous analysis of doxofylline and montelukast sodium in a combined form. Pharm Methods 2(4): 223-228.

Fini, A., Bergamante, V. and Ceschel, G. C. (2011). Mucoadhesive gels designed for the controlled release of chlorhexidine in the oral cavity. Pharmaceutics 3(4): 665-679.

Ghori, M. U., Mahdi, M. H., Smith, A. M. and Conway, B. R. (2015). Nasal drug delivery systems: an overview. AJPS 3(5): 110-119.

Gizurarson, S. (2015). The effect of cilia and the mucociliary clearance on successful drug delivery. Biol Pharm Bull 38(4): 497-506.

Illum, L. (2002). Nasal drug delivery: new developments and strategies. Drug Discov Today 7(23): 1184-1189.

Jadhav, K. R., Gambhire, M. N., Shaikh, I. M., Kadam, V. J. and Pisal, S. S. (2007). Nasal drug delivery system-factors affecting and applications. Curr Drug ther 2(1): 27-38.

Khairnar, G. and Sayyad, F. (2010). Development of buccal drug delivery system based on mucoadhesive polymers. International Journal of Pharm Tech Research 2(1): 719-735.

Knorr, B., Holland, S., Rogers, J. D., Nguyen, H. H. and Reiss, T. F. (2000). Montelukast adult (10-mg film-coated tablet) and pediatric (5-mg chewable tablet) dose selections. J Allergy Clin Immun 106(3): S171-S178.

Kreft, M. E., Jerman, U. D., Lasič, E., Rižner, T. L., Hevir-Kene, N., Peternel, L. and Kristan, K. (2015). The characterization of the human nasal epithelial cell line

RPMI 2650 under different culture conditions and their optimization for an appropriate in vitro nasal model. Pharmaceut Res 32(2): 665-679.

Lagos, J. A. and Marshall, G. D. (2007). Montelukast in the management of allergic rhinitis. Ther Clin Risk Manag 3(2): 327.

Menaka, M. and Pandey, V. P. (2014). Formulation development and evaluation of cinnarizine nasal spray. JPHS 2(4): 339-346.

Min, Y.-G. (2010). The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy, asthma & immunology research 2(2): 65-76.

Okumu, A., DiMaso, M. and Löbenberg, R. (2008). Dynamic dissolution testing to establish *in vitro/in vivo* correlations for montelukast sodium, a poorly soluble drug. Pharm. Res 25(12): 2778-2785.

Okumu, A., DiMaso, M. and Löbenberg, R. (2008). Dynamic dissolution testing to establish *in vitro/in vivo* correlations for montelukast sodium, a poorly soluble drug. Pharm. Res 25(12): 2778-2785.

Pardeshi, C. V. and Belgamwar, V. S. (2016). Controlled synthesis of N, N, N-trimethyl chitosan for modulated bioadhesion and nasal membrane permeability. Int J Biol Macromol 82: 933-944.

Patil-Gadhe, A., Kyadarkunte, A., Patole, M. and Pokharkar, V. (2014). Montelukast-loaded nanostructured lipid carriers: part II pulmonary drug delivery and in vitro–in vivo aerosol performance. Eur J Pharm Biopharm 88(1): 169-177.

Patil-Gadhe, A. and Pokharkar, V. (2014). Montelukast-loaded nanostructured lipid carriers: part I oral bioavailability improvement. Eur J Pharm Biopharm 88(1): 160-168.

Patil, V. B., Kalkotwar, R. S., Patel, A., Tathe, S. and Jadhav, V. B. (2012). Evaluation and quality control of nasal spray. JDDT 1(2): 1-4.

Pawankar, R., Mori, S., Ozu, C. and Kimura, S. (2011). Overview on the pathomechanisms of allergic rhinitis. Asia Pacific Allergy 1(3): 157-167.

Peters-Golden, M., Gleason, M. and Togias, A. (2006). Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. Clinical & Experimental Allergy 36(6): 689-703.

Pharmacopeia, U. (2016). USP 39-NF 34.

Philip, G., Malmstrom, K., Hampel, F. C., Weinstein, S. F., LaForce, C. F., Ratner, P. H., Malice, M. P. and Reiss, T. F. (2009). Montelukast for treating seasonal allergic rhinitis: a randomized, double-blind, placebo-controlled trial performed in the spring. Clin Exp Allergy 39(10): 1622-1622.

Pozzoli, M., Ong, H. X., Morgan, L., Sukkar, M., Traini, D., Young, P. M. and Sonvico, F. (2016). Application of RPMI 2650 nasal cell model to a 3D printed apparatus for the testing of drug deposition and permeation of nasal products. Eur J Pharm Biopharm 107: 223-233.

Rahamatullah, S., Thakur, R. R. S., Garland, M. J., Woolfson, A. D. and Donnelly, R. F. (2011). Mucoadhesive drug delivery systems. J Pharm Bioallied Sci 3(1): 89.

Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J. and Minor, L. (2016). Cell viability assays.

Rowe, R. C., Sheskey, P. J. and Weller, P. J. (2006). Handbook of pharmaceutical excipients.

Salopek, B., Krasić, D. and Filipović, S. (1992). Measurement and application of zeta-potential, Rudarsko-geološko-naftni fakultet.

Saravanan, M., Reddy, P. P., Naidu, M. N., Babu, J. M., Srivastava, A. K., Kumar, T. L., Sekhar, B. V. V. N. C. and Satyanarayana, B. (2008). Identification, synthesis, isolation and spectral characterization of potential impurities of montelukast sodium. J
Pharmaceut Biomed 48(3): 708-715.

Suzuki, Y. and Makino, Y. (1999). Mucosal drug delivery using cellulose derivatives as a functional polymer. Journal of controlled release 62(1): 101-107.

