Universidade de Lisboa

Faculdade de Farmácia

Investigation of tocophersolan (TPGS) - Pluronic F127 mixed micellar thermosensitive systems and its nasal applicability

Maria Inês Francisco Livramento

Trabalho de Campo orientado pelo Professor Doutor Sipos Bence, Professor Auxiliar, Instituto de Tecnologia Farmacêutica e Assuntos Regulamentares, Faculdade de Farmácia da Universidade de Szeged, e coorientado pelo Professor Doutor João Lopes, Professor Associado, da Faculdade de Farmácia da Universidade de Lisboa.

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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Declaração Código de Conduta

Declaro ter desenvolvido e elaborado o presente trabalho em consonância com o Código de Conduta e de Boas Práticas da Universidade de Lisboa. Mais concretamente, afirmo não ter incorrido em qualquer das variedades de fraude académica, que aqui declaro conhecer, e que atendi à exigida referenciação de frases, extratos, imagens e outras formas de trabalho intelectual, assumindo na íntegra as responsabilidades da autoria.

Resumo

Cerca de 24 milhões de pessoas em todo o mundo sofrem de esquizofrenia, sendo a doença de saúde mental com mais custos por pessoa globalmente. Os tratamentos para a esquizofrenia congelaram no tempo, por isso, neste projeto foi desenvolvida uma nova tecnologia de formulação da risperidona pela administração nasal, pois tem a vantagem de melhorar a biodisponibilidade do medicamento e reduzir os efeitos adversos a ele associados. A risperidona tem a desvantagem de ser insolúvel em água, assim desenvolveu-se uma formulação que contém TPGS e Pluronic F127 como constituintes de micelas poliméricas termossensíveis. Estas são nanotransportadores que protegem o fármaco hidrofóbico no seu núcleo hidrofóbico, aumentando a solubilização devido à sua camada externa hidrofílica. Para além disso, libertam o fármaco a uma temperatura específica. Para avaliar a viabilidade da formulação determinou-se o valor de 29ºC como o LCST das micelas sem o fármaco, resultado positivo, uma vez que se encontra acima da temperatura ambiente e abaixo da temperatura da cavidade nasal. A risperidona não alterou a estabilidade das micelas, pois o LCST foi o mesmo. A caracterização da mistura das micelas com a risperidona revelou resultados favoráveis, sendo que o tamanho e a distribuição das mesmas cumpriram com os valores de referência. A estabilidade da formulação foi testada em diferentes pH e viscosidade crescente, demonstrando-se que estes parâmetros não a afetam. Os estudos de diluição demonstraram que o valor da LCST aumentou a partir de diluições de 25 vezes, mas os resultados são aceitáveis dado que a cavidade nasal suporta diluições entre 15 a 20 vezes. O soro fisiológico é um excipiente adequado para formulações nasais em concentrações abaixo de 2M, pois, em concentrações mais altas, compromete a estabilidade devido ao aumento da LCST, como demonstrado nos testes de força iónica. Os estudos de mucoadesão revelaram-se positivos uma vez que a adesão à mucosa nasal foi forte, mas não o suficiente para ser permanente, o que favorece a libertação eficaz do fármaco neste local. Estudos *in vitro* de libertação e permeabilidade da risperidona demonstraram um perfil melhorado da eficácia da risperidona quando encapsulada nas micelas. A formulação apresenta uma possível opção de tratamento para a esquizofrenia.

Palavras-chave: esquizofrenia; risperidona; micelas poliméricas, termossensível, administração nasal

Abstract

Approximately 24 million people worldwide have schizophrenia and it is the most costly mental health condition per person globally. Schizophrenia treatment options were freezed in time, but to get around this fact, the aim of this project was to develop and improve new risperidone formulation and technology to achieve nasal administration, which has the advantage of improving the bioavailability of the drug and reduce the side effects associated to it. Risperidone has the disadvantage of being an insoluble drug. A formulation containing TPGS and Pluronic F127 as constituents of mixed micellar thermosensitive systems was developed. These are nanocarriers that can protect the hydrophobic drug in the hydrophobic nucleus and enhance the solubilisation due to its hydrophilic shell, and by being thermosensitive, the drug is released at a certain temperature. To evaluate the potential viability of the formulation, several tests were carried. It was determined that blank mixture polymeric micelles's LCST value is of 29ºC, which is good because it is above ambient temperature and below nasal cavity temperature. Risperidone did not change the micelles' stability as the LCST remained the same and its characterisation showed great results, where polymeric micelles size and distribution were positive. By testing the variation of the formulation stability, it was possible to conclude that pH from 5 to 7 and viscosity do not interfere with the stability of the formulation. Dilution studies showed that LCST increased at dilutions beyond 25-fold, but it is acceptable given nasal cavity dilution ranges of 15 to 20-fold. Physiological saline is a suitable excipient for nasal formulations below concentrations of 2M as it compromises stability due to a LCST raise on ionic strength tests. Mucoadhesion studies revealed strong but non-permanent adherence to nasal mucosa, which supports effective drug release. *in vitro* release and permeability studies demonstrated enhanced profile of risperidone when encapsulated in the micelles. Overall, the formulation presents promising potential for being a new treatment option for schizophrenia.

Keywords: schizophrenia; risperidone; polymeric micelle; thermosensitive; nasal administration

Abreviaturas

APD	New-generation Antipsychotic Drugs
BBB	Blood-Brain Barrier
CBT	Cognitive Behaviour Therapy
CMC	Critical Micelle Concentration
CMT	Critical Micellar Temperature
CNS	Central Nervous System
CST	Critical Solution Temperature
DBT	Dialectical Behaviour Therapy
DMDD	Disruptive Mood Dysregulation Disorder
EPS	Extrapyramidal Side Effects
GNP	Gross National Product
LCST	Lower Critical Solution Temperature
NIMH	National Institute of Mental Health
SGA	Second-generation Antipsychotic Drugs
SNES	Simulated Nasal Electrolyte Solution
TPGS	D-α-tocopheryl polyethylene glycol 1000 succinate
UCST	Upper Critical Solution Temperature
WHO	World Health Organization

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1 Introduction

1.1 Aim of the project

Between January and April of 2024, this work was developed under the Erasmus+ Programme at the Faculty of Pharmacy of the University of Szeged, in Szeged, Hungary. This project aimed to develop a stable formulation with risperidone that contains TPGS and Pluronic F127 as constituents of mixed micellar thermosensitive systems that is suitable for nasal administration. In this behalf it could enhance the bioavailability of risperidone and improve pharmacological treatment in schizophrenia.

Nasal drug delivery has been studied by the Institute of Pharmaceutical Technology and Regulatory Affairs's team as a great administration route, due to the advantages of nasal administration compared with the disadvantages of first-pass effect due to the metabolism of oral administration, or the poor compliance associated with injections.

Polymeric micelles were prepared, as the nanocarrier system, checking important factors like the micellar size, and thermosensitive behaviour in nasal conditions.

Advancing therapeutic systems to enhance drug bioavailability in schizophrenia treatments involves optimising physical, chemical and technological properties APIs. This approach improves the efficacy and safety of treatments, promotes better adherence to therapy, and enhances patients' quality of life.

1.2 Mental health and psychiatric diseases outcome

Health is considered by WHO as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, where everyone has the right to enjoy the highest attainable standard of health, without discrimination. (1) Mental health is an integral and essential component of health, so it means that there is no health without it. (2) Mental Health and its conditions suffered a lot of stigma in the past, and even though there's a lot of progress and demystification on the topic, it remains societies where healthcare systems do not adequately support people with mental illnesses and neglect them. Citing Dévora Kestel, Director of the Department of Mental Health and Substance Use of the World Health Organization, "millions of

people around the world suffer in silence, experience human rights violations or are negatively affected in their daily lives.”. (3)

In 2001, WHO published a report: The World Health Report 2001: Mental Health – New Understanding, New Hope, to increase global awareness of mental health issues, promote research on the topic, encourage stakeholders to advocate mental health, and develop and implement evidence-based policies and practices, and with a huge importance, to improve access to mental health services. (3)

WHO published the Comprehensive Mental Health Action Plan 2013-2030, to develop objectives for WHO’s Member States to achieve until 2030, with different options for their members to implement, again to promote mental well-being, prevent mental disorders, provide care, enhance recovery, promote human rights and reduce the mortality, morbidity and disability for persons with mental disorders. (4)

In 2020, it was possible to analyse advances achieved through WHO's Mental Health Atlas and which goals Member States have implemented. The Action Plan 2013-2030 pointed out that “80% of countries will have developed or updated their policy or plan for mental health in line with international and regional human rights instruments (by 2020)” but just “99 countries, 51% of WHO Member States” had done that. In providing care services, “Service coverage for mental health conditions will have increased at least by half (by 2020)” but “a global median of 29% of persons with psychosis and a global median of 40% of persons with depression are receiving mental health services”. When analysing objective 3 to implement strategies for promotion and prevention in mental health-based settings and objective 4 to strengthen information systems, evidence, and research for mental health, numbers do not look promising. (5)

The progress has been slow and there's still a long way to go, and to add to this, in most countries Mental Health is utilised as a tool of business, resulting in a heavy and negative impact on people’s mental health and maintaining the inadequacy of health services. (3)

Mental Health is defined as a state of mental well-being that enables people to cope with the stresses of life, realise their abilities, learn well and work well, and contribute to their communities. On having balanced mental health, humans are able to create positive connections, empathise with others, gain an education, feel confident to make

healthy, informed, and complex choices, adapt to change, find purpose in life, and feel good. (6)

Consistent with point 1. of Article 25 of the Universal Declaration of Human Rights "Everyone has the right to a standard of living adequate for the health and well-being of himself and of his family, including food, clothing, housing and medical care and necessary social services (...)". (7) However, according to the 2022 WHO's World Mental Health Report, most people with mental health conditions do not have access to effective care, this happens due to services and supports that are not available, or have lack of capacity, or cannot be accessed or are unaffordable, besides the major fact that widespread stigma and discrimination limits people seeking help, which also constitutes a violation of human rights. (3)

In 2019, 970 million people worldwide were living with a mental disorder, which means 1 in every 8 people. The COVID-19 pandemic has brought destabilisation in this field, leading to a 26% and 28% increase, respectively for anxiety and major depressive disorders in just one year. (8) This public health emergency has introduced several mental health stressors, significantly exacerbating social inequalities and exposing weaknesses in healthcare services. Many people have avoided seeking medical help out of fear of infection or death. Also, the risk factors for mental health conditions have increased: anxiety about the virus's potential impact, stress from quarantines and distancing measures, unemployment and financial insecurity; misinformation and uncertainty about the future, or depression stemming from social isolation and disconnection. (3)

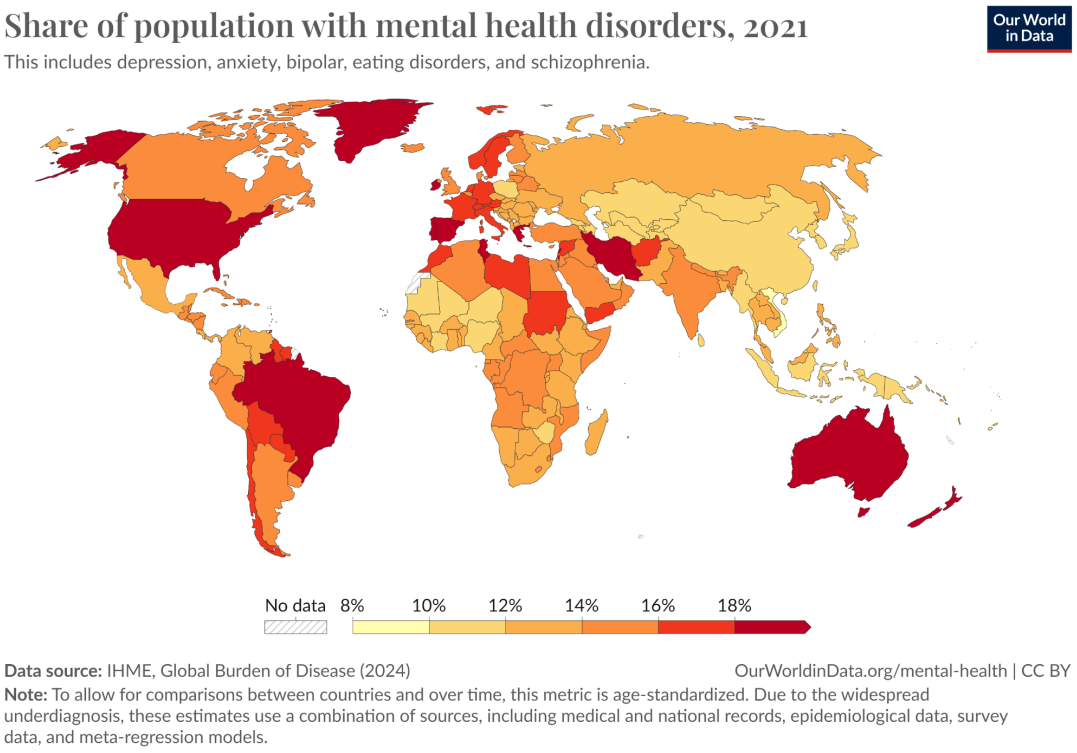

Figure 1: Share of population with mental health disorders, 2021, (adapted from [9]).

