DEVELOPMENT OF pH-SENSITIVE INTRANASAL PREGABALIN GEL AND ITS PHARMACOKINETIC EVALUATION

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology)

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OCTOBER 2021

ABSTRACT

Intranasal drug delivery study is one of the promising methods of administration for central nervous system (CNS) drugs. It brings drugs directly to the brain through olfactory and trigeminal nerve. Any CNS drugs delivered via oral and intravenous have the potential to be delivered via nasal route. The main advantage of using intranasal route is to lessen the peripheral side effects by reducing the dose administered. Nasal route also brings its own challenge which is high mucociliary clearance rate. To solve the challenge, this study attempted an intranasal formulation using pH-sensitive polymer. Pregabalin was used as the model drug. Simple and robust analytical method for quantitation of Pregabalin has been developed and validated by using HPLC with UV detector. Validation criteria including specificity, linearity, limit of quantification (LOQ), limit of detection (LOD), precision and accuracy were met with International Conference on Harmonisation (ICH) standard. The method was linear with correlation coefficient (r^2) 0.992 and limit of quantitation (LOQ) of 15 µg/ml. The method was applied to commercial Pregabalin (Pregabalin Sandoz) for assay analysis with 100.37 ±2.94 % accuracy. The formulation of pH-sensitive intranasal Pregabalin gel has been characterized by compatibility study, $pH_{sol\text{-}gel}$ and gelation time test, viscosity study, drug assay test, clarity test and *in-vitro* dissolution test. The formulation consists of 0.35%, 0.4% and 0.45% of Carbopol 940 with distilled water and Pregabalin. Compatibility study had shown no interaction within the formulation. From *in-vitro* dissolution test, there was no significant difference of drug release between the formulations ($p>0.05$) because of the slight difference in percentage of Carbopol 940 used. For bioanalytical method development, Aspirin had been used as the internal standard (IS) because it did not interact with Pregabalin and bioanalytical sample (brain and plasma). The HPLC parameters also had been optimized and suitable extraction solvent (Acetonitrile) was selected. The developed method was validated according to Bioanalytical Method Validation Guidance for Industry from United States Food and Drug Administration (US FDA) and met with the required criteria. The validated criteria included specificity, calibration curve, lower limit of quantification (LLOQ), recovery, precision and accuracy. Linearity of the method was measured by r^2 with the result higher than 0.99 for both plasma and brain. LLOQ for the method was 25 and 75 µg/mL for plasma and brain respectively. The validated method had been applied for pharmacokinetic analysis in rat's plasma and brain. Brain $AUC_{0\text{-}4}$ for intranasal route was higher compared to oral route. At first sampling point (15 minutes), Pregabalin concentration in the brain of pH-sensitive intranasal Pregabalin gel (0.4% Carbopol 940) was highest compared to intranasal drop and oral solution. In conclusion, pH-sensitive intranasal Pregabalin gel has better Pregabalin delivery to the brain compared to normal intranasal drop solution and oral Pregabalin.