Tas, C., Özkan, Y., Savaser, A. and Baykara, T. (2003). In vitro release studies of chlorpheniramine maleate from gels prepared by different cellulose derivatives. Farmaco Sci 58(8): 605-611.

Touitou, E. and Illum, L. (2013). Nasal drug delivery. Drug Deliv Transl Res: 1-3.

Ugwoke, M. I., Agu, R. U., Verbeke, N. and Kinget, R. (2005). Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives. Adv Drug Deliver Rev 57(11): 1640-1665.

Washington, N., Steele, R. J. C., Jackson, S. J., Bush, D., Mason, J., Gill, D. A., Pitt, K. and Rawlins, D. A. (2000). Determination of baseline human nasal pH and the effect of intranasally administered buffers. Int J Pharm 198(2): 139-146.

Worthey, J. A. (1985). Visual Clarity with a Black-and-white Scene. J Illum Eng Soc 14(2): 643-648.

Yuan, Y. and Lee, T. R. (2013). Contact angle and wetting properties. Surface science techniques, Springer: 3-34.

Zheng, W. (2010). Surface wetting characteristics of rubbed polyimide thin films, INTECH Open Access Publisher.

Table 1: Physical and chemical characterizations of the MTS nasal sprays (mean ± SD, n=3).

Formulation	pH	Particle size (nm)		Zeta potential (mV)*	Viscosity (cps)	Contact angle (degree)	Surface tension (mN/m)
		Z-average	PDI				
No polymer	7.2 ± 0.1	53.01	0.13	-28.50	5.7 ± 0.2	66.9 ± 1.3	0.5 ± 0.1
HPC 5	7.5 ± 0.1	70.57	0.14	-24.63	6.2 ± 0.0	23.7 ± 3.7	31.3 ± 0.7
HPC 10	7.5 ± 0.0	78.48	0.17	-21.37	6.7 ± 0.0	24.3 ± 2.3	32.6 ± 0.3
HPC 50	7.4 ± 0.0	80.52	0.38	-9.68	8.0 ± 0.0	29.0 ± 4.4	33.1 ± 0.1
HPC 100	7.4 ± 0.1	93.21	0.27	-6.33	11.4 ± 0.1	36.8 ± 3.1	34.5 ± 0.1
HPC 300	7.4 ± 0.0	96.77	0.23	-5.09	46.8 ± 0.1	42.8 ± 3.5	35.3 ± 0.2
HPC 500	7.3 ± 0.0	100.09	0.38	-3.66	318.7 ± 1.3	43.5 ± 4.6	35.4 ± 0.6
C940 5	6.8 ± 0.0	84.25	0.15	-32.37	6.8 ± 0.0	17.6 ± 1.8	33.2 ± 0.5
C940 10	6.7 ± 0.0	94.02	0.16	-37.80	7.3 ± 0.0	20.9 ± 1.4	34.0 ± 0.5
C940 50	6.6 ± 0.1	100.37	0.14	-44.30	18.2 ± 0.1	27.0 ± 2.9	34.4 ± 0.3
C940 100	6.6 ± 0.0	103.14	0.31	-43.97	115.1 ± 0.9	31.8 ± 0.9	35.5 ± 0.1
C940 150	6.5 ± 0.0	267.17	0.64	-43.30	NA	34.5 ± 0.3	37.8 ± 0.8

* Indicates significant difference between HPC and C940 formulations $p<0.05$, NA is not applicable.

Table 2. Laser diffraction particle size analysis of montelukast nasal sprays (mean ± SD, n=3).

	Diameter (µm)				
Polymer concentration (%w/v)	**Dv 10**	**Dv 50**	**Dv 90**	**VMD**	**Span**
0	43.5 ± 4.4	87.7 ± 4.8	135.3 ± 0.3	88.8 ± 3.2	1.1 ± 0.1
HPC 5	35.4 ± 1.7	61.7 ± 0.7	205.7 ± 0.6	100.9 ± 1.0	1.2 ± 0.2
HPC 10	42.1 ± 1.1	94.7 ± 1.9	213.2 ± 0.3	116.5 ± 0.9	1.7 ± 0.1
HPC 50	133.7 ± 2.9	279.8 ± 0.2	557.2 ± 2.1	323.5 ± 1.7	1.2 ± 0.3
HPC 100	307.7 ± 2.4	557.5 ± 1.1	762.6 ± 1.6	542.6 ± 2.1	0.8 ± 0.1
HPC 300	324.8 ± 0.6	582.3 ± 1.1	827.3 ± 1.6	578.1 ± 1.1	0.5 ± 0.4
C 940 5	35.0 ± 0.1	71.8 ± 0.3	115.4 ± 0.1	74.1 ± 0.2	1.1 ± 0.1
C 940 10	38.8 ± 2.0	87.8 ± 0.5	143.0 ± 0.5	89.8 ± 1.0	0.9 ± 0.2
C 940 50	48.9 ± 2.7	92.2 ± 2.6	153.0 ± 0.6	98.0 ± 2.0	1.1 ± 0.2
C 940 100	65.8 ± 2.9	98.6 ± 1.1	155.8 ± 1.8	106.7 ± 1.9	1.1 ± 0.0
C 940 150	81.8 ± 0.9	108.7 ± 0.6	172.5 ± 1.1	121.0 ± 0.8	0.9 ± 0.1

Dv10 is the 10% of the cumulative undersized (volume) fraction; Dv50 is the 50% of the cumulative undersized (volume) fraction; Dv90 is the 90% of the cumulative undersized (volume) fraction; VMD is the volume mean diameter; and Span refers to relative span (Dv90-Dv10)/Dv50.

Table 3. Texture profiles of MTS nasal sprays (mean ± SD, n=3).