By observing Figure 1, it is possible to gain a better understanding of the global distribution of mental illness. Europe shows very concerning levels of these conditions, like the United States of America, Brazil, Australia and Iran. Although mental disorders are common in all countries, they can be more common in high-income countries. The variations of the prevalence could be explained by demographic factors, war conflicts and sociocultural factors. (9)

Mental disorders are described as clinically significant disturbances in an individual's cognition, emotional regulation, or behaviour that reflect a dysfunction in the psychological, biological, or developmental processes underlying mental and behavioural functioning. Examples of mental disorders include depression, anxiety disorder, conduct disorder, bipolar disorder, and other psychosis. (10)

People with severe mental health conditions die on average 10 to 20 years earlier than the general population, because these diseases are major contributors to preventable diseases, especially cardiovascular disease, respiratory disease and infection, which are more common in people with mental health conditions. Moreover, individuals with mental health conditions are more susceptible to the adverse effects of

medications. When they are directed to mental health services, their physical health is often at risk of being neglected. (3)

Beyond the impact these conditions have on the individual, they can have an enormous impact on society but, still, are undertreated and under-resourced. In 2019, 71% of people with psychosis didn't receive mental health services and, on average, on the total health budgets, just 2% went to mental health. (3)

Schizophrenia was found to be the most costly mental health condition per person globally. (3) In 2013, the disease was ranked among the top 25 leading causes of disability in the world (3), and according to the National Institute of Mental Health (NIMH), it is now in the top 15. It has a massive impact on patient's lives, not forgetting the families who also suffer from the consequences of the disease. (11)

In 2019, it was estimated that 40 million people had bipolar disorder (1 in 150 adults globally). Likewise, schizophrenia, bipolar disorder affect working-age populations, and employment can be an enormous source of stress. (3)

Children are a vulnerable population on the mental health topic too. Once they do not have a choice about their social environment and a voice that is taken seriously, they get vulnerable to tutors who have the duty of protecting them and giving them tools to become a healthy and educated adult. If children are not provided with a healthy environment and proper care, they are at risk of developing mental health disorders.

It is very consensual that before the age of five, the foundation for health, competence and education is set for later stages of life (12). Around 8% of the world's young children (aged 5–9 years) and 14% of the world's adolescents (aged 10–19 years) live with a mental disorder. In childhood, children are learning how to deal with their emotions, developing their personality and character. Anger and irritability are normal when they are facing feelings of frustration, so it is not uncommon for a child to have tantrums, and episodes of crying, kicking, stomping, hitting, and pushing that last five to ten minutes as many as nine times per week. However, it is important to be aware when tantrums get frequent and extreme or continue to occur while a child gets older. (13)

Disruptive Mood Dysregulation Disorder (DMDD) is a condition that makes children or adolescents experience ongoing irritability, anger, and frequent, intense temper outbursts and tantrums. Children who face this disorder have poor control over their

emotions, they experience their feelings powerfully and have difficulty reading facial expressions, perceiving neutral faces as negatively and slightly negative faces as severely judgmental. DMDD can be considered a risk factor for mental health, as youth with this condition face an increased risk of developing anxiety and depression in the future. To deal with this condition the goal is to support children by regulating their emotions and mood. This can be achieved with psychotherapy, using methods like Cognitive Behaviour Therapy (CBT), Parent management training and Dialectical Behaviour Therapy (DBT). When these options are not available or effective, pharmacological treatment will be the second choice, unless providers recommend both psychotherapy and medication. There are no protocols or standard treatment for DMDD. However, stimulants, antidepressants and atypical antipsychotic medication can be prescribed in this situations to decrease irritability in youths. (14)

1.3 Schizophrenia

According to ICD-11, schizophrenia is characterised by disturbances in multiple mental modalities, including thinking, perception, self-experience, cognition, volition, affect, and behaviour. It is a severe psychiatric disorder that adversely affects the patient's life due to core symptoms, like persistent delusions, persistent hallucinations, disorganised thinking, and experiences of influence, passivity, or control. At least two of these symptoms must have persisted for at least one month for a diagnosis of schizophrenia to be assigned, and the symptoms shouldn't be triggered by another medical condition or the use of substances or medication. (10) It is important to keep in mind that beyond the symptoms that impact the lives of these individuals, their life expectancy is 10 to 20 years below that of the general population. (8)

Schizophrenia affects approximately 24 million people worldwide, and it has a huge socioeconomic impact too. (8) It is usual to diagnose schizophrenia in the late teens and early 20s, as we can observe in Figure 3, which corresponds to working-age populations that face increased unemployment because of their mental health condition, this fact is an indirect financial cost for society. (3)

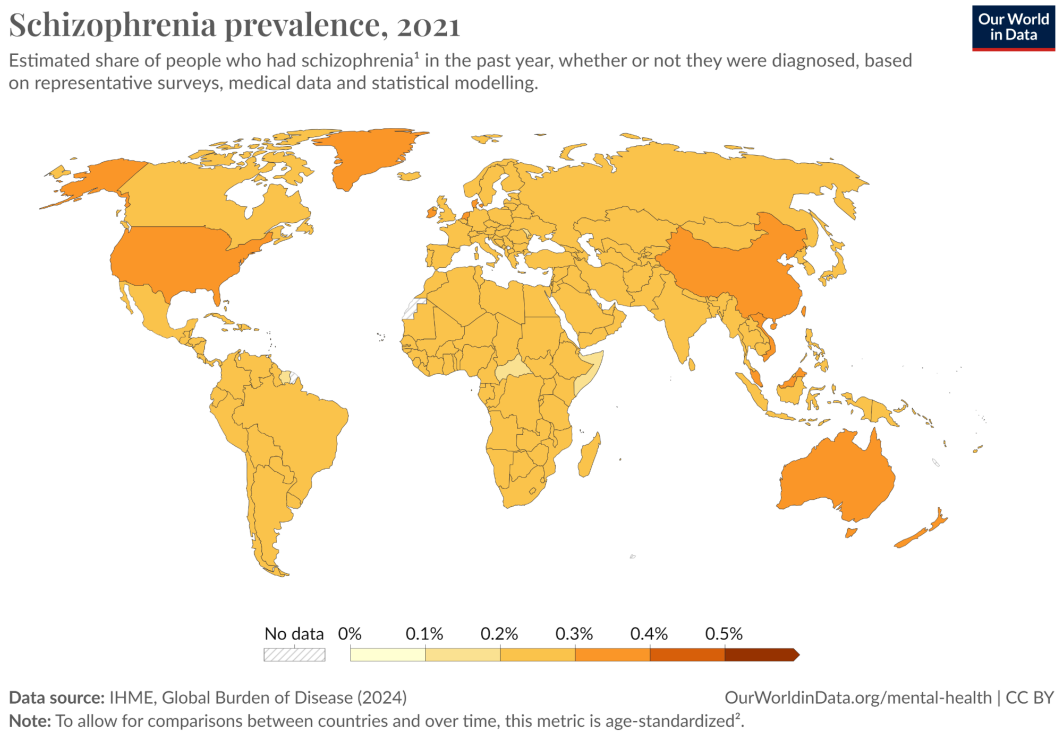

Figure 2: Estimated share of people who had schizophrenia in the past year, whether or not they were diagnosed, based on representative surveys, medical data and statistical modelling, (adapted from [15]).

Before understanding the quantitative impact of schizophrenia in society, it is important to clarify that these costs can be divided into two important groups, direct and indirect costs. Direct costs include the cost of hospitalisation, outpatient follow-up, residential and daycare, pharmaceutical interventions, laboratory testing, and social security payments. Indirect costs correspond to the impact of the patient's disability to work or the loss of productivity, indirect costs associated with time, and services caregivers contribute to their patients, some family members have to leave or sacrifice their own careers. (16)

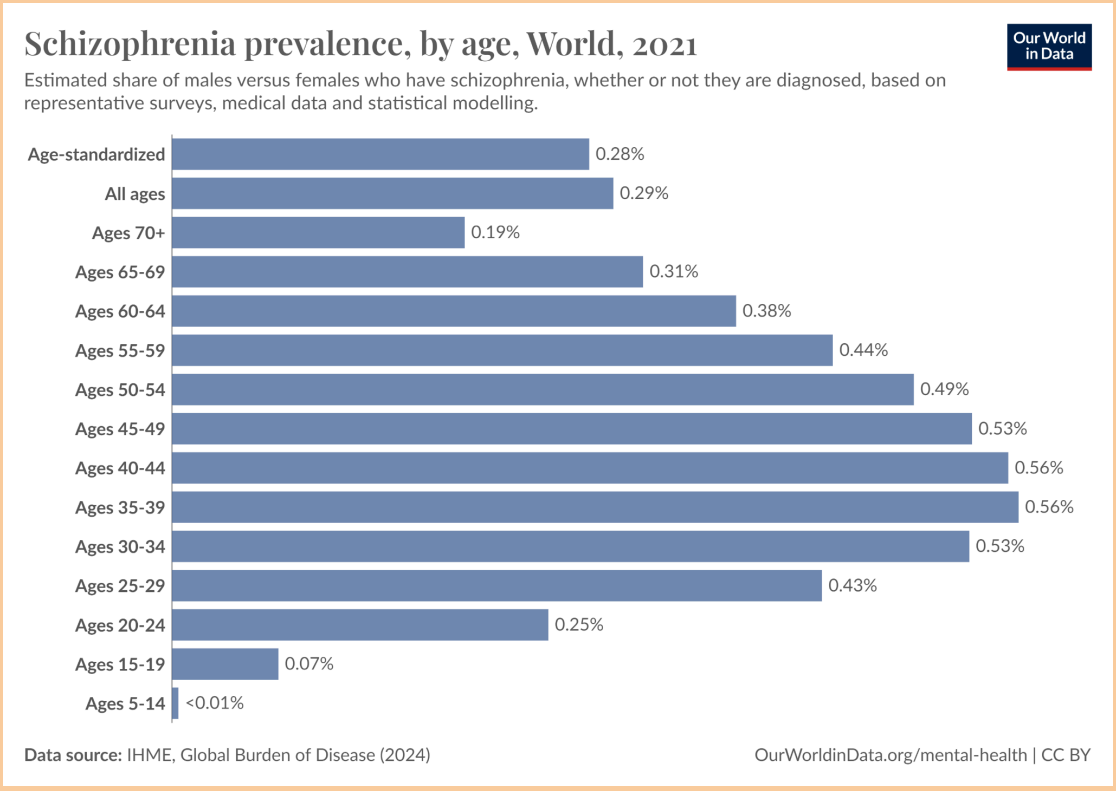

Figure 3: Estimated share of males versus females who have schizophrenia, whether or not they are diagnosed, based on representative surveys, medical data and statistical modelling, (adapted from [17]).