خلاصة البحث

توصيل الأدوية عن طريق الأنف إحدى الطرق لاعطاء أدوية الجهاز العصبي المركزي حيث يتم توصيلها مباشرة إلى الدماغ. أي أدوية تستخدم للجهاز العصبي المركزي التي تستخدم عن طريق الفم أو الوريد يمكن أن يتم استخدامها عبر الأنف. الميزة الرئيسية لاستخدام الدواء عن طريق الأنف هي تقليل الآثار الجانبية عن طريق تقليل الجرعة المعطاة. توصيل الدواء عن طريق الأنف أيضًا لديه التحدي الخاص به وهو معدل إزالة المخاطية المخاطية المرتفعة. لحل هذا التحدي ، تم استخدام تركيبه دوائيه داخل الأنف باستخدام بوليمر حساس لدرجة الحموضة. لقد تم استخدام البريجابالين وتم تطوير طريقة تحليلية لتقدير كمية البريجابالين والتحقق من صحتها باستخدام HPLC. تم تطبيق الطريقة على البريجابالين التجاري لتحليل المقايسة بدقة 100.37 ± 2.94٪. تتميز تركيبة هلام بريجابالين داخل الأنف الحساس للأس الهيدروجيني بدراسة التوافق واختبار زمن الهلام والجيل الهلامي ودراسة اللزوجة واختبار فحص الأدوية واختبار الوضوح واختبار الذوبان في المختبر. تتكون التركيبة من 0.35٪ و 0.4٪ و 0.45٪ من كاربوبول 940 مخلوط بالماء المقطر والبريجابالين. أظهرت دراسة التوافق عدم وجود تفاعل داخل المستحضر. من اختبار الذوبان في المختبر، لم يكن هناك اختلاف كبير في إطلاق الدواء بين التركيبات بسبب الاختلاف الطفيف في النسبة المئوية للكاربوبول 940 المستخدم. لتطوير طريقة التحليل الحيوي، تم استخدام الأسبرين كمعيار داخلي لأنه لم يتفاعل مع البريجابالين وعينة التحليل الحيوي (الدماغ والبلازما). كما تم تحسين معلمات HPLC واختيار مذيب استخلاص مناسب (أسيتونتريل). تم التحقق من صحة الطريقة التي تم تطويرها وفقًا لإرشادات التحقق من صحة طريقة التحليل الحيوي للصناعة من إدارة الغذاء والدواء الأمريكية وتلبية المعايير المطلوبة. تضمنت المعايير التي تم التحقق من صحتها، الخصوصية ومنحنى المعايرة والحد الأدنى للتقدير الكمي (LLOQ) والاستعادة والدقة والدقة. كان LLOQ للطريقة 25 و 75 ميكروغرام / مل للبلازما والدماغ على التوالي. تم تطبيق الطريقة التي تم التحقق من صحتها لتحليل الحرائك الدوائية في بلازما الفئران والدماغ. كان الدماغ AUC_{0-4} للطريق الأنفي أعلى مقارنة بالطريق الفموي. في نقطة أخذ العينات الأولى (15 دقيقة)، كان تركيز البريجابالين في دماغ هلام بريجابالين داخل الأنف حساساً لدرجة الحموضة (0.4 ٪ كاربوبول 940) أعلى مقارنة مع القطرات الأنفية والمحلول الفموي. في الختام، يحتوي هلام بريجابالين داخل الأنف الحساس لدرجة الحموضة على توصيل أفضل للبريجابالين إلى الدماغ مقارنة بمحلول قطرة الأنف الطبيعي و بريجابالين عن طريق الفم.

APPROVAL PAGE

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

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DECLARATION

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

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ACKNOWLEDGEMENT

First and foremost, I would like to present my gratitude to Allah, The Almighty for giving me strength, mentally and physically to get through this Master's Degree journey.

I am also grateful for having parents who trust me to start this journey and support me throughout the years. Indeed, without their trust I will never reach this point of life.

My utmost gratitude goes to my kind and supportive supervisor, Assoc Prof Dr Farahidah Mohamad for accepting me as her student and guiding me until the end of this journey. A special thanks to my co-supervisor, Dr Bappaditya Chatterjee for teaching and supporting me especially in formulation and analytical part, and also guiding me to publish scientific articles during the study. Not to be forgotten, my co-supervisor, Dr Helal Uddin for giving me knowledge and experience for analytical chemistry part. It is indeed the best learning journey for me.

I would also give my utmost respect and thanks to dedicated staffs from Pharmaceutical Technology Department, Sr Yanti, Sr Zai, Br Hanif and Br Dzadil. And also to staffs from Pharmaceutical Chemistry Department, my second department, Sr Fatimah, Sr Zihan, and Br Razif. Next to staffs from Basic Medical Science Department, for giving me chance and training during animal study part, Sr Rusianti, Sr Sri, Sr Ayu and Br Zack.

Lastly, very special thanks for all my supporting friends for guiding me through proposal defense until thesis submission, for keeping up my spirit, Fakhrul, Luqman and Azri. And also my labmates, Sr May, Sr Irda, Sr Anjali, Br Ibrahim, Br Khateer and others. May Allah grant upon all of you the best rewards in this world and hereafter.