Polymer concentration (%w/v)	Work of adhesion (µJ)	
	Hydroxypropyl cellulose	**Carbomer940**
0	0.5 ± 0.1	0.5 ± 0.1
0.005	0.9 ± 0.1†	0.2 ± 0.1
0.01	0.9 ± 0.1†	1.0 ± 0.2†
0.05	1.1 ± 0.1†	3.6 ± 0.4†
0.10	1.3 ± 0.4†	8.1 ± 0.9†
0.15	NA	27.1 ± 0.8†
0.30	1.8 ± 0.6†	NA
0.50	3.9 ± 0.7†	NA

†Indicates significant difference between the solution without polymers and the formulation with HPC or C940 $p<0.05$**.** NA is not applicable**.**

Table 4. %Drug content and Stability (mean ± SD, n=3).

Month	0	1	2	3
HPC 5	92.7 ± 1.5	70.4 ± 0.6	67.3 ± 0.8	60.9 ± 0.8
HPC 10	91.9 ± 0.4	86.39 ± 0.9	81.4 ± 0.4	75.5 ± 0.6
HPC 50	90.3 ± 0.5	75.7 ± 0.6	72.3 ± 2.9	70.6 ± 1.6
HPC 100	91.7 ± 0.9	85.9 ± 0.8	82.9 ± 3.3	78.5 ± 2.5
HPC 300	91.1 ± 1.4	87.7 ± 1.4	80.0 ± 2.3	75.1 ± 1.7
HPC 500	91.8 ± 0.5	60.6 ± 0.5	50.7 ± 0.5	48.6 ± 0.7
C940 5	97.2 ± 3.7	93.3 ± 1.5	91.9 ± 0.4	89.8 ± 0.6
C940 10	97.9 ± 2.9	96.9 ± 0.8	95.2 ± 2.9	94.8 ± 1.8
C940 50	92.7 ± 2.6	90.6 ± 2.5	91.8 ± 1.9	80.7 ± 1.4
C940 100	86.7 ± 4.2	80.7 ± 1.5	74.7 ± 1. 0	69.8 ± 1.2
C940 150	84.5 ± 0.1	78. 5 ± 0.8	71.37 ± 0.18	60.8 ± 0.4

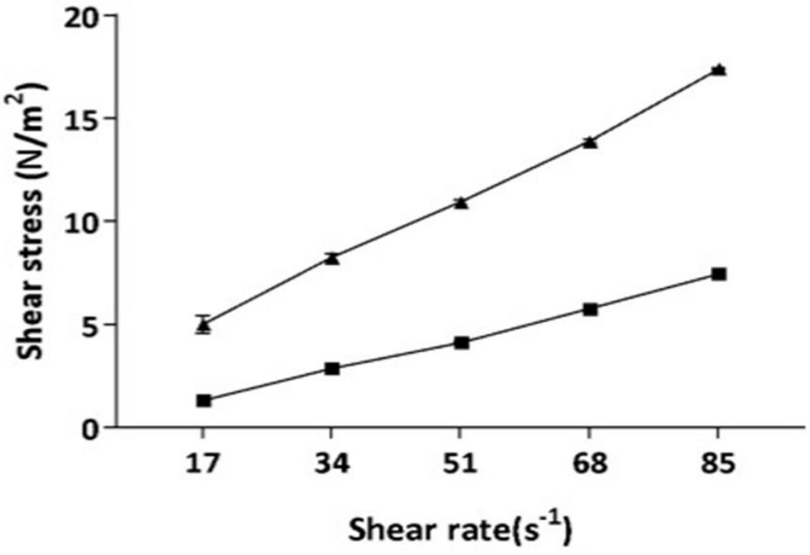


Figure 1. Rheologic analysis of the MTS nasal sprays with 0.05% w/v HPC (■) and 0.05% w/v C940 (▲), (mean ± SD, n=3).

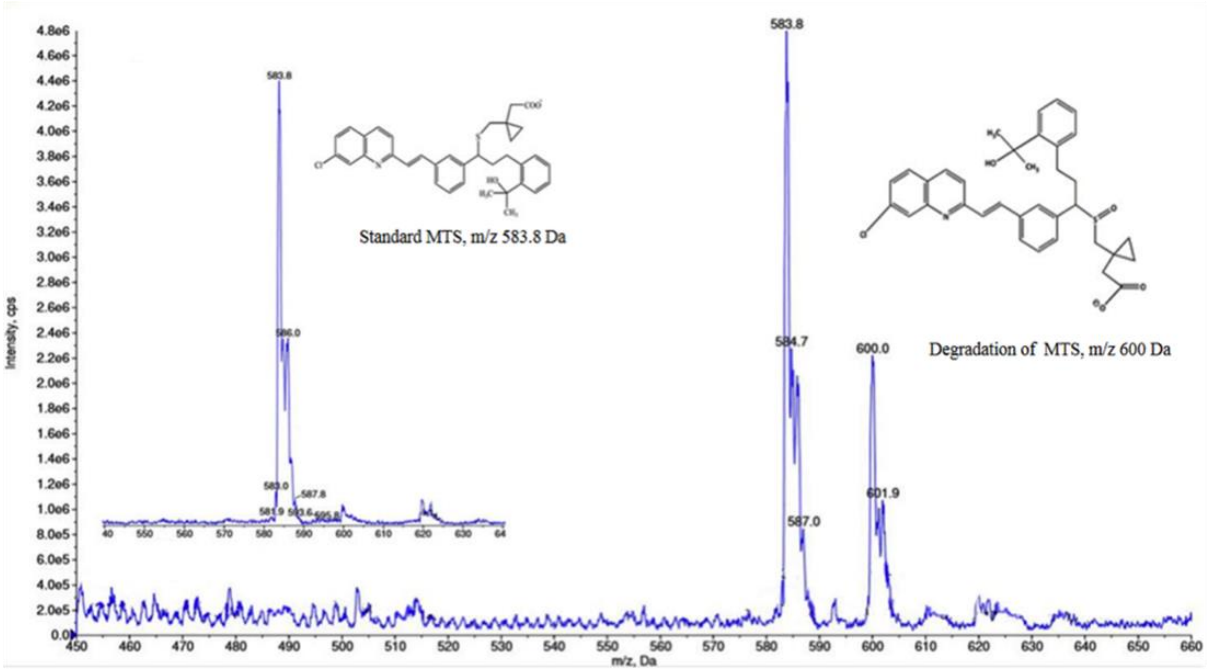


Figure 2. MS-MS spectra of standard MTS at concentration 1µg/mL and crystal from MTS formulation.