The WHO estimated that in Western nations, the direct costs (medical or non-medical) associated with schizophrenia range from 1.6% to 2.6% of overall healthcare expenditures, which in turn account for between 7% and 12% of the Gross National Product (GNP). Specifically in the United States, the economic burden of schizophrenia is found to be more than US$60 billion per year. According to the study, based on a total of 56 articles, 80% were conducted in high-income countries, the annual costs of schizophrenia were estimated to be between US$94 million to US$102 billion, which means 0.02%-5.46% of Gross Domestic Product (GDP). The study concludes that schizophrenia has a substantial economic burden on society, mainly driven by high indirect costs. (11) Having this in mind it is important to improve this reality and find solutions to improve the schizophrenia situation. By controlling symptoms of schizophrenia, patients can lower their risk of mental impairment and as a consequence, they can feel more included in society. By enhancing their ability to work and improving their productivity, individuals may

require less support from caregivers. It would be very important to expand psychological therapies and psychoeducational approaches for both patients. Additionally, raising awareness about schizophrenia and promoting public health education about this condition could be highly beneficial. (16)

Schizophrenia is a complex disease with causes that involve a combination of genetic and environmental factors. It has a strong genetic basis, with heritability estimated at around 80%. Despite the fact that it was not identified any single gene or variant as the sole cause, there were identified 270 schizophrenia-associated loci which include genes involved in dopamine synthesis, calcium channel regulation, immunity, and glutamate neuroreceptors. (18) Genetics has a clear influence on schizophrenia, but non-genetic factors contribute to the illness, and evidence suggests ways to find potential subgroups of subjects at higher risk. It is essential to recognise the importance of environmental influences, such as pregnancy and birth complications (bleeding during pregnancy, emergency caesarean section or exposure to viruses and other infectious agents contracted during pregnancy), advanced parental age, trauma and social adversities and social class and isolation. None of these risk factors alone are sufficient to develop schizophrenia, but rather, it is the interaction between genetic and environmental factors that is key. (19)

The symptoms of schizophrenia can be divided into positive symptoms (delusions and hallucinations) which tend to relapse and remit; negative symptoms (impaired motivation, reduction in spontaneous speech, and social withdrawal) and cognitive impairment (problems with a person's ability to think, learn, remember, use judgement, and make decisions), and both of these last two types of symptoms tend to be chronic and can impact long-term social function. (20)

1.3.1 Schizophrenia treatment

1952 marked the psychopharmacology era since the first-generation antipsychotic (FGAs) chlorpromazine was introduced in the market. While chlorpromazine, haloperidol, or fluphenazine have been proven effective in relieving positive symptoms of the disease, they are less effective in addressing negative symptoms. (21) They are also known as typical new-generation Antipsychotic Drugs (APDs) and have a high affinity for and act as full antagonists at D2 receptors, and are associated with many side effects, especially Extrapyramidal Side Effects (EPS) and

hyperprolactinemia. (22) EPS are the most common adverse drug effects patients experience with agents that block the receptor of dopamine within the mesolimbic and mesocortical pathways of the brain. EPS symptoms consist of dystonia, akathisia, drug-induced parkinsonism and tardive dyskinesia, interfering with the quality of life of the patients, which can lead to abandonment of therapy, and may result in disease relapse and re-hospitalisation. (23)

Continued interest in advancing this field led to the development of clozapine in the early 1960s, which showed good efficacy and a lack of EPS. However, its use is complicated by the risk of agranulocytosis, leaving individuals vulnerable to infections. Intending to develop safer profile drugs, the early 1990s saw the additional introduction of newer drugs in the market, known as second-generation antipsychotics (SGA), such as olanzapine, risperidone, quetiapine, ziprasidone, and aripiprazole, which became the mainstay of schizophrenia treatment, because, evidence showed that core illness symptoms were improved significantly more with olanzapine and risperidone than haloperidol. Olanzapine improved negative symptoms significantly more than haloperidol, and risperidone and aripiprazole improved negative symptoms significantly more than haloperidol. Additionally, rates of patients reporting adverse events were 11 to 20 percent higher with haloperidol compared to aripiprazole, risperidone, and ziprasidone, besides, evidence indicates a higher rate of withdrawal from the study due to adverse events with haloperidol. (21,24)

However, in some reviews, it was concluded that SGAs didn't fulfil the expectations of EPS-free antipsychotic drugs. Studies are not clear to confirm the superiority in efficacy and tolerability, and SGAs in general have lower liability to cause EPS than FGAs but with great variations within the class. (21)

The evidence on FGAs and SGAs shows a lot of heterogeneity and limited clinical trial data, so it is not possible to note preference between these medication classes. The selection of antipsychotic medication should depend on several factors such as drug formulation, drug interactions and metabolism, pharmacokinetic properties, side-effect profile and patient's treatment-related preferences and prior treatment responses. The initiation of treatment with antipsychotic medication has the goal of reducing acute symptoms to return individuals to their baseline level of functioning. Maintenance treatment will serve to prevent recurrence of symptoms and maximise quality of life. (25)

On September 26th, in the latest updates, FDA approved a new Drug for Treatment of Schizophrenia with a new mechanism of action. (26) Xanomeline and trospium chloride are the APIs of the new drug that was studied in two 5-week clinical studies. Xanomeline seems to activate specific muscarinic receptors in specific areas of the brain related to schizophrenia symptoms due to the presence of too many or too few important chemical messengers. Xanomeline may help in adjusting the levels of these important chemical messengers. On the other hand, Trospium chloride may specify the work of xanomeline to the brain, by avoiding this medicine of working outside the brain. (27) The data is still limited and it is important to collect more information about the safety and efficacy profile of this drug relative to others. However, it is a great development on schizophrenia treatment and it promises to improve patients lives by reducing not just the positive, but also the negative symptoms, while reducing the side effects. (28)

1.4 Risperidone

Risperidone is indicated in the treatment of schizophrenia and other mental health disorders such as bipolar disorder, aggressive or agitated behaviour in children (over 5 years) or young people with learning disabilities, aggressive or agitated behaviour in adults with Alzheimer's disease, and it is one of the most widely used SGAs. It is called a serotonin/dopamine antagonist because it is a strong postsynaptic dopamine receptor antagonist and it also acts as a 5-HT2A antagonist. (29) Risperidone works by reducing activity in the dopaminergic and serotonergic pathways in the brain because it has a high binding affinity for serotonergic 5-HTA receptors and binds to D2 receptors with a low affinity, which is contrary to first-generation antipsychotic drugs, which can justify the reduction in EPS. (30)

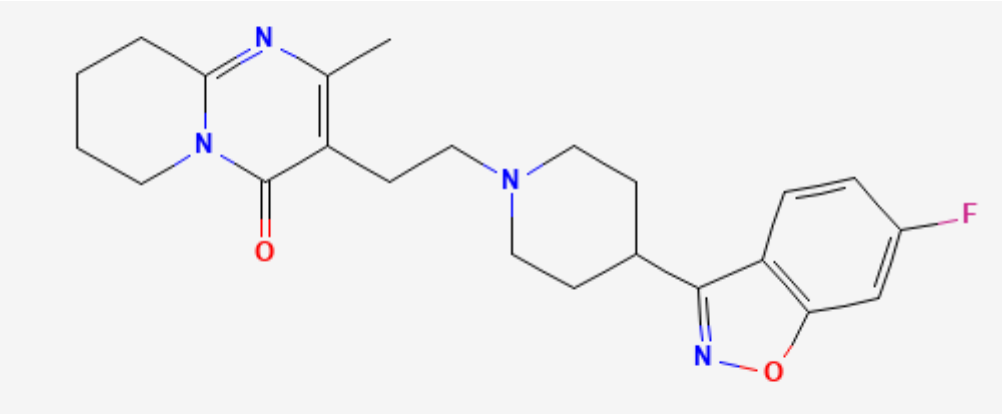

Figure 4 : Chemical structure of risperidone, (adapted from [31]).

Risperidone exists in oral formulations, such as tablets, orally disintegrating tablets, and liquid formulation, and in long-acting injections, as risperidone microspheres. (30) Risperidone is metabolised by hepatic cytochrome P450 2D6 isoenzyme in 9-hidroxy-risperidone, which has the same pharmacological activity as risperidone. Risperidone's bioavailability in oral formulation is 70%. (30) Risperidone is rapidly distributed in the body, with a volume of distribution 1-2L/kg. Risperidone and 9-hydrxy-risperidone link to albumin and alpha-1-acid glycoprotein at 90% and 77% rate, respectively. On the excretion topic, 70% of the dose is excreted in the urine (35-45% being inactive metabolites) and 14% through faeces a week after the administration. (30) After oral administration in patients with psychotic diseases, risperidone has an elimination half-life around 3 hours, compared with 24 hours of the half-elimination of 9-hydroxy-risperidone. (30)

Table 1: Physical and chemical properties of risperidone. (30, 32)

Definition	3-[2-[4-(6-Fluoro-1,2-benzisoxazol-3-il)-piperidinil]etil]-2- -metil-6,7,8,9-tetra-hidro-4H-pirido[1,2-a]-4-pirimidinona.
Chemical Formula	C23H27FN4O2
ATC Code	N05AX08
Appearance	White or almost white powder
Molecular Weight (Mr)	410.5
Solubility	Practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in ethanol (96 per cent) . It dissolves in dilute acid solutions. It shows polymorphism.
Melting Point	170 ºC
logP	3.27
logS	-3.4
pka (Strongest Basic)	8.76
Maximum Absorbance	238 nm

1.5 Effect of solubilisation

It has been estimated that 40% of drugs approved in the market and almost 90% of drugs in development have a poorly soluble characterisation. The research of modified formulations and strategies to overcome the low solubilisation of existing drugs can show a great solution to this problem by improving formulations and

enhancing therapeutic excellence. (33) Plentiful solubilisation techniques are utilised to promote the aqueous solubility of poorly soluble drugs. The conventional approaches are explained in Table 2. (34)

Table 2. Conventional approaches of solubilisation techniques. (34)

Solubilisation techniques	Concept
Micronisation - Milling; - Supercritical fluid technology; - Microprecipitation - Microcrystallisation.	Convert particles into uniform small sizes (<5 μm in diameter). Particle size is a direct factor that affects the solubilisation of drugs. If the size is reduced, the surface area increases and the dissolution enhances.
Inclusion complexes - Kneading method; - Physical mixing; - Co-precipitation method; - Solvent evaporation method.	Inclusion of a nonpolar molecule into the cavity of another. Cyclodextrins are the most commonly used. They have a hollow and lipophilic core cavity that makes it possible attach lipophilic molecules in a variety of intermolecular interactions
Solid dispersions - Hot melt method; - Hot-melt extrusion; - Solvent evaporation method; - Spray drying	Solid dispersions are solid materials with a hydrophilic matrix and a hydrophobic drug.
Prodrugs	Inactive with improved aqueous solubility compound that can be converted into the active parent drug via biotransformation
Co-Crystallisation - Evaporation; - Sublimation; - Melt growth; - Slurry preparation.	A co-crystal consists in a crystalline structure where two or more stoichiometric amounts of noncovalent forces hold two or more electrically neutral substances together
Supercritical Fluid Technology	Considered as a green technology. The most common solvents used by industries in the preparation of soluble versions of drugs are toxic. The supercritical solution technology overcomes this problem, because it is free of organic solvents and heavy metals.