Finally, I would sincerely thanks to everyone involved in my Master's journey directly and indirectly. Thank you very much.

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LIST OF ABBREVIATION

%RSD	Percentage of Relative Standard Distribution
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ACN	Acetonitrile
AMV	Analytical Method Validation
AMV	Analytical Method Validation
API	Active Pharmaceutical Ingredient
ATR-IR	Attenuated Total Reflectance Infra-red
BBB	Blood Brain Barrier
BCS	Biopharmaceutical Classification System
CL	Clearance Rate
CNS	Central Nervous System
CPP	Cell Penetrating Peptides
CSF	cerebrospinal fluid
CV%	Correlation of Variation Percentage
DPN	Diabetic Peripheral Neuropathy
EDTA	Ethylenediamine Tetraacetic Acid
fMRI	Functional Magnetic Resonance Imaging
GABA	gamma-aminobutyric acid
GBP	Gabapentin
GC-MS	Gas Chromatography with Mass Spectometry
GIT	gastrointestinal tract
HPLC	High Performance Liquid Chromatography
HQC	High Quality Control

IACUC-IIUM	Institutional Animal Care & Use Committee
ICF	Intracellular Fluid
IS	Internal Standard
ISF	Interstitial Fluid
K_e	elimination rate constant
LLE	Liquid-liquid extraction
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LOQ	Limit of Quantification
LQC	Low Quality Control
MCC	Mucociliary Clearance
MQC	Medium Quality Control
MS	Mass Spectroscopy
PBS	Phosphate buffer saline
PD	Pharmacodynamics
PET	Positron Emission Tomography
PK	Pharmacokinetics
PPT	Protein precipitation
PRG	Pregabalin
QC	Quality Control
R%	Percentage of Extraction Efficiency
R_2	Correlation coefficient
RMT	Receptor Mediated Transport
SLE	Systemic Lupus Erythematosus
SPE	Solid Phase Extraction

T%	Percentage of transmission
TCA	Tricylic agents
TEA	Triethylamine
TEOA	Triethanolamine
UV	Ultraviolet
V_d	Volume of Distribution

CHAPTER ONE

INTRODUCTION

1.1 GENERAL OVERVIEW

As modern technologies expand, pharmaceutical technology also continuously expand with new findings and breakthroughs every year. Drug delivery is one of them, particularly. Route of administration of the drug consists of intranasal, topical, oral and parenteral which includes intramuscular, intravenous and subcutaneous injection. In order to meet the needs for each route of administration, drug delivery study is consistently refined to solve the challenges faced with each route.

The problems and challenges faced by each of the route of administration are different. Oral drug delivery must undergo first pass effect and enzymatic degradation before it reaches the systemic circulation. In addition, minimum level of lipophilicity and concern for colon normal flora changes also need to be considered in oral drug delivery (Sosnik & Augustine, 2016). Once the drugs are delivered into the systemic circulation, another challenge for a drug intended to reach the brain, is the blood-brain barrier (BBB). BBB is composed of endothelial cells with tight junctions within them which cover up the brain capillaries and perivascular elements such as end-feet of astrocytes, pericytes and perivascular neuron (Alexander, 2018). Thus, many physicochemical parameters of the drug need to be assessed before releasing it into the systemic circulation intended to target the brain, as the reachability is very limited. Intravenous administration is also not left without flaws – even though pharmacokinetically the absorption reaches 100%. This is because the drug will be rapidly delivered to the organs as fast as the rate of injection which can possibly cause

toxic effects and high risk of infection (Maddison, Page, & Dyke, 2008; Ruiz & Montoto, 2018).

On the other hand, for intranasal drug delivery, common problem faced by both oral and parenteral drug deliveries is no longer major concern. The main concern for intranasal drug delivery is the rapid mucociliary clearance rate which causes high variability of drug absorption. As for conventional intranasal drug delivery such as nasal drop and spray, the clearance rate is generally very high even though it is also depends on the exact anatomical part of the intranasal, but the anatomical part is not affecting much. The main problem for the conventional intranasal drug delivery is lack of mucoadhesive properties to ensure the formulation retains long enough to be absorbed (Illum, 2003; Rohrer, Lupo, & Bernkop-Schnürch, 2018).