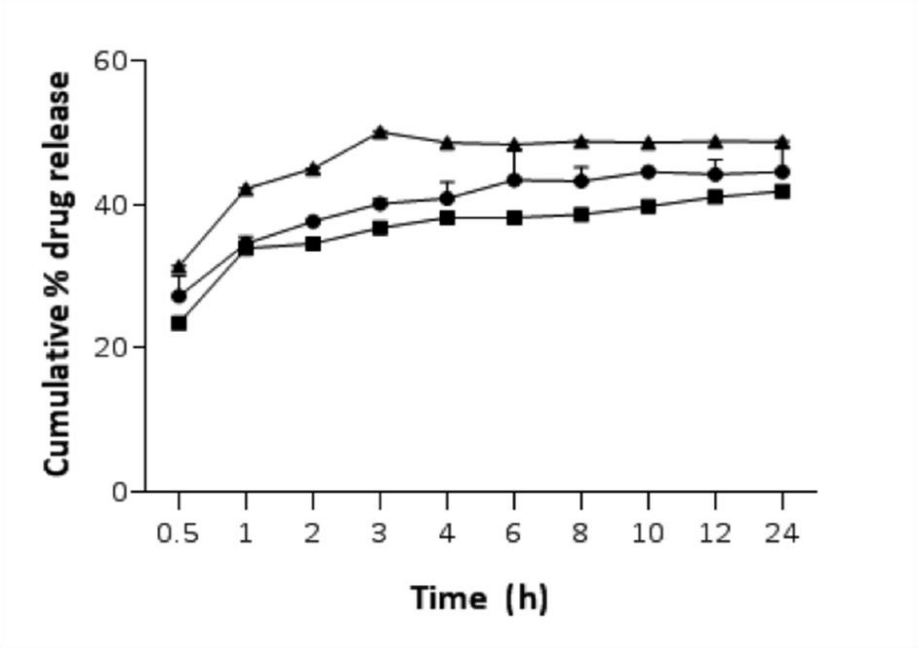


Figure 3. % Cumulative drug release using the MTS solution (●), the formulation containing 0.01% w/v HPC (■) and the formulation containing 0.01% w/v C940 (▲), (mean ± SD, n=3).

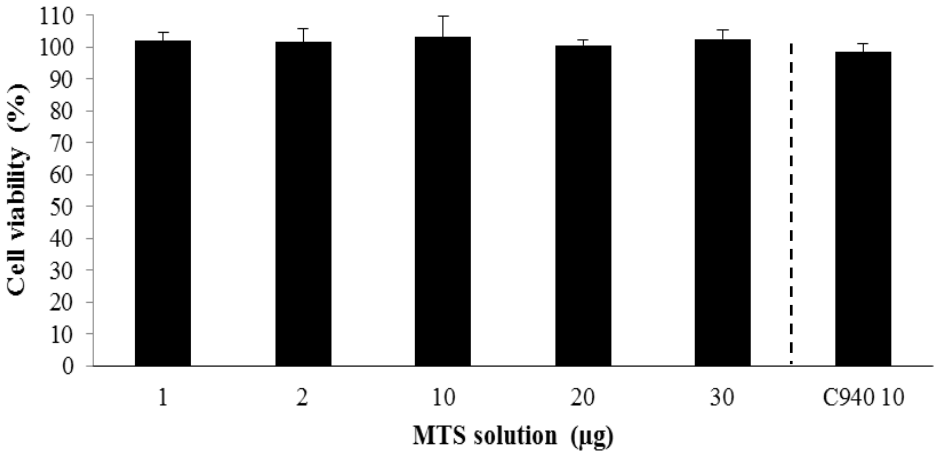


Figure 4. Nasal epithelium cell viability incubated with the dose of 1 – 30 ug MTS and the formulation C940 10 on RPMI 2650 cells, (mean ± SD, n=3**)**.

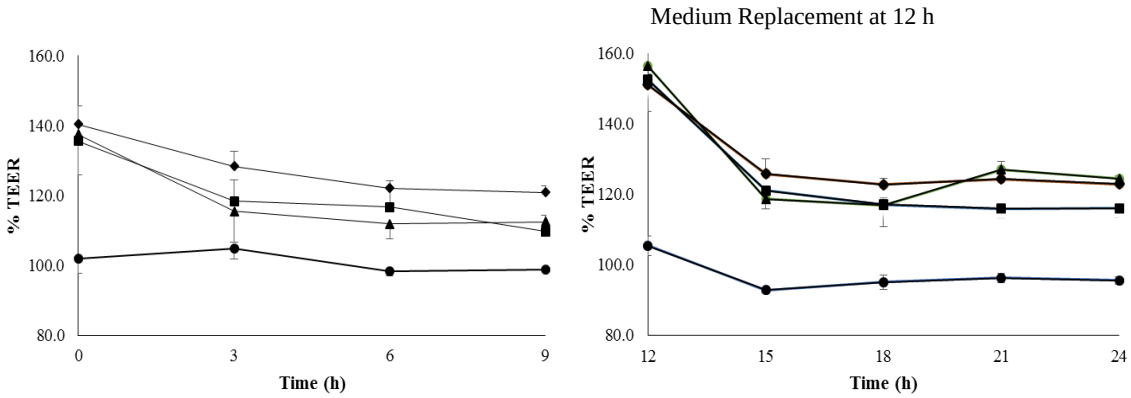


Figure 5. % Transepithelial electrical resistance (TEER) of RPMI 2650 cells cultured in the medium with the formulation containing 0.01% w/v C940 (◆), MTS solution (■), the solution without MTS (▲) and control (●) using LLI conditions 12 h, (mean ± SD; n = 3).