Nanotechnology plays a fundamental role in enhancing drug solubilization through the formulation of nanocarriers. The most commonly used are nanoemulsions, dendrimers, micelles, liposomes, solid lipid nanoparticles, polymeric nanoparticles, inorganic nanoparticles, and carbon nanotubes. (34) These drug delivery systems can retain, evade, target, and release its drug load with controllable and well-regulated kinetics to the diseased cells, while preserving the healthy tissues. (35)

1.6 Polymeric micelles

Nanosystems are used for solving various issues related to drug delivery. Their significant potential lies in optimising formulations by increasing the water-solubility of drugs and improving the stability of APIs. An increased drug retention in tissues, higher cellular internalisation, and targeted drug delivery and controlled drug release will improve the efficacy of therapies and their safety too. (36)

Polymeric micelles are nano-sized drug delivery systems that stand out for their core-shell structure building from the self-assembly of amphiphilic block copolymers as associated colloids in the form of micelles above the Critical Micellar Concentration (CMC) (concentration at which surfactant molecules in a solution begin to aggregate to form micelles). (37) Critical Micelle Temperature (CMT) is another parameter of polymeric micelles and corresponds to the temperature at which micelles start to form. (38)

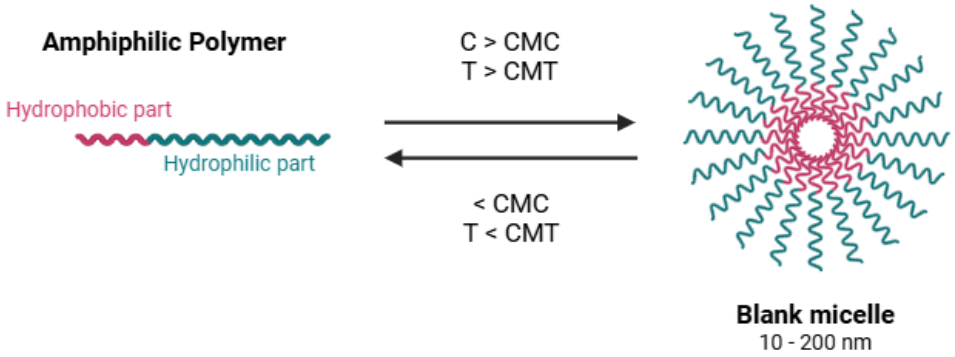

Figure 5: CMC and CMT roles on polymeric micelle formation. Created in BioRender.com

The drugs can be encapsulated into their micellar cores in a molecularly dispersed form. (37) This feature provides an advantage in improving the water solubility for hydrophobic APIs, because the drug will be protected in its hydrophobic core, and the hydrophilic shell will support and stabilise the core in the aqueous medium, enhancing the water solubility of the polymers. (36)

Micelles can be formed from more than one copolymer. They are called mixed micelles, which means the formation of polymeric micelles from two or more co-polymers with or without small molecular weight surfactants. Mixed polymeric micelles can be a better choice due to their high resistance to the metabolic effect and elimination of different organs, which means long-term stability of the particle in the circulation and reduction of liver-dependent metabolic effects. (37)

With the development of nanotechnology, smart nanoparticles that are thermosensitive can be developed. Thermosensitive nanoparticles were designed to release the drugs at the desired site by stimulation of the temperature. It is employed on chemotherapeutic drugs that will act on the desired tissues while preserving the healthy tissues at normal temperature. Nanoparticles that incorporate thermosensitive polymers will be sensitive to a change in temperature and their conformation will modify resulting in them releasing their load in a controlled way. (35)

Thermosensitive polymeric solutions have an important characteristic, the Critical Solution Temperature (CST). There are two types of this parameter: The Upper Critical Solution Temperature (UCST) and the most commonly used the Lower Critical Solution Temperature (LCST). (35) LCST is the critical temperature at which polymeric micelles decrease in size and get a uniform manner. Below LCST micelles will have different size distributions. The LCST micellar corona suffers mechanical distortions and the encapsulated active substances can be quickly released because of water infiltration in the micellar core. (38)

Some parameters for characterising polymeric micelles are important to have in mind and to understand this investigation project. First is the Z-average, the average size of each micelle in the mixture. The size of drug delivery systems influences pharmacokinetics, tissue distribution, and clearance, and the smaller the particle size, the better the bioavailability of the drug. Second is the Polydispersity Index (PdI), which is used to measure the extent of polydispersity of the sample that will

characterise the size distribution of the polymeric micelle. A PdI close to 0 indicates a narrow size distribution (monodisperse) desirable for consistent and predictable performance, while a higher PdI suggests a broader distribution of particle sizes (polydisperse). (39) Zeta potential is also important to keep in mind as it is defined as the number of charges it carries. A high absolute zeta potential indicates a high degree of electrostatic repulsion between particles, it decides the dispersion control as it reflects the degree of repulsion between adjacent particles, which helps in preventing particle aggregation or coagulation cryoprotectant concentration. (40)

1.6.1 Pluronic F-127 and TPGS

Pluronic F-127 is a hydrogel biomaterial with the basic tri-block structure polyoxyethylene-polyoxypropylenepolyoxyethylene (PEO-PPO-PEO), where PEO is hydrophilic and PPO is lipophilic. Between 37ºC, the hydrophobic PPO block dehydrates and crosslinks with the hydrophilic PEO block forming spherical microgels. The safety, bio adhesiveness, stability, and capacity to form gels at low concentration at body temperature contribute to the appeal of Pluronics. (41) However, F127 has little effect on P-gp efflux pump and practically does not transport across the membrane due to its relatively hydrophilic property. (42)

D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble anionic derivative of vitamin E, resulting from the conjugation of vitamin E succinate with polyethylene glycol (PEG). The advantages of this component are solubilisation of poorly soluble drugs, enhancing the cellular uptake of the drugs and prolonging the blood circulation time of the drugs. It is a potent biological response modifier that inhibits P-gp by reducing drug efflux from cells and facilitating drug transport across cellular barriers, to highlight the brain endothelium. However, the CMC of TPGS is relatively high which may make TPGS micelles dissociate in the plasma. (42)

The strengths of one compensate for the weaknesses of the other; thus, the combination of both could prove highly beneficial in facilitating the transport of the API across the Blood-Brain Barrier (BBB). (42)

1.7 Nasal administration and delivery

The administration route is a very important factor to consider when planning a drug formulation. Not all drugs can achieve the Central Nervous System (CNS) in a

considerative therapeutic concentration because of their molecular nature. (43) BBB is a complex multicellular structure with extremely low permeability. This fact restricts the movement of molecules between the blood and the neural tissues to make sure homeostasis is maintained. Due to the tight junctions of the BBB, the passage of drugs into the brain when they are administered via oral or injection routes is highly limited. Nasal drug delivery has shown great results because it is a systemic route of administration that increases drug absorption and bioavailability, and at the same time it minimises adverse side effects of medication. (44) There are some pathways where drugs can reach the brain through nasal administration: blood circulation, trigeminal nerve, olfactory nerve, and olfactory mucosal epithelial pathways. The nasal mucosa is a highly vascularised large surface with rich capillary network, where low-molecular-weight lipophilic drugs can be absorbed. Moreover, this is a very innervated area with olfactory nerves and trigeminal nerves, which are two possible nasal pathways for drugs to reach the CNS. Finally, through the olfactory mucosal epithelial, it is possible for drugs to reach the BBB due to the transcellular and paracellular transport. After the molecules cross the basement membrane close to axons in the lamina propria they can be transported to the cerebrospinal fluid or the lymphatic and systemic circulation. (37) Due to these four possibilities, there will be a rapid bypass of the BBB where the intact carrier or drug can travel directly to the brain. (43) Another advantage of the nasal route that enhances bioavailability is that the API is protected from the first-pass metabolism in the liver, and side effects will be reduced. (45)

There are some important factors that molecules must meet to guarantee a proper nasal absorption:

- Drug molecular weight and size;
- Drug solubility in the nasal secretions;
- Drugs with high lipid solubility have good compatibility with the nasal mucosa;
- Drug viscosity. (37)

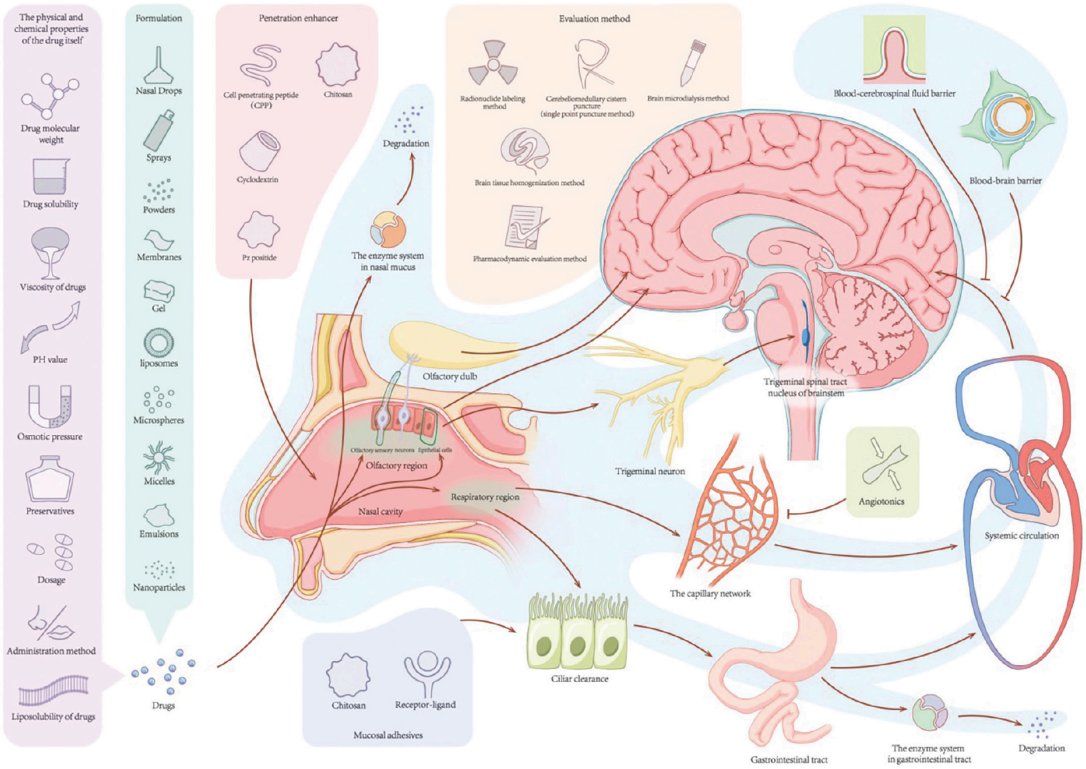

Figure 6: Nasal anatomy, nose-to-brain drug delivery system and factors that affect the absorption of drugs into the brain through the nose, (adapted from [44])

2 Materials and Methods

2.1 Materials

Pluronic F-127 (F127 (PEG_{95}-PPG_{62}-PEG_{95} (poly(ethylene-glycol)-*block*-poly(propylene-glycol)-*block*-poly(ethylene-glycol)); average molecular weight: 12,500 Da) and tocophersolan (TPGS, D-α-tocopheryl polyethylene glycol 1000 succinate) were used as polymeric micelle forming agents and were acquired from Sigma-Aldrich Co., Ltd. (Budapest, Hungary). Risperidone (RIS, 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one) was utilised as a model active substance and also bought from Sigma-Aldrich Co., Ltd. Hydroxypropyl-methylcellulose (HPMC, average molecular weight: 10 kDa, viscosity 80 – 120 cP, 2%w/v in water (20 °C)),

and chemicals for simulated nasal electrolyte solution (SNES) and saline solutions were also acquired from Sigma-Aldrich Co., Ltd. SNES was composed via the following: 2.98 g/l of potassium chloride, 8.77 g/l of sodium chloride, 0.59 g/l of anhydrous calcium chloride in 1000 ml of purified water, adjusted to a pH of 5.6.

2.2 Formulation of thermosensitive polymeric micelles

At first, 5 mg of RIS was dissolved in 0.5 ml of ethanol, which solution was mixed with 1 - 1 ml of 20 mg/ml TPGS and Pluronic F127 each. The mixture was kept under constant stirring for an hour at ambient temperature and with a stirring speed of 500 rpm. The mixture then was placed in round-bottom flasks and the solvents were evaporated via rotary evaporation using a Büchi R-210 (Büchi, Flawil, Switzerland) rotation vacuum evaporator. The thin film was hydrated via 10 ml of purified water at ambient temperature. If needed, samples were freeze dried. Freeze-drying of solid products was carried out using ScanVac CoolSafe 100–9 (LaboGene, ApS, Lynge, Denmark) laboratory apparatus. Vials were filled with 1.5 ml of polymeric micelles solution, then freeze-dried at −40 °C and 0.013 mbar for 12 h. Secondary drying was carried out at 25 °C and 0.013 mbar for 3 h.