Thus, this research focused on preparation of sol-gel pregabalin (PRG) intended for delivery to the brain via intranasal route, as an approach to solve the problem as stated above.

1.1.1 Challenges of Drug Delivery to the Brain

Brain as one of the organs in human anatomy is the most protected among the other organs both physically; by skull and cerebrospinal fluid, and chemically; by blood brain barrier (BBB). BBB provides very stringent control for the neural tissues in the brain to undergo their normal physiologic activities by inhibiting any foreign substances from entering the brain in order to keep the ionic concentration balance, and no harmful matters like endotoxin and pathogens breach into the tissues (Daneman, 2012). It is constructed by cerebrovascular endothelium covered by tight junction and other supportive cells such as end-feet of astrocytes, pericytes and discontinuous basal lamina (Krol, 2012). Pakkenberg and Gundersen found out that only small molecules below 43

kDa (~6 nm) can pass through the basal lamina, meanwhile the large molecules above 460 kDa (~10 nm) are exempted (Pakkenberg & Gundersen, 1997). However, not all drugs below 43 kDa can pass through BBB as there were 4 drugs as small as 400 Da that had no measurable brain uptake because P-glycoprotein brain-to-blood efflux pump located at BBB decreased concentration of drug in the brain especially for lipophilic and small molecules drugs (Hoosain et al., 2015). Hence, we can conclude that ability to get through BBB is different depending on the particular molecules themselves.

However, BBB like many other tissues itself can be compromised due to many factors such as age-related neurological diseases – Alzheimer's, systemic lupus erythematosus (SLE), Parkinson's -, head injury, infections and even ischemia (Alexander, 2018; Angelov et al., 2009; Ingebrigtsen, Waterloo, Jacobsen, Langbakk, & Romner, 1999; Kaur & Ling, 2008). There were some studies which indicate that in neurodegenerative and neuroinflammatory conditions, the complement system was induced which will produce pro-inflammatory byproducts that may deregulate BBB and its functions (Esen et al., 2017). Hence, proper techniques and methods of administration, together with suitable drug delivery which can fit into either normal or abnormal settings of BBB needs to be designed to ensure that therapy is successful (Doolittle, Muldoon, Culp, & Neuwelt, 2014).

With all the information gained and researches done on BBB to date, many methods have been invented and designed for drug delivery, especially brain-targeted drugs. All in all, BBB is responsible as the guardian of the brain but it is also the main challenge for brain-targeted drugs to achieve their purposes (Wei, Chen, Ying & Lu, 2014).

1.1.2 Strategies to Overcome Challenges of Drug Delivery to the Brain

The main concern for drug delivery to the brain is BBB. In order to bypass the BBB, the cells surrounding it need to be permeated so that the drugs can pass through and reach the brain tissues. The conventional methods of bypassing BBB utilizes paracellular or transcellular transport by enhancing their permeability. Paracellular transport can be used by osmotic (utilization of the osmotic pressure by altering the osmolarity of environment of BBB) or chemical (induction of the temporary inflammation by vasodilation of BBB) disruption of BBB. It is suggested that the transcellular transport used some modifications towards the drugs to enable them to bypass BBB, but it was found that modulation of receptor-mediated transport (RMT) also can done so that the pathway to the brain tissue can be easily opened (Hersh et al., 2016). Apart from conventional methods, several novel techniques have also been established to enhance the permeability of the BBB such as nanoparticles, hyperthermia, RMT, cell penetrating peptides (CPP) and cell-mediated delivery (Zhang, Xu & Liu, 2015). Nanoparticles-assisted drug delivery is counted as one of the non-invasive technique to bypass BBB by using solid colloidal particles with the size 1-1000 nm commonly used as carrier for the drug (Zhou, Peng, Seven, & Leblanc, 2018). However, the process to design nanoparticles and bind them to the drugs need special equipment and specific characterization process such as porosity, surface area and particle size. Thus, to keep the procedure simple, other method need to be considered (Esquivel-Castro, Ibarra-Alonso, Oliva, & Martínez-Luévanos, 2018; Samimi, Maghsoudnia, Eftekhari, & Dorkoosh, 2019).