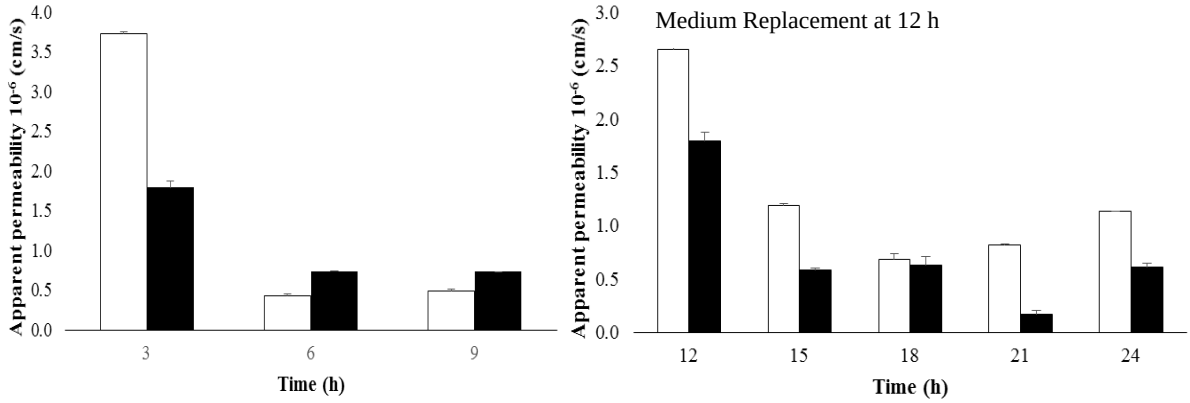
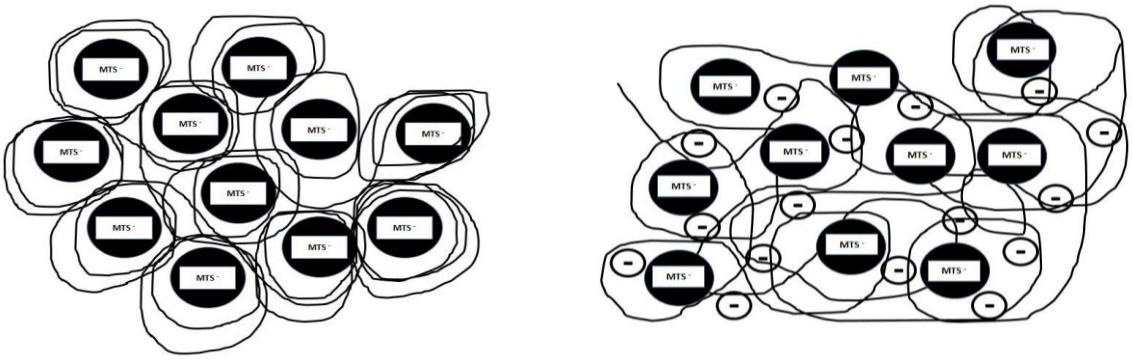


Figure 6. Comparison between permeability of MTS solution and the formulation containing 0.01% w/v C940 in a nasal epithelial cell monolayer. Apparent permeability (P_{app}) of MTS solution (□) and the formulation containing 0.01% w/v C940 (■) from apical to basolateral (AP-BL), (mean ± SD; n=3).



(a) **(b)**

Figure 7. Schematic of HPC-coated (a) and C940-coated (b) the MTS in nasal spray formulation.

Figures Legends

Figure 1 Rheologic analysis of the MTS nasal sprays with 0.05% w/v HPC (■) and 0.05% w/v C940 (▲), (mean ± SD, n=3).

Figure 2 MS-MS spectra of standard MTS at concentration 1µg/mL and crystal from MTS formulation.

Figure 3 % Cumulative drug release using MTS solution (●), the formulation containing 0.01 % w/v HPC (■) and the formulation containing 0.01% w/v C940 (▲), (mean ± SD, n=3).

Figure 4 Nasal epithelium cell viability incubated with the dose of 1 – 30 ug MTS and the formulation C940 10 on RPMI 2650 cells, (mean ± SD, n=3**)**.

Figure 5 % Transepithelial electrical resistance (TEER) of RPMI 2650 cells cultured in the medium with the formulation containing 0.01% w/v C940 (◆), MTS solution (■), the solution without MTS (▲) and control (●) using LLI conditions 12 h, (mean ± SD; n = 3).

Figure 6 Comparison between permeability of MTS solution and the formulation containing 0.01% w/v C940 in a nasal epithelial cell monolayer. Apparent permeability (P_{app}) of MTS solution (□) and the formulation containing 0.01% w/v C940 (■) from apical to basolateral (AP-BL), (mean ± SD; n=3).

Figure 7 Schematic of HPC-coated (a) and C940-coated (b) the MTS in nasal spray formulation.