2.3 Quantitative analysis of risperidone via high-performance liquid chromatography

The quantification of RIS was performed via an Agilent Infinity HPLC (Agilent Technologies, Santa Clara, CA, USA). The stationary phase was a Kinetex® C18 column (5 µm, 150 mm × 4.6 mm (Phenomenex, Torrance, CA, USA). The mobile phases were the following: (A) acidic purified water (0.1% w/v formic acid) and (B) acidic acetonitrile (0.1% w/v formic acid). 10 µl was the injection volume. The separation was performed isocratically for 6 min. The chromatograms were detected at 280 ± 4 nm using a UV–Vis diode array detector. The retention time was 2.65 min. The chromatograms were evaluated using ChemStation B.04.03. Software (Agilent Technologies, Santa Clara, CA, USA). The limit of detection (LOD) and limit of quantification (LOQ) of RIS were 4.78 and 15.75 ppm, respectively. The calibration was performed between 5 to 100 µg/ml, where the determined coefficient of determination (R2) value was 0.9999.

2.4 Dynamic light scattering measurements

To determine micelle size (expressed as the average hydrodynamic diameter), micelle size distribution (as polydispersity index) and zeta potential, dynamic light scattering (DLS) measurements were performed via the Malvern Nano ZS Zetasizer (Malvern Instruments, Worcestershire, UK). For the blank mixed micellar systems, the samples were placed in folded capillary cells and left inside during the whole measurement process. The average hydrodynamic diameter was registered at every 1 °C increment between 25 – 40 °C. Between each measurement, 2 min lag time was provided for proper heating. A refractive index of 1.335 was used. For the risperidone-loaded polymeric micelles, a refractive index of 1.677 was used. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.5 Determination of encapsulation efficiency

The encapsulation efficiency (EE) was measured via the indirect method. After dissolving the samples in purified water, the RIS-loaded micelles were separated from the aqueous media via centrifugation utilising a Hermle Z323 K high-performance refrigerated centrifuge (Hermle AG, Gosheim, Germany) at 13,500 rpm, 4 °C for 30 min. The supernatant was diluted 5-fold with methanol and quantitative measurements took place via HPLC. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD. The EE was calculated via the following equation:

$$EE\ (\%) = \frac{initial\ RIS\ (mg) - measured\ RIS\ in\ the\ supernatant\ (mg)}{initial\ RIS\ (mg)}\ x\ 100$$

2.6 Determination of thermodynamic solubility

The saturation method was applied to determine the solubility of the RIS-loaded polymeric micelles. Freeze-dried samples were dissolved in 1 ml of purified water followed by the addition of further samples until visible saturation. The system was kept stirred for 72 h (25 °C, 250 rpm) and covered with parafilm to reduce evaporation. Then, the solutions were filtered through a 0.22 μm pore-sized PES (polyethersulfone) membrane. The filtrate's RIS concentration was measured via

HPLC. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.7 Colloidal stability of polymeric micelles against various conditions

2.7.1 Colloidal stability against pH

Freeze-dried samples were dissolved in purified water at various pH conditions (5.0 – 7.0 with an increment of 0.5). pH setting was performed via the addition of 1 n hydrochloric acid or 0.05 M sodium hydroxide solution. Then micelle size and micelle size distribution were measured via dynamic light scattering from 25 to 40 °C with an increment of 1 °C. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.7.2 Colloidal stability against dilution

Freeze-dried samples were dissolved in simulated nasal electrolyte solution at the target concentration of 1 mg/ml of risperidone. Then, they were further dissolved to 2-,5-,10-, 25- and 50-fold with SNES. Micelle size and micelle size distribution were measured via dynamic light scattering from 25 to 40 °C with an increment of 1 °C. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.7.3 Colloidal stability against ionic strength

Freeze-dried samples were dissolved in various sodium chloride solutions in the ionic strength range of the following: 0.25 – 0.50 – 0.75 – 1.0 – 2.0 M. Micelle size and micelle size distribution were measured via dynamic light scattering from 25 to 40 °C with an increment of 1 °C. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.7.4 Colloidal stability against viscosity

Freeze-dried samples were dissolved in various hydroxypropyl-methylcellulose solutions in the viscosity range of 5 – 25 cP with an increment of 5 cP. Micelle size and micelle size distribution were measured via dynamic light scattering from 25 to

40 °C with an increment of 1 °C. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.8 Stability tests at ambient temperature

Freeze-dried samples were prepared and a part of them was dissolved in SNES. Weekly measurements were performed with the freeze-dried powders and the dissolved solutions. DLS measurements were performed to determine micelle size and size distribution at 25 and 36.5 °C. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.9 In vitro nasal applicability studies

2.9.1 In vitro mucoadhesion study

Tensile tests were performed with a TA-XT Plus Texture analyser (Metron Ltd., Budapest, Hungary) equipped with a 5 kg load cell and a cylinder probe with a diameter of 1 cm. Polymeric micelle solutions were placed in contact with a filter paper disc with 25 mm diameter wetted with 50 µl of an 8% w/w porcine mucin (Mucin type III, Merck Ltd., Budapest, Hungary) dispersion in SNES (pH 5.6). 20 µl of the samples was attached to the filter paper fixed on the cylinder probe and placed in contact with the mucin dispersion. A 2500 mN preload was used for 3 min, then, the cylinder probe with the solutions was moved upwards at a prefixed speed of 2.5 mm × min−1 to separate the attaching surfaces. Adhesive force (F, mN) and adhesive work (A, mN × mm) were applied for the evaluation of the mucoadhesivity of the nanoemulsions. All measurements were carried out 5 times with individual batches (n = 5), and the results are expressed as the average ± SD.

2.9.2 In vitro drug release study

The modified paddle method was used to determine the drug release tendency of the formulation and the reference RIS suspension. The formulation was placed in dialysis tubes (Spectra/Por® Dialysis Membrane with a 3.5 kD MWCO value (Spectrum Laboratories Inc., Rancho Dominguez, CA,USA)). The tubes were placed in 50 ml of SNES. The temperature was set at 36.5 °C and the paddle was rotated at 100 rpm. At predetermined time points, 250 µl of aliquots were taken up to 60 min. The

quantification of RIS was performed via HPLC. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

2.9.3 In vitro drug permeation study

A modified Side-bi-Side® horizontal diffusion cell was applied to determine the passive diffusion of RIS in the formulation. A regenerated cellulose membrane (Whatman™ (0.45 μm, 25 mm)) was impregnated with isopropyl myristate with a surface of 0.785 cm^2 and used as the diffusion barrier between the acceptor and donor compartments. Both compartments' volumes were 9.0 ml and the temperature was set at 36.5 °C. The donor phase consisted of SNES and the acceptor phase was a pH 7.4 PBS. At predetermined time points, 50 μl of aliquots were taken from the acceptor chamber, and then concentration was measured via HPLC. The taken aliquots were immediately replaced with the same volume of pH 7.4 PBS. Flux (J), as the cumulative permeability per time point, was calculated via the following equation:

$$J = \frac{m_t}{A \, x \, t}$$

where m_t is the permeated drug amount at t time and A is the surface of the membrane. All measurements were carried out in triplicate with individual batches (n = 3), and the results are expressed as the average ± SD.

3 Results and Discussion

3.1 Determination of the low critical solution temperature of blank mixed polymeric micelles

LCST of blank polymeric micelles, which means the polymeric micelle without adding risperidone, was measured in the range of 25 to 40ºC. LCST needs to fit the nasal drug delivery criteria, by being below the general nasal cavity temperature (36.5ºC), which is slightly below body temperature, and above the ambient temperature (25ºC), so it is possible to distinguish the storage stability and the administration conditions. By checking Graphic 1, it is possible to determine that

LCST is 29ºC because the polymeric micelle size is considerably bigger below this temperature. It can be observed that the size remains equal above 29ºC.

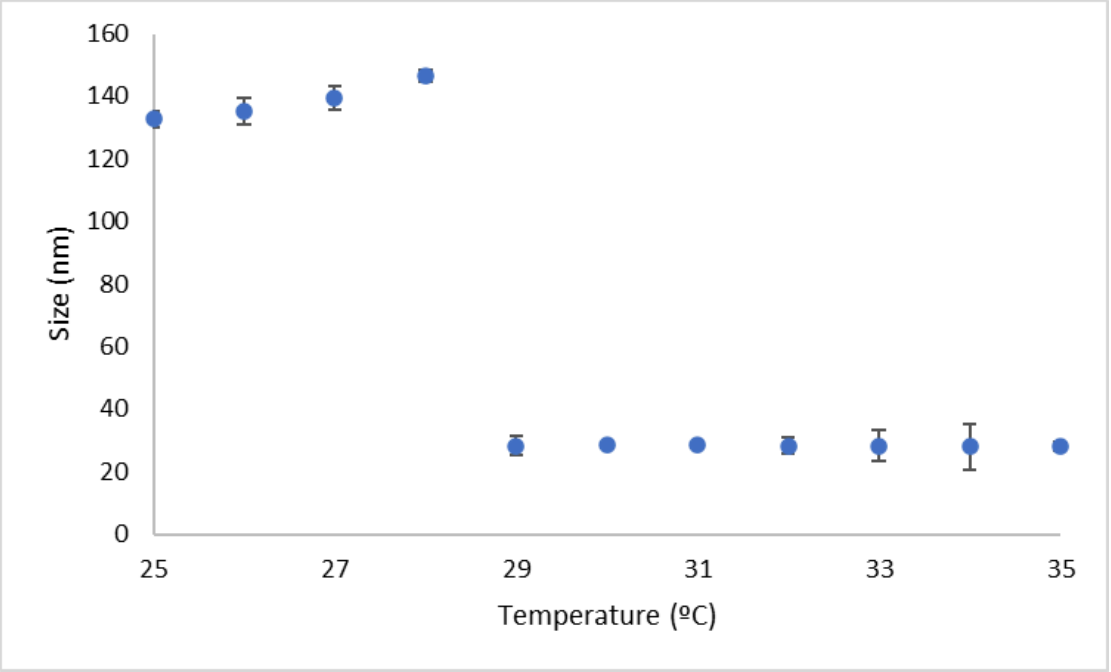

Figure 7: Determination of the LCST of the blank mixed polymeric micelles.

3.2 Characterisation of the effect of drug loading on the thermosensitive polymeric micelles

The next step was to characterise the drug-loading effect on the polymeric micelle. After adding risperidone to the micelle system, the LCST was investigated again at the same temperature ranges and conditions, and it remained at 29ºC, so it is possible to affirm that drug loading did not change the thermosensitive behaviour of the micelle system.

Table 3 shows more parameters that were measured to characterise this polymeric micelle mixture. At ambient temperature, the micelle Z-Average, or Average Hydrodynamic Diameter, was 137.4 nm, considerably bigger when compared with 28.1 nm at nasal cavity temperature. Both of these sizes fit the criteria of polymeric micelle size (10 - 200 nm), but it is always important to keep in mind that the smaller the size of the micelles, the better the formulations' bioavailability.

The PdI was measured at 25ºC and 36.5ºC. At nasal cavity temperature PdI is lower than the reference value of 0.300, making sure that polymeric micelles will have a monodisperse distribution in the formulation.

Zeta Potential shows a value of -2.14, which is almost around 0, which could be a negative factor because of particle aggregation. Although, in this case, it does not play a major role due to the non-ionic nature of this polymeric formulation.

Table 3: Characterisation of drug-loaded polymeric micelles.

Characterisation	Results	Reference Values/Observations
LCST (ºC)	29	-
d (nm, 25ºC)	137.4 ± 4.9	10 - 200 nm
d (nm, 36.5ºC)	28.1 ± 2.6	10 - 200 nm
PdI (25ºC)	0.368 ± 0.027	<0.3
PdI (36.5ºC)	0.064 ± 0.009	<0.3
Zeta potential (mV)	-2.14 ± 0.27	
pH	6.17 ± 0.38	
S (mg/ml) - Risperidone	0.017 ± 0.006	Thermodynamic solubility
S (mg/ml) - Polymeric micelle	4.586 ± 0.177	Thermodynamic solubility
Encapsulation efficiency (%)	82.3± 3.7	

Risperidone was efficiently loaded into the core of the polymeric micelle with an encapsulation efficiency reaching 82.31% of the amount of drug in the feed.