Meanwhile hyperthermia has been studied specifically to kill glioma cells by increasing the surrounding temperature (41-43 ºC) as it is more sensitive towards sudden increase in temperature than normal cells. During the process, it was found out

that the integrity of BBB was disrupted temporarily which indicate possibility to administer drugs through this site (Zhang, Xu, & Liu, 2015). As the name suggested, each of them possess their own principal advantages but carries the same role which is to enhance the permeability of BBB.

1.1.3 Brain Targeting Intranasal Delivery

One of the possible approaches to minimize the challenges in brain-targeted drug delivery is by using intranasal mode of administration. Intranasal drug delivery can solve the problems faced by brain-targeted drug as it can offer different pathway which bypassed BBB. As illustrated in Figure 1.1, nasal mucosa has unique neural connection with the brain in which pathogens and toxic metals can directly enter the brain by using trigeminal and olfactory pathway due to the connection from external environment to the brain (Hanson & Frey, 2008). It is only these recent years, the same pathway has been acknowledged for therapeutic applications (Agrawal et al., 2018). This pathway has been first developed by Frey by applying the administration for neurotrophic factors to the central nervous system (CNS) (Frey, 1991).

For olfactory pathway, the proposed mechanism via intraneuronal depends on axonal transport which requires hours to days to reach the brain and via extraneuronal depends on bulk flow transport via perineural channels which requires several minutes to arrive to the brain (Frey, 2002). Later, trigeminal pathway has also been discovered as another pathway which specifically targeted caudal (posterior part) brain region as depicted in Figure 1.1 and Figure 1.2 (Thorne, Pronk, Padmanabhan & Frey, 2004). Thus, by intranasal pathway, the BBB can be bypassed through the mechanisms stated as shown in Figure 1.

In addition, it can bypass the first pass effect (hepatic metabolism) and gastric acid degradation which are the common challenges in enteral route of administration. Hence, by selecting intranasal administration for the drug, problems faced by enteral and most parenteral drug administration can be avoided.

Main advantage of intranasal drug delivery is reduction of drug dosage because it delivers the drug directly to the brain. In dose-response relationship, the higher the administered dose, the stronger the intended effect and also the unwanted side effect (Moffett, El-Masri, & Fowler, 2007). Thus, by reducing the administered dose, the unwanted side effects also can be reduced.

As shown in Figure 1.1, the drug distributed directly from nasal cavity to the brain bypassed BBB. This increases the unbound fraction of drug in intracellular fluid (ICF) and interstitial fluid (ISF) of the brain compared to the plasma. It is generally accepted that unbound fraction is responsible in exerting the physiological effects (Hammarlund-Udenaes et al., 2008). Some other factors also affected drug-brain distribution such as protein binding which affecting bound-unbound drugs and drug efflux transporters which have an effect on drug concentration in the brain (Srinivas, Maffuid, & Kashuba, 2018).