Proceedings

Physico-chemical properties of formulated montelukast sodium nasal spray

Physico-chemical properties of montelukast sodium nasal spray formulation

Thunyaporn Jullaphant[1] and Teerapol Srichana[2]

Abstract

Montelukast sodium (MTS) is leukotriene receptor antagonist indicated for prophylaxis and treatment of asthma and is widely used treatment for systemic and local diseases of the upper respiratory tract. However, MTS has many adverse effects including cough, fever, bronchitis, agitation, aggression, anxiousness, hallucinations, depression, insomnia, restlessness, suicidal ideation and liver dysfunction. Therefore administration of MTS locally can result in significant reduction in dose and possibly avoid systemic side effects. In this study, we prepared MTS nasal spray formulation using hydroxypropyl cellulose (HPC) as a mucoadhesive agent from 0.03% to 0.5%w/v. The single dose of MTS was 240 ng. The prepared formulations were evaluated for their physicochemical properties including pH, viscosity, contact angle, droplet size and mucoadhesiveness. pH of all the formulations was in the range of 7 to 7.4. The viscosity was found to be 2 – 4 cPs which was suitable for sprays. The contact angle was in the range of 40 – 60^{o} indicating good wettability. The average droplet size was in the range of 60 – 80 μm which was suitable for nasal sprays. The mucoadhesive force was in the range 0.021 – 0.15 N.mm. Overall physicochemical properties of all formulations met the requirement for nasal drug delivery.

Key words: montelukast sodium hydroxypropyl cellulose nasal sprays

[1,2] Drug Delivery System Excellence Center (DDSEC), Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hatyai, Songkla 90112,Thailand. E-mail: Thunya.new387@gmail.com

Introduction

Allergic rhinitis (AR) is an inflammatory disease of the upper respiratory tract characterized by watery rhinorrhea, nasal itching, nasal obstruction and sneezing. Montelukast sodium (MTS) is the Leukotriene receptor antagonist used for relieving symptoms of AR. MTS binds with high affinity and selectivity to Cysteinyl leukotriene receptor 1(CysLT1) receptor and inhibits physiologic actions of Leukotrine D4 at the CysLT1 receptor without any agonist activity. However, MTS is associated with many systemic adverse effects including fever, agitation, aggression, anxiousness, hallucinations, depression, insomnia, restlessness, suicidal ideation and liver dysfunction [1]. Therefore administration of MTS locally can result in significant reduction in dose and possibly avoid systemic side effects. Nasal drug delivery is widely used treatment of systemic and local diseases of the upper respiratory tract. Although nasal delivery has many benefits [2], one of the limitations is the nasal mucociliary clearance (MCC) due to the presence of ciliated cells in the nasal epithelium cells used to transport substance in the nasal cavity [3-5]. The strategies to overcome the MCC is using the carrier materials with good mucoadhesive properties to modulate the MCC that enables prolonged contact of drug with the mucosa and increased drug absorption. The objective of this study was to prepare MTS nasal spray using hydroxypropyl cellulose (HPC) as a mucoadhesive

agent [2, 6]. The prepared formulations were evaluated for their physicochemical properties.

Materials and Methods

Materials

Montelukast sodium was purchased from Sigma - Aldrich, Singapore. Hydroxypropyl cellulose (KrucelTM) was obtained from Bronson and Jacobs International co. ltd., (Bangkok, Thailand). Potassium dihydrogen phosphate (KH_2PO_4) and sodium monohydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) were obtained from Ajax Finechem Pty Ltd (Bangkok, Thailand). All the chemical and reagents were of analytical grade and used as obtained.

Methods

Preparation of the polymer solution

The nasal spray formulations were prepared by varying the amount of HPC. HPC stock solution was prepared by dissolving 1 g of HPC in distilled water followed by stirring overnight at room temperature and filtering through cotton wool. The volume was adjusted to 100 mL with distilled water. HPC stock solution was diluted to obtain concentrations in the range of 0.03% – 0.5% w/v.

Preparation of the formulation

The dose of MTS was calculated as 0.240 μg/dose. MTS 1 mg was dissolved in phosphate buffer at pH 8 and was adjusted to 25 mL volumetric flask. Then the

solution 1.2 mL was poured slowly into the polymers solution 7.8 mL with continuous stirring to form the homogenous solution. The volume was adjusted to 10 mL with distilled water.

Evaluation of physicochemical properties

Physicochemical properties were determined to screen the appropriate formulations for nasal spray development. The pH of MTS nasal sprays was measured using the pH meter (FE20/FG, Mettler Toledo, Switzerland). The desired pH range is 5.5 – 8 [7, 8]. The viscosity of MTS nasal spray formulations was measured using Brookfield viscometer (model DV-III, Brookfield Engineering Laboratories, USA). The viscosity measurement was carried out with a rotational speed at 250 rpm. The temperature was controlled at 35 °C. The acceptable criteria is 1.2 – 5 cPs [9]. The contact angle was measured by drop shape analysis using contact angle meter (OCA 15 EC Data physics instruments GmbH, Germany) to determine the spreadability of formulations on the microscopic glass slide which is wrapped with dialysis membrane representing nasal epithelial cells. The acceptable criteria is between 30^{o} - to 50^{o} [10]**.** The mucoadhesive property was measured using texture analyzer (Stable Micro system TA-XT Plus Texture Analyzer Surrey, England). This parameter was determined by a method adopted by Pardeshi et al. (2016) with some modifications. The load cell was set with a capacity equivalent to 5 kg. The dialysis membrane previously hydrated overnight with 0.3% (w/v) mucin dispersion was attached to the upper probe of the

instrument and drop of sample solution was kept below that. The upper probe was then lowered at a speed of 0.1 mm/s to touch the surface of the solution. A force of 0.25 N was applied for 5 min and the probe was lifted upwards at a similar rate (0.1 mm/s). The surface area of exposed mucous membrane was 1.13 cm^2. The mucoadhesive force (detachment stress, mN) required to detach each polymer compact from membrane was considered as an index of mucoadhesiveness. The area under the curve was calculated by multiplying force (N) and distance resulting from the work of adhesion (N·mm) value [11]. Greater the value work of adhesion, greater was the mucoadhesive property. Droplet size of MTS formulations was analyzed at room temperature using the real-time laser diffraction technique (Spraytec®; Malvern Instrument, Worcestershire, UK). Prior to measure, 5 doses were discharged to waste. Then 5 puffs were sprayed as two seconds interval per puff. The desired range between 10 – 100 μm [12]. All the experiments were performed in triplicate and the data was as represented in mean ± SD. Statistical analysis was performed using Student t-test and significance was set as $p< 0.05$.