Solubility was also investigated and it was found that the solubility of the system of risperidone in the polymeric micelle (4.586 mg/ml) was 269.77 times higher compared with the solubility of risperidone by itself (0.017 mg/ml). It confirms that nanocarrier systems are a great option to increase the bioavailability of drugs in the organism, as it is a good nano technique of solubilisation. In the case of polymeric micelles, it is explained with the encapsulation of the hydroponic API in the micelle core, which is also hydrophobic and is protected by its hydrophilic shell, supporting and stabilising the core in the aqueous medium and enhancing the water solubility. All these characteristics reinforce the formulation's potential efficacy.

3.3 Stability of polymeric micelle against pH values

It is important to check the stability of the formulation against the various pH values because the drug will face different pH values across its pathway in the organism, as the pH of the nasal cavity (5.5 - 6.5) and blood (7.35 - 7.45) are different. Table 4 shows that the LCST values that were obtained in different pH remained the same.

Table 4: LCST values in different pH.

pH	LCST (ºC)
5	29
5.5	29
6	29
6.5	29
7	29

The effect of pH on the micelle size and the micelle distribution was investigated and the results can be analysed in Table 5 and Table 6, respectively.

In Table 5, size was measured in different temperatures and from pH 5 to pH 7. It was not observed considerable changes in this parameter. The same can be said for PdI which was also measured as shown in Table 6.

Table 5: pH effect on polymeric micelles size.

pH	5	5.5	6	6.5	7
Temperature (ºC)	**Micelle Size (nm)**				
25	138.4 ± 7.4	142.4 ± 5.7	139.7 ± 6.2	145.9 ± 9.3	138.5 ± 1.2
26	141.2 ± 11.2	137.5 ± 4.1	136.5 ± 4.9	140.4 ± 6.1	137.5 ± 5.7
27	129.7 ± 6.4	141.1 ± 9.5	136.2 ± 10.6	143.2 ± 14.1	139.2 ± 8.8
28	135.6 ± 7.2	136.6 ± 7.4	140.5 ± 7.8	141.2 ± 6.8	138.1 ± 7.1
29	29.3 ± 1.2	29.4 ± 1.5	28.6 ± 1.4	28.1 ± 1.2	27.9 ± 0.9
30	28.6 ± 1.5	29.1 ± 1.4	28.4 ± 2.4	28.2 ± 1.6	27.6 ± 1.3
31	28.6 ± 1.3	28.3 ± 1.5	27.9 ± 1.6	28.4 ± 2.1	27.8 ± 1.1
32	29.1 ± 1.2	28.4 ± 1.3	28.3 ± 1.2	28.1 ± 0.7	27.2 ± 0.6
33	28.4 ± 1.4	28.5 ± 0.9	28.4 ± 1.6	27.6 ± 0.5	27.8 ± 0.8
34	28.5 ± 1.5	28.4 ± 1.6	28.2 ± 1.1	27.5 ± 1.1	27.9 ± 1.5
35	28.5 ± 1.3	28.6 ± 0.6	27.6 ± 1.9	27.1 ± 1.4	27.6 ± 1.3
36	28.3 ± 1.2	27.9 ± 1.2	27.7 ± 1.4	27.2 ± 1.3	28.3 ± 1.1
37	28.5 ± 1.1	27.7 ± 1.6	27.5 ± 1.3	27.3 ± 0.5	28.2 ± 1
38	28.4 ± 1.5	27.9 ± 1.3	27.9 ± 1.5	27.6 ± 0.8	27 ± 1.6
39	28.6 ± 1.5	27.8 ± 1.8	27.6 ± 0.9	27.5 ± 0.9	27.1 ± 1.3
40	28.6 ± 1.2	28 ± 1.4	27.8 ± 1.6	27.3 ± 1.4	26.9 ± 0.4

Table 6: pH effect on PdI of polymeric micelles.

pH	5	5.5	6	6.5	7
Temperature (ºC)	**PdI**				
25	0.413 ± 0.098	0.376 ± 0.074	0.315 ± 0.041	0.331 ± 0.055	0.328 ± 0.026
26	0.398 ± 0.085	0.351 ± 0.066	0.364 ± 0.026	0.319 ± 0.081	0.316 ± 0.049
27	0.364 ± 0.073	0.414 ± 0.096	0.324 ± 0.008	0.321 ± 0.064	0.374 ± 0.044
28	0.345 ± 0.053	0.364 ± 0.045	0.349 ± 0.038	0.309 ± 0.049	0.322 ± 0.028
29	0.086 ± 0.009	0.074 ± 0.013	0.089 ± 0.009	0.065 ± 0.007	0.054 ± 0.002
30	0.084 ± 0.014	0.054 ± 0.011	0.068 ± 0.019	0.063 ± 0.015	0.067 ± 0.009
31	0.104 ± 0.008	0.098 ± 0.018	0.098 ± 0.005	0.074 ± 0.01	0.103 ± 0.005
32	0.143 ± 0.005	0.14 ± 0.003	0.128 ± 0.005	0.101 ± 0.009	0.107 ± 0.004
33	0.105 ± 0.006	0.085 ± 0.012	0.141 ± 0.009	0.132 ± 0.005	0.098 ± 0.002
34	0.119 ± 0.019	0.043 ± 0.008	0.082 ± 0.002	0.131 ± 0.004	0.088 ± 0.005
35	0.054 ± 0.015	0.049 ± 0.003	0.077 ± 0.009	0.119 ± 0.009	0.103 ± 0.009
36	0.068 ± 0.01	0.084 ± 0.001	0.092 ± 0.004	0.095 ± 0.008	0.085 ± 0.005
37	0.049 ± 0.008	0.035 ± 0.004	0.094 ± 0.005	0.107 ± 0.004	0.081 ± 0.004
38	0.089 ± 0.005	0.098 ± 0.003	0.104 ± 0.008	0.106 ± 0.014	0.079 ± 0.006
39	0.068 ± 0.014	0.103 ± 0.006	0.129 ± 0.01	0.116 ± 0.011	0.093 ± 0.008
40	0.054 ± 0.004	0.111 ± 0.011	0.099 ± 0.06	0.078 ± 0.09	0.092 ± 0.01

It can be concluded that pH does not influence formulations' stability as the LCST values are the same between pH 5 and 7, plus the size decreased in all studied pH values. When comparing the results with Table 3, which are the results of the in-water dissolved formulation, it can be seen that they are similar so no changes in the stability were observed at different pH values.

3.4 Stability of polymeric micelle against dilution

The next step was to investigate the stability of the particle size and distribution against volume expansion. In Table 7 it can be observed that LCST increased when the formulation suffered dilutions from 25-fold.

Table 7: LCST variation against dilution.

Dilution	LCST (ºC)
2x	29
5x	29
10x	29
25x	31
50x	33
100x	33

The results in Table 8 show that Z-Average does not vary considerably. The same profile can be observed for PdI in Table 9. The fact that LCST increases with the dilution expresses that it will need a higher temperature to be released from the polymeric micelle and the stability of the formulation is compromised.

Table 8: Stability of polymeric micelles' size against dilution

Dilution	2x	5x	10x	25x	50x	100x
Temperature (°C)	**Micelle Size (nm)**					
25	153.1 ± 7.6	136.4 ± 9.9	149.3 ± 8.3	149.5 ± 7.9	144.4 ± 3.9	153.2 ± 11.2
26	142.4 ± 4.1	135.1 ± 4.3	143.5 ± 4.7	143.7 ± 6.2	145.3 ± 11	146.1 ± 7.4
27	145.3 ± 4.6	130.8 ± 5.6	138.4 ± 10.6	139.7 ± 9.1	135.1 ± 7.1	148.9 ± 6
28	137.9 ± 5.5	136.7 ± 6	142.4 ± 3.5	142.3 ± 4.3	138.4 ± 6.8	139.5 ± 9.8
29	28.5 ± 1.2	28.1 ± 1.5	28.3 ± 0.4	145.5 ± 10.2	141.4 ± 9.4	143.3 ± 3.7
30	28.9 ± 4.1	28.4 ± 1.2	27.8 ± 0.3	142.9 ± 5.8	136.4 ± 5.3	146.7 ± 10.9
31	28.4 ± 2.1	28.5 ± 1.1	28.9 ± 0.5	28.5 ± 0.4	139.9 ± 8.7	144.1 ± 5.5
32	28.3 ± 1.6	27.9 ± 0.6	28.5 ± 0.4	28.3 ± 1.9	140.5 ± 4.1	147.4 ± 8
33	28.2 ± 1.4	28.6 ± 0.1	28.3 ± 1.5	28.2 ± 1	28.1 ± 0.5	27.4 ± 1.5
34	28.4 ± 1.5	28.4 ± 1.5	28.4 ± 1.2	28.4 ± 1.2	28.3 ± 1.4	27.3 ± 1.9
35	28.1 ± 1.2	28.2 ± 1.4	28.1 ± 0.3	28.6 ± 1.8	27.4 ± 0.8	28 ± 0.7
36	28.4 ± 0.9	28.5 ± 1.2	28.4 ± 0.6	28.4 ± 0.3	27.9 ± 1.1	28.1 ± 1.3
37	28.2 ± 1.6	28.8 ± 1.4	28.6 ± 2.1	27.4 ± 1.6	28.3 ± 1.7	27.3 ± 1.7
38	28.3 ± 1.6	28.5 ± 1.6	28.3 ± 1.6	27.9 ± 1.1	27.6 ± 0.9	27.1 ± 1.2
39	28.6 ± 1.4	27.6 ± 1.2	28.5 ± 1.1	28.1 ± 0.6	27.4 ± 1.2	27.3 ± 0.5
40	28.6 ± 1.5	27.9 ± 1.3	28.1 ± 0.5	27.6 ± 1.3	27.9 ± 0.6	27.4 ± 0.2

Table 9: Stability of polymeric micelles' PdI against dilution

Dilution	**2x**	**5x**	**10x**	**25x**	**50x**	**100x**
Temperature (°C)	**PdI**					
25	0.315 ± 0.065	0.329 ± 0.023	0.417 ± 0.047	0.424 ± 0.018	0.343 ± 0.014	0.42 ± 0.026
26	0.364 ± 0.054	0.445 ± 0.072	0.335 ± 0.011	0.332 ± 0.056	0.427 ± 0.089	0.331 ± 0.077
27	0.322 ± 0.015	0.338 ± 0.034	0.446 ± 0.064	0.395 ± 0.095	0.37 ± 0.037	0.38 ± 0.042
28	0.31 ± 0.087	0.379 ± 0.091	0.36 ± 0.082	0.439 ± 0.029	0.337 ± 0.053	0.429 ± 0.009
29	0.141 ± 0.015	0.137 ± 0.009	0.145 ± 0.011	0.325 ± 0.073	0.441 ± 0.02	0.344 ± 0.0083
30	0.132 ± 0.007	0.131 ± 0.018	0.125 ± 0.013	0.41 ± 0.044	0.355 ± 0.068	0.451 ± 0.031
31	0.114 ± 0.019	0.125 ± 0.006	0.087 ± 0.021	0.105 ± 0.02	0.399 ± 0.01	0.325 ± 0.055
32	0.125 ± 0.011	0.096 ± 0.022	0.097 ± 0.007	0.114 ± 0.008	0.334 ± 0.092	0.322 ± 0.051
33	0.078 ± 0.021	0.106 ± 0.014	0.096 ± 0.019	0.1 ± 0.015	0.124 ± 0.012	0.126 ± 0.01
34	0.097 ± 0.01	0.112 ± 0.008	0.091 ± 0.022	0.112 ± 0.018	0.114 ± 0.015	0.131 ± 0.017
35	0.076 ± 0.014	0.093 ± 0.015	0.078 ± 0.006	0.13 ± 0.011	0.108 ± 0.022	0.127 ± 0.019
36	0.121 ± 0.017	0.099 ± 0.01	0.085 ± 0.017	0.132 ± 0.006	0.116 ± 0.007	0.122 ± 0.022
37	0.115 ± 0.008	0.104 ± 0.021	0.086 ± 0.02	0.127 ± 0.021	0.111 ± 0.002	0.125 ± 0.012
38	0.101 ± 0.022	0.116 ± 0.017	0.086 ± 0.015	0.122 ± 0.013	0.098 ± 0.008	0.13 ± 0.015
39	0.094 ± 0.013	0.085 ± 0.005	0.101 ± 0.014	0.125 ± 0.019	0.114 ± 0.014	0.115 ± 0.021
40	0.088 ± 0.02	0.089 ± 0.019	0.094 ± 0.02	0.129 ± 0.009	0.127 ± 0.019	0.123 ± 0.012

This study is very important because during circulation in human blood vessels, the particles will suffer dilutions, and studying particle size stability will reinforce the security and efficiency of the formulation. Observing these results, even though the LCST value increased, they are acceptable since polymeric micelles can experience dilutions of 15-fold to 20-fold in the nasal cavity, and it can be seen that the desired size can still be achieved.