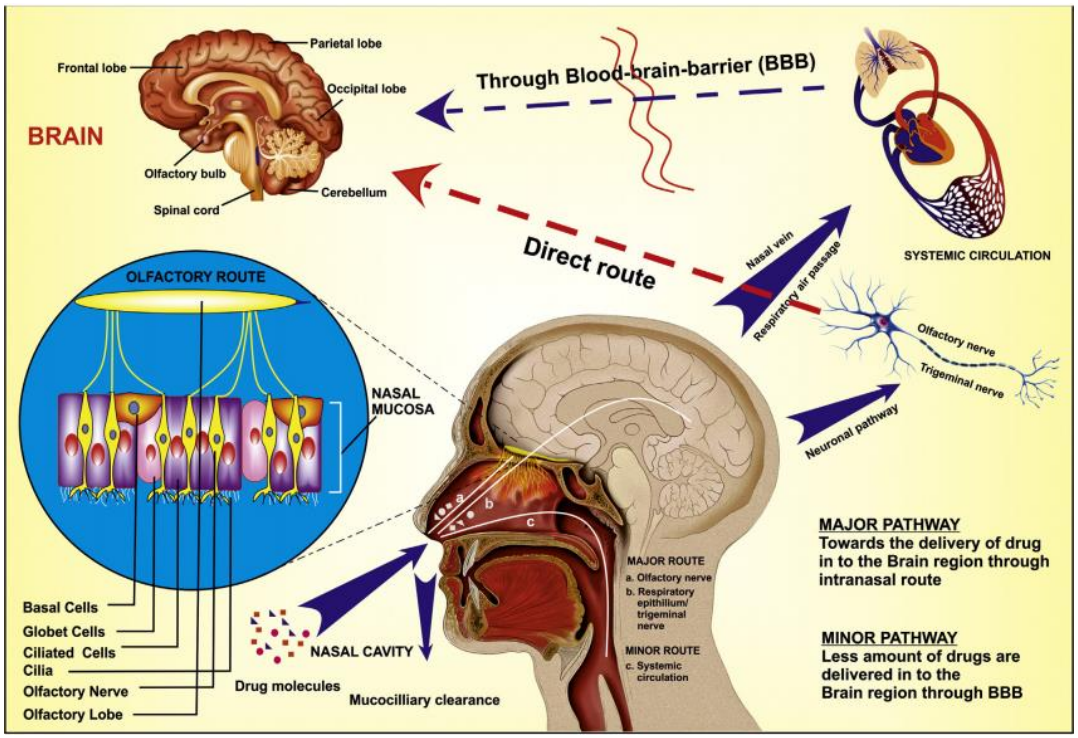

Figure 1.1 Nose to brain drug delivery mainly through the neural pathway via olfactory and trigeminal nerve (adapted from Agrawal et al., 2018)

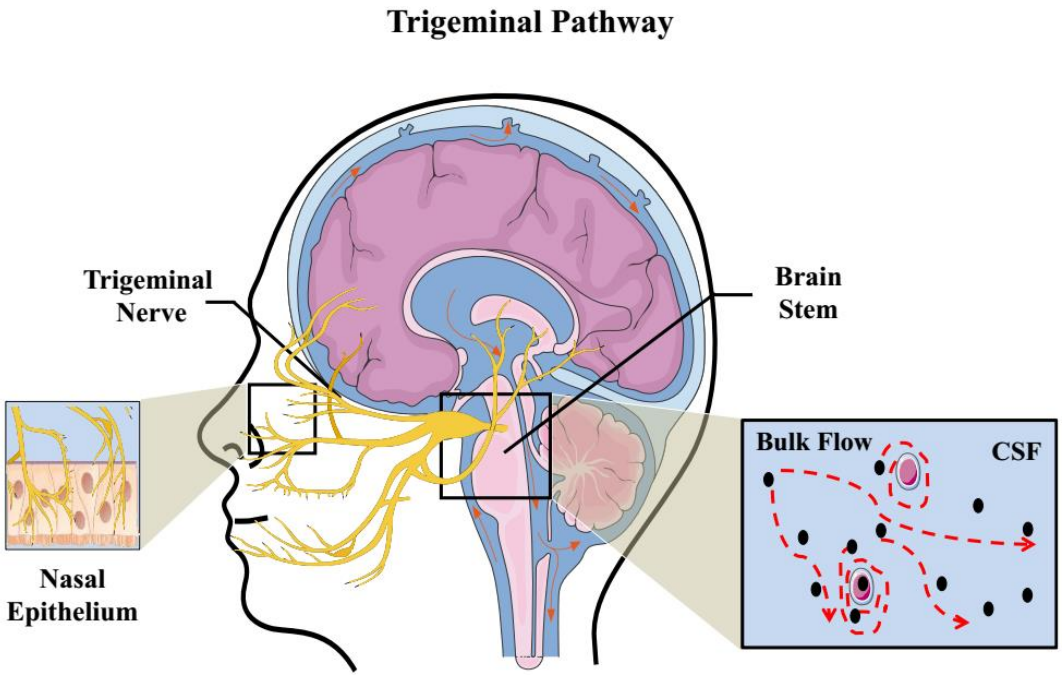

Figure 1.2 Trigeminal pathway for drug delivery (adapted from Samaridou & Alonso, 2017)