Results

No precipitation was observed in any formulation after mixing of MTS with the polymer and other excipients. The formulations give a milky solution. The normal pH of human nasal mucosa is in the range of 5-6.5 however it can tolerate about 4-7.5 [8].

The pH of prepared formulation should be in the range of tolerable pH to prevent the nasal irritation. The result of pH measurement from HPC formulations were in the range of 7.1 – 7.5. The viscosity for nasal sprays should be in the range 1.25 – 5.0 cPs. The viscosity of formulations was found dependent on the HPC concentration.

For the concentrations 0.03125, 0.0625, 0.125, 0.25 and 0.50% w/v, the viscosity was 2.68, 2.69, 2.72, 2.76 and 3.80 cP, respectively. Therefore the viscosity of all formulations was in the range suitable for nasal sprays. The results of measurement of the contact angle determine the wettability of the formulations. If the formulation has the contact angle degree less than 30^{o}, it has high degree of wetting property. The contact angle of purified water from the experiment was found to be 49.9^{o}. The formulation with HPC concentrations 0.03125, 0.0625, 0.125, 0.25 and 0.5 % w/v had value of contact angle 49.7^{o}, 49.2^{o}, 48.3^{o}, 44.7^{o} and 39.2^{o}, respectively showing all formulations with good wettability. The degree of wettability was decreased when the polymer concentration was increased. The suitable droplet size of nasal sprays should be in the range of 10 – 100 μm. The droplet size of the formulation with polymer concentrations 0.03125, 0.0625, 0.125, 0.25 and 0.5 % w/v was determined as 64.69, 66.79, 68.58, 71.48 and 75.29 μm, respectively depicting that the formulation will retain in the nasal cavity and not enter to the lung. The mucoadhesion value can be reported as the value of work of adhesion. The value of mucoadhesion was found dependent on the concentration of polymers as shown in the Fig.3. The work of

adhesion for the formulations with the polymer concentrations 0.03125, 0.0625, 0.125, 0.25 and 0.5 % w/v was 0.021, 0.085, 0.086, 0.108 and 0.156 N.mm, respectively.

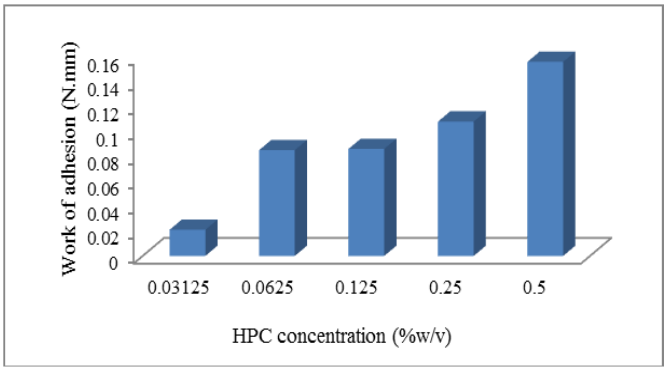


Figure 3. Mucoadhesion value of the formulation with different HPC concentrations

Discussion

The HPC was chosen for this nasal formulation because it is a nonionic cellulose that does not interrupt other excipients due to ionic interactions. Moreover HPC is freely soluble in water below 38 ^{o}C and can be obtained as the thickening agent depending on the concentration. Aqueous solution of HPC is stable at pH 6.0 – 8.0, with the viscosity of formulations being relatively unaffected [13]. The pH of nasal formulation should be adjusted to appropriate due to 3 reasons. The first reason is the avoidance of nasal irritation, second one is to obtain efficient drug absorption and the third one is to prevent the growth of pathogenic bacteria in the nasal cavity [14, 15].

The viscosity is an important factors of the nasal formulations because the contact time between the drug and the nasal mucosa is increased by higher viscosity of the formulation therefore increasing the time for absorption [2]. HPC is the soluble cellulose derivatives and mucoadhesive polymer having viscosity dependent on the concentration of HPC in the nasal formulations. The advantages from the viscosity of HPC include prolonging the residence time of drug in nasal cavity, to sustain the release of drug, act as absorption enhancer and effectively increase intranasal bioavailability [2]. However the suitable viscosity of the solution for nasal sprays is in the range of 1.25 – 5 cPs. If the viscosity is higher than 5 cPs, the formulation will be difficult to spray and the droplet size will be larger than 100 μm. The evaluation of mucoadhesive potential was performed by using texture analyzer. The dialysis membrane with 0.3% mucin is present in the nasal epithelial cell set as the upper probe. The applied force was 0.25 N used to contact the nasal formulations for 5 mins and the probe lifted upwards. The mucoadhesion was reported from the work of adhesion (N.mm) which was derived from the recorded force and the distance curve. This result can be explained by different mucoadhesive theories. However it should be noticed that this situation is based on sufficient cohesiveness of the dialysis membrane saturated with 0.3% mucin and the solution of each formulations. On the other hand, in this experiment, there is no electrostatic attraction between dialysis membrane saturated with 0.3% mucin (negative charge) and the formulation with

HPC (no charge) resulting in low work of adhesion [16]. The contact angle is the determination of wettability of the formulation. The contactangle (less than 90o) increase the contact surface area between the formulation and nasal epithelial cell, resulting in increased wettability [10]. When the wettability was increased it influence the absorption of solution in the nasal epithelial cell. The results show that as the concentration of polymers was increased, the contact angle show a decreasing trend. The applicability of laser diffraction (Spraytec®) for characterizing the droplet-size distribution from nasal sprays was determined to understand the relationship between physical properties of nasal formulations and their spray characteristics. Therefore droplet size and spray pattern studies are thought to be important characterization techniques in evaluating product performance. Droplet size may also be useful in predicting nasal deposition [17]. If the droplet size smaller than 5 μm, it may enter the lung. If the droplet is larger than 100 μm, it forms a drop that couldn't spread thoroughly in the nasal turbinate or it may drip drop from the area of nasal cavity.