3.5 Stability of polymeric micelle against ionic strength

The aim of investigating the stability of polymeric micelles against ionic strength was carried out to understand how the ionic forces could affect the formulation by adding physiological saline to it and confirm if NaCl could perform as an excipient for this nasal formulation. Analysing Table 10, it can be observed that LCST increases when ionic strength increases. NaCl is used to mimic physiological conditions and is known due to its stability properties in polymeric micelles. It can work as an excipient for nasal formulations but regarding our results, when LCST increases too much the formulation can lose stability, like when the ionic strength is 2M. For that reason, to have an optimised formulation and to maintain the LCST values, the levels of NaCl should be added in a controlled way to the formulation making sure that ionic forces do not bypass 1 M.

Table 10: Ionic strength effect on LCST values.

Ionic strength (M)	LCST (ºC)
0.25	29
0.5	29
0.75	29
1	31
2	35

Table 11: Ionic strength effect on polymeric micelles' size.

Ionic Strength (M)	0.25	0.5	0.75	1	2
Temperature (ºC)	**Micelle Size (nm)**				
25	137.7 ± 6.4	137.8 ± 4.7	159.4 ± 7.5	146.1 ± 1.1	148.5 ± 3.5
26	150.6 ± 8.5	138.8 ± 5.2	134.4 ± 1.9	148.9 ± 2.9	157.8 ± 4.9
27	135.7 ± 1.4	156 ± 2.5	151.2 ± 5.6	153.8 ± 6.6	142 ± 5.7
28	149 ± 3.5	148.1 ± 3.4	156.3 ± 7.2	159.2 ± 4.5	156.4 ± 3
29	27.5 ± 1.2	28.6 ± 3.9	27.6 ± 2.4	159.4 ± 1.9	148.9 ± 1.2
30	28.1 ± 2.5	27.1 ± 1.8	28.2 ± 4.3	151 ± 3.7	133.9 ± 3.7
31	26.9 ± 3.7	29 ± 4.1	27 ± 0.6	28.7 ± 4.1	149.6 ± 4.5
32	28.7 ± 4	27.7 ± 2.3	28.8 ± 3	27.4 ± 1.7	158.2 ± 6.3
33	27.2 ± 2.9	28.3 ± 4.1	26.9 ± 1.7	29.1 ± 3.8	139.3 ± 0.5
34	28.9 ± 0.6	28.9 ± 1.5	27.3 ± 3.4	28.3 ± 0.8	138.6 ± 4.2
35	27.8 ± 1.7	26.8 ± 3.2	29 ± 2.5	27.9 ± 2.2	28.9 ± 0.9
36	28.4 ± 2.5	27.5 ± 0.8	28.5 ± 1.7	28.5 ± 4.1	27.3 ± 3.2
37	27 ± 3.8	28.2 ± 4.4	27.1 ± 2.2	26.8 ± 1.5	29.2 ± 2.1
38	29.1 ± 1.2	27.9 ± 1.6	28.9 ± 4.3	27.6 ± 3.6	27.7 ± 4.2
39	28 ± 4.2	28 ± 0.9	28.1 ± 1.9	28 ± 1.1	28.1 ± 1.6
40	27.3 ± 2.3	27.4 ± 3.1	27.8 ± 3.4	27.2 ± 2.9	26.9 ± 3.3

Table 12: Ionic strength effect on polymeric micelles' distribution.

Ionic Strength (M)	0.25	0.5	0.75	1	2
Temperature (ºC)	**PdI**				
25	0.325 ± 0.067	0.402 ± 0.068	0.413 ± 0.044	0.355 ± 0.037	0.323 ± 0.034
26	0.412 ± 0.055	0.349 ± 0.049	0.305 ± 0.009	0.309 ± 0.057	0.36 ± 0.016
27	0.345 ± 0.009	0.358 ± 0.051	0.364 ± 0.063	0.346 ± 0.011	0.349 ± 0.027
28	0.379 ± 0.043	0.364 ± 0.022	0.317 ± 0.057	0.317 ± 0.031	0.314 ± 0.034
29	0.045 ± 0.008	0.098 ± 0.014	0.125 ± 0.014	0.409 ± 0.019	0.366 ± 0.051
30	0.122 ± 0.023	0.034 ± 0.003	0.156 ± 0.019	0.378 ± 0.025	0.398 ± 0.032
31	0.112 ± 0.002	0.143 ± 0.017	0.061 ± 0.002	0.05 ± 0.019	0.072 ± 0.011
32	0.099 ± 0.011	0.011 ± 0.005	0.103 ± 0.003	0.081 ± 0.022	0.115 ± 0.024
33	0.076 ± 0.019	0.16 ± 0.02	0.112 ± 0.034	0.128 ± 0.006	0.048 ± 0.005
34	0.034 ± 0.007	0.086 ± 0.006	0.145 ± 0.019	0.067 ± 0.013	0.14 ± 0.003
35	0.011 ± 0.002	0.07 ± 0.002	0.099 ± 0.014	0.034 ± 0.016	0.09 ± 0.017
36	0.128 ± 0.016	0.054 ± 0.004	0.154 ± 0.027	0.05 ± 0.008	0.034 ± 0.005
37	0.14 ± 0.012	0.125 ± 0.015	0.048 ± 0.011	0.111 ± 0.1	0.122 ± 0.006
38	0.067 ± 0.001	0.143 ± 0.023	0.112 ± 0.03	0.015 ± 0.003	0.081 ± 0.027
39	0.156 ± 0.018	0.045 ± 0.003	0.131 ± 0.013	0.142 ± 0.017	0.127 ± 0.031
40	0.112 ± 0.021	0.112 ± 0.019	0.16 ± 0.011	0.056 ± 0.012	0.155 ± 0.017

In Tables 11 and 12 it can be analysed the micelle size and distribution, respectively. In both cases, the values of micelle size and PdI do not improve but, at the same time, do not have a big variation with the increase of ionic strength, compared with the results of Table 3, about the in-water dissolved formulation.

3.6 Stability of polymeric micelle against added viscosity

The next study is about adding viscosity enhancers in the formulation to understand the behaviour of polymeric micelles against these conditions. Viscosity will be important to optimise the application of the polymeric micelles in the nasal mucosa and to ensure the integrity of the formulation under several conditions.

The results in this field were very optimistic. By evaluating Table 13 it can be said that LCST did not change and the polymeric micelle size was reduced, as can be observed in Table 14, and polymeric micelles present a good distribution as the results in Table 15 are almost < 0.300. Thus, the addition of viscosity enhancers did not affect the stability of the formulation.

Table 13: Effect of viscosity on LCST values.

Viscosity Agent (cP)	LCST (ºC)
5	29
10	29
15	29
20	29
25	29

Table 14: Viscosity effect on polymeric micelles' size.

Viscosity (cP)	5	10	15	20	25
Temperature (ºC)	**Micelle Size (nm)**				
25	134.2 ± 4.9	132.5 ± 6.1	138.9 ± 4.8	152.3 ± 4.5	146.7 ± 12.3
26	139.7 ± 7.5	149 ± 2.7	144.1 ± 4.2	135.4 ± 6.7	149.8 ± 7.5
27	153.6 ± 6.8	155.3 ± 1.2	150.2 ± 6.9	153.5 ± 0.5	141.4 ± 6.8
28	140.1 ± 4.1	145.8 ± 6.4	147.9 ± 7.7	149.3 ± 6.4	133.8 ± 9.4
29	28.5 ± 1.8	29.2 ± 0.5	28.6 ± 3.6	27.8 ± 1.4	29.2 ± 2.2
30	27.7 ± 3.1	27.5 ± 3.2	27.5 ± 0.2	27.9 ± 2.7	27.5 ± 2.6
31	28.7 ± 4.2	28.6 ± 1.9	27 ± 4.5	27.2 ± 3.1	27.3 ± 1.1
32	28.5 ± 2.4	28.7 ± 0.6	27.9 ± 2.3	27.5 ± 1.9	29.2 ± 3.7
33	28.1 ± 1.5	27.2 ± 4.2	28.8 ± 3.1	27.9 ± 2.9	27.8 ± 0.7
34	28.1 ± 4.3	29 ± 1.3	27 ± 2.5	27.2 ± 0.7	28.7 ± 3.5
35	27.7 ± 2.1	27.5 ± 2.7	28.4 ± 1.9	27.5 ± 3.2	27.6 ± 1.6
36	27.9 ± 0.8	29.2 ± 4.1	27.5 ± 3.7	28.5 ± 1.1	27.4 ± 4.2
37	28.3 ± 3.7	28.5 ± 3.8	28.4 ± 1.4	28.4 ± 4.2	27.6 ± 2.3
38	28.3 ± 2.3	27.2 ± 1.5	28.6 ± 4.1	28.5 ± 2.5	28.1 ± 0.6
39	28.7 ± 1.1	28.4 ± 0.8	27.8 ± 2.1	26.7 ± 1.3	28.6 ± 3.4
40	27.9 ± 4.1	28.6 ± 3.5	28.9 ± 3.9	28.2 ± 0.8	27.5 ± 1.4

Table 15: Viscosity effect on polymeric micelles' distribution.

Viscosity (cP)	5	10	15	20	25
Temperature (ºC)	**PdI**				
25	0.48 ± 0.071	0.462 ± 0.046	0.334 ± 0.006	0.37 ± 0.062	0.425 ± 0.031
26	0.39 ± 0.039	0.421 ± 0.037	0.446 ± 0.094	0.435 ± 0.029	0.355 ± 0.047
27	0.371 ± 0.031	0.335 ± 0.022	0.462 ± 0.051	0.54 ± 0.037	0.47 ± 0.058
28	0.329 ± 0.068	0.472 ± 0.019	0.423 ± 0.034	0.467 ± 0.017	0.331 ± 0.009
29	0.066 ± 0.004	0.059 ± 0.022	0.087 ± 0.014	0.142 ± 0.007	0.12 ± 0.011
30	0.034 ± 0.015	0.078 ± 0.001	0.092 ± 0.017	0.132 ± 0.015	0.143 ± 0.018
31	0.0115 ± 0.013	0.048 ± 0.012	0.133 ± 0.009	0.099 ± 0.018	0.056 ± 0.003
32	0.071 ± 0.019	0.145 ± 0.003	0.141 ± 0.006	0.079 ± 0.002	0.087 ± 0.031
33	0.15 ± 0.009	0.119 ± 0.018	0.056 ± 0.013	0.064 ± 0.008	0.01 ± 0.007
34	0.013 ± 0.005	0.098 ± 0.021	0.015 ± 0.001	0.14 ± 0.001	0.12 ± 0.013
35	0.092 ± 0.002	0.087 ± 0.02	0.066 ± 0.012	0.078 ± 0.013	0.044 ± 0.005
36	0.054 ± 0.013	0.099 ± 0.014	0.131 ± 0.003	0.133 ± 0.007	0.051 ± 0.003
37	0.13 ± 0.014	0.041 ± 0.006	0.154 ± 0.01	0.097 ± 0.004	0.155 ± 0.025
38	0.101 ± 0.002	0.11 ± 0.006	0.078 ± 0.013	0.119 ± 0.02	0.154 ± 0.034
39	0.09 ± 0.007	0.123 ± 0.03	0.142 ± 0.014	0.061 ± 0.013	0.111 ± 0.039
40	0.14 ± 0.01	0.054 ± 0.014	0.039 ± 0.007	0.01 ± 0.002	0.118 ± 0.004

3.7 Stability of polymeric micelle during time

Freeze-dried samples were analysed for 1 month to evaluate the formulation stability through the variation of Z-Average and PdI values during one month. In Table 16 it is possible to understand that at ambient temperature, the particle size increased by 14.3 nm, while in nasal cavity temperature it increased by 1 nm in 14 days. Although the particle size in ambient temperature varied considerably, it met the requirement of polymeric micelles sizes (10 nm - 200 nm). About PdI, at ambient temperature, it increased by 0.049, and at body temperature, PdI increased by 0.016 in 14 days, and it remained <0.300, the reference of adequate PdI value.