Conclusions and Recommendations

The nasal sprays are the challenge for nasal route administration because of their many advantages including self-administration, non-invasive and painless, suitable for children and adult, patient convenience and improved compliance. Moreover, nasal administration gives rapid onset of therapeutic effects with the rate of absorption

comparable to IV medication, avoidance of first pass effect and destruction in GI tract and the use of low dose can possibly reduce the side effects. Montelukast sodium nasal spray is designed to use for local effect. From aforementioned study, the physicochemical properties of all nasal formulations were in the range suitable for nasal sprays [1, 2]. In the further experiments, the formulation could be tested with the nasal epithelial cells to evaluate the toxicity of the formulation to the cells and the opening of nasal epithelial tight junction to confirm that the drug will not permeate to the blood circulation and also not crossing blood brain barrier resulting in the avoidance of systemic side effects.

Acknowledgement

The author would like to acknowledge the financial support by Discipline of Excellence in Pharmacy phase II and Drug Delivery System Excellence Center (DDSEC), Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

References

Benninger, M.S. and H. Waters, Montelukast: Pharmacology, safety, tolerability and efficacy. Clin Med, 2009. 1: p. 1253–1261.

Appasaheb, P.S. and S.D. Manohar, A Review on intranasal drug delivery system. JAPER, 2013. 3: p. 333 - 346.

Illum, L., Nasal drug delivery: new developments and strategies. Research focus, 2002. 7: p. 1184 - 1189.

Türker, S., Onur, E., Özer, Y., Nasal route and drug delivery systems. Pharm World Sci, 2004. 26: p. 137 - 142.

Ugwoke, M.I., Agu, R. U., Verbeke, N., Kinget, R., Nasal mucoadhesive drug delivery. Adv Drug Deliv Rev, 2005. 57(11): p. 1640-1665.

Suzuki, Y. and Y. Makino, Mucosal drug delivery using cellulose derivatives as a functional polymer. J control release, 1999. 62 p. 101-107.

Bittera, C., K.S. Zimmermanna, and C. Surbera, Nasal drug delivery in humans. Curr Probl Dermatol, 2011. 40: p. 20–35.

Wadell, C., Nasal drug delivery. Comprehensive summaries of uppsala dissertations from the Faculty of Pharmacy 278, 2002.

Trivedi, J.S. and R.P. Quinn, *Document made available under the Patent Cooperation Treaty (PCT)*. 2006. p. 1-15.

Yuan, Y. and T.R. Lee, Contact Angle and Wetting Properties. 2013. 51: p. 3-34.

References

Bitter, C., Z.K. Zimmermanna, and C. Surbera, Nasal drug delivery in humans. Curr Probl Dermatol., 2011. 40: p. 20-35.

Frank, D.O., et al., Effects of anatomy and particle size on nasal sprays and nebulizers. Otolaryngol Head Neck Surg, 2012. 146(2): p. 313-319.

Rowe, R.C., Sheskey, P. J., Cook, W. G., Fenton, M. E., Handbook of Pharmaceutical Excipients. 2012. 6: p. 159-161, 317-322.

Bankar, P.V., et al., Evaluation and quality control of nasal spray. Journal of drug delivery & therapeutics, 2012. 2(4): p. 1-4.

Behl, C.R., et al., Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv drug deliver rev, 1998. 29: p. 89-116.

Xu, X., et al., Preparation and in vitro characterization of thermosensitive and mucoadhesive hydrogels for nasal delivery of phenylephrine hydrochloride. Eur J Pharm Biopharm, 2014. 88(3): p. 998 - 1004.

Makidon, P.E., et al., Characterization of stability and nasal delivery systems for immunization with nanoemulsion-based vaccines. Journal of aerosol medicine and pulmonary drug delivery, 2010. 23: p. 77-89.

VITAE

Name Miss Thunyaporn Jullaphant

Student ID 5810720006

Education Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Pharmacy	Prince of Songkla University	2010

Scholarship Awards during Enrolment

1. Thesis Grant, Education fee and Salary:

   The Discipline of Excellence in Pharmacy, phase 2, Faculty of Pharmaceutical Sciences, Prince of Songkla University.

   The Drug Delivery System Excellence Center, Prince of Songkla University.

2. Thesis Grant:

   Faculty of Pharmaceutical Sciences, Prince of Songkla University.

   The Graduate School, Prince of Songkla University.

3. Conference schlolarship:

   The Nanotec-PSU Excellence Center on Drug Delivery System, Prince of Songkla University.

Work-Position and Address

Pharmacist and store manager of Walgreens Boots Alliance company, Big C Hatyai branch, Songkhla Thailand, 2011-2015

List of Publication and Proceeding

1. Jullaphant, T., Nakpeng, T. and Srichana, T. 2017. Formulation development and *in vitro* evaluation of montelukast nasal sprays.

   (Manuscript was submitted to Drug development and industrial pharmacy)

2. Jullaphant, T. and Srichana, T. Physico-chemical properties of montelukast sodium nasal spray formulation. The 40th National Graduate Research Conference, Oct 20-2, 2016, Prince of Songkla University, Hatyai, Thailand.