Table 16: 1 Month stability test on freeze-dried powder state.

Days	Z-Average* (nm) - 25ºC	Z-Average* (nm) - 36.5ºC	PdI* - 25ºC	PdI* - 36.5ºC
0	135.4 ± 6.7	28.3 ± 2.4	0.348 ± 0.028	0.039 ± 0.012
7	139.5 ± 5.4	28.7 ± 1.5	0.366 ± 0.057	0.048 ± 0.009
14	143.4 ± 2.8	29.3 ± 0.6	0.371 ± 0.063	0.055 ± 0.01
21	138.6 ± 3.6	28.6 ± 2.8	0.318 ± 0.019	0.05 ± 0.003
28	149.7 ± 4.5	29.1 ± 1.2	0.397 ± 0.049	0.044 ± 0.007

* Data means SD (n = 3 independent formulations).

Beyond freeze-dried samples, the formulation was analysed in the liquid state. On Table 17 it is possible to understand that at ambient temperature, the particle size increased by 41.7 nm, a much greater increase than the freeze-dried power state. At nasal cavity temperature it increased 9.4 nm in 21 days. Although the particle size showed a considerable variation, it met the requirement of polymeric micelles sizes (10 nm - 200 nm). About PdI, at ambient temperature, it increased by 0.064 in 21 days, and at body temperature, PdI increased by 0.052, and it remained <0.300, the reference of adequate PdI value.

Table 17: 1 month stability test on liquid state.

Days	Z-Average* (nm) - 25ºC	Z-Average* (nm) - 36.5ºC	PdI* - 25ºC	PdI* - 36.5ºC
0	137.5 ± 6.1	28.1 ± 0.2	0.355 ± 0.034	0.051 ± 0.008
7	163 ± 2.8	28.3 ± 3.7	0.379 ± 0.011	0.068 ± 0.016
14	158.4 ± 5.9	32.3 ± 5.6	0.402 ± 0.067	0.108 ± 0.23
21	167.8 ± 3.7	37.5 ± 1.3	0.419 ± 0.029	0.129 ± 0.022
28	179.2 ± 11.3	26.8 ± 4.5	0.399 ± 0.058	0.133 ± 0.004

Comparing both formulation states, freeze-dried powder showed better and more consistent results, which can be explained by the freeze-drying technology. It is a lyophilisation process that allows the development of dry or solid-state presentations by three steps: freezing, primary drying and secondary drying steps, preserving structural integrity and enabling rapid rehydration. (46)

3.8 Mucoadhesion study

Mucoadhesion study is a crucial parameter to confirm the formulation absorption in the nasal mucosa. This test will mimic the nasal mucosa conditions as the mucoadhesive force represents the adhesive properties of the nasal surface. On this hand, the higher the value of this parameter, the stronger force will be required to separate polymeric micelles from the nasal cavity, This is important to increase the contact of the formulation in the nasal mucosa and increase the bioavailability by increasing the contact with this surface and so on the absorption of polymeric micelles. The values are high enough to assure this connection but not too much to be permanent which will not affect the rapid drug release profile.

Mucoadhesive work is correlated with the distance it requires from complete separation between the nasal mucosa and formulation and the same can be said about the results.

Table 18: Mucoadhesion studies of mucoadhesive force and mucoadhesive work.

Mucoadhesive force (mN)	841.128 ± 42,565
Mucoadhesive work (mN x mm)	70.354 ± 5,809

3.9 *in vitro* drug release study

in vitro drug release assays are made to predict stability and drug release *in vivo* of the drug-loaded polymeric micelle. By checking the results in Graphic 2, the drug release profile of Risperidone was enhanced after encapsulation into the polymeric micelle.

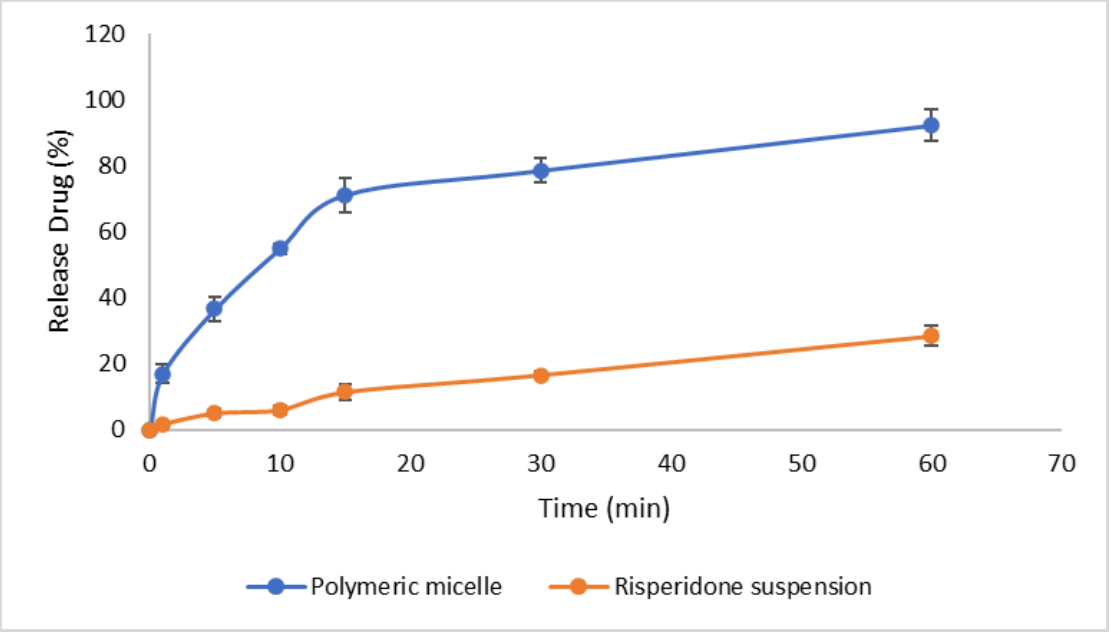

Figure 8: *in vitro* drug release determination of the polymeric micelle formulation profile comparing with risperidone profile reference.

3.10 *in vitro* drug permeability studies

The same result was observed in the *in vitro drug* permeability studies. These assays measure the rate at which a drug molecule can cross a simulated biological membrane. It was observed that risperidone presents an enhanced profile when encapsulated than in a solution.

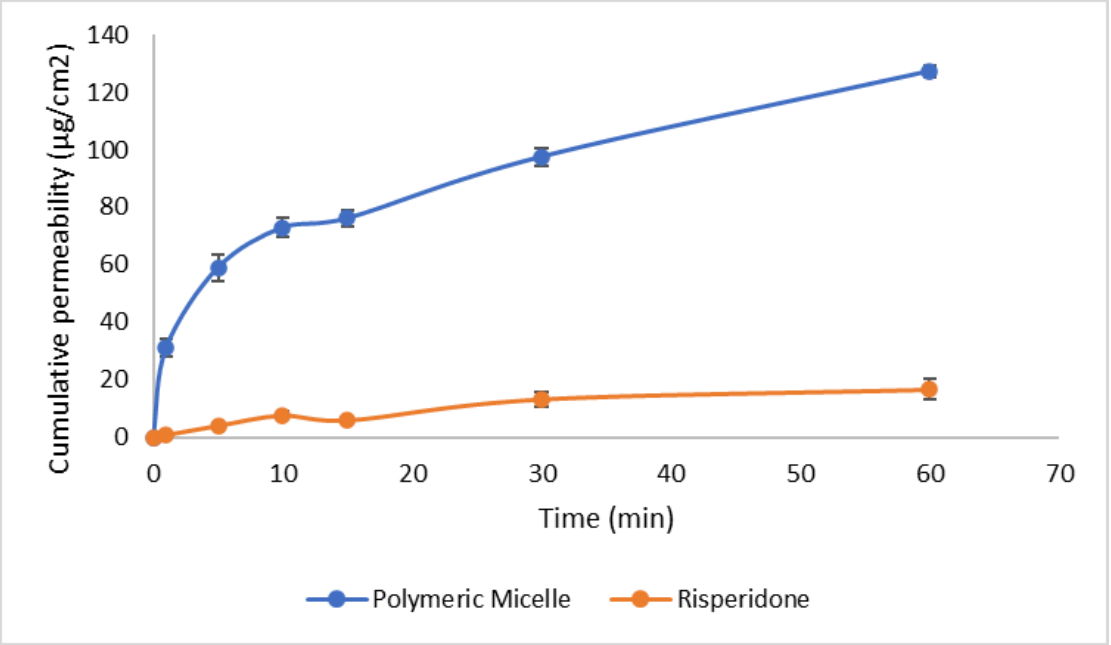

Figure 9: *in vitro* drug permeability determination of the polymeric micelle formulation profile comparing with risperidone profile reference.

Both of these last two studies confirm that nanocarriers, in this case, polymeric micelles, are a great option for administering insoluble drugs via the nasal route, because of their technology already explained throughout this project.

4 Conclusions

Schizophrenia affects approximately 24 million people worldwide and it is the most costly mental health condition per person globally. It is a severe psychiatric disorder that adversely affects the patient's life due to core symptoms, like persistent delusions, persistent hallucinations, disorganised thinking, and experiences of influence, passivity, or control. It is normally diagnosed in late teens and early 20s which leads to schizophrenia being on the top 15 leading causes of disability worldwide.

Schizophrenia treatments were freezed in time for decades and this year a new drug was finally approved. However, with the access data that existed before Xanomeline and trospium chloride approval, schizophrenia treatments were based on FGAs and SGAs, drugs that present some several side effects.

With this project it was aimed to develop and improve new risperidone (SGA) administration technologies through the nasal route due to several advantages of this option. Nasal administration promotes better drug bioavailability while reducing the side effects due to less metabolisation, like avoiding first-pass metabolism in the liver. Nasal mucosa is a highly vascularised large surface area with rich capillary network and innervated area with the olfactory nerves and trigeminal nerves, which are direct pathways to the Brain.

To achieve this goal the investigation focused on developing a formulation that contains TPGS and the Pluronic F127 as constituents of mixed micellar thermosensitive systems and how they could enhance the bioavailability of risperidone and improve pharmacological treatment in schizophrenia.

Polymeric micelles are nano-sized drug delivery systems that because of its technology they can carry risperidone improving the solubilisation of this insoluble drug. In this case it was utilised thermosensitive nanoparticles so the drug just can be released at a specific temperature. Several studies were conducted to test the viability, security and effectiveness of the formulation. First it was found the LCST (29ºC) of the blank mixture of polymeric micelles, which was good because it is above ambient temperature and below nasal cavity temperature. Then the drug-loaded polymeric micelles were characterised, and LCST value remained the same, so the introduction of Risperidone did not change the stability of the micelles. Next steps were to study

the formulation against different pH, several dilutions, the increase of ionic strengths and viscosity. LCST in pH and viscosity studies remained the same, so it can be concluded that these two parameters do not compromise the formulation stability. On dilution studies, LCST suffered changes when the concentration decreased, from dilutions of 25-fold LCST increased from 29ºC to 33ºC. However, the results are acceptable since polymeric micelles can experience dilutions of 15-fold to 20-fold in the nasal cavity. Ionic strength tests showed that physiological saline can be used as an excipient for nasal formulation, but higher than 2M it will not be good for the stability of the formulation, as the LCST increased greatly. The results of mucoadhesion studies were outstanding as the values of mucoadhesive forces and mucoadhesive work were high enough to assure the connection between the polymeric micelles and the nasal mucosa but not too much to be permanent so it does not affect the rapid drug release profile. In the end were carried out the in vitro drug release study and the in vitro drug permeability study where the drug release profile of Risperidone was enhanced after encapsulation into the polymeric micelle. It can be concluded that this formulation has a big potential of being used in clinical trials of phase I and eventually be out in the market.

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