Universidade de Lisboa

Faculdade de Farmácia

Effect of cellulose derivatives on the characteristics and nasal application of vinpocetine-loaded polymeric micelles

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Trabalho de Campo orientado pelo Professor Doutor Bence Sipos, Professor Auxiliar, Instituto de Tecnologia Farmacêutica e Assuntos Regulamentares, Faculdade de Farmácia, 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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It's the closing of a chapter but the opening of a new one.

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:

Este trabalho teve como objetivo o desenvolvimento e a otimização de um sistema de administração nasal utilizando micelas poliméricas carregadas com vinpocetina. A vinpocetina, um fármaco utilizado para melhorar a função cerebral particularmente em doenças neurodegenerativas como a do Alzheimer, enfrenta desafios relacionados com a sua fraca solubilidade em água e baixa biodisponibilidade. Foi avaliada a forma como diferentes derivados de celulose - especificamente hidroxipropilmetilcelulose (HPMC), carboximetilcelulose (CMC), metilcelulose (MC) e hidroxietilcelulose (HEC) - afetam as propriedades destas micelas e a sua aplicação na administração nasal. Estas formulações foram submetidas a um processo de secagem por aspersão, formando micelas com vinpocetina, que foram objeto de vários testes.

O produto obtido através do processo de secagem por aspersão foi caracterizado, determinando o tamanho das partículas e o teor do fármaco. As micelas foram também estudadas no estado líquido e, neste caso, foram determinados o seu tamanho, a distribuição, o potencial zeta, a eficiência de encapsulação e a solubilidade termodinâmica. Foram ainda efetuados estudos de aplicabilidade nasal *in vitro*, com estudos de libertação e permeação do fármaco.

A caraterização das partículas mostrou que as formulações com HEC e HPMC apresentavam as distribuições de tamanho de partículas mais uniformes. A eficiência de encapsulamento foi mais elevada com HEC a 0.5%, demonstrando um encapsulamento quase completo do fármaco. Já os estudos de libertação do fármaco *in vitro* indicaram que todas as formulações apresentaram uma libertação rápida nos primeiros 15-20 minutos, crucial para a administração nasal. Os testes de permeabilidade ao fármaco demonstraram ainda que concentrações mais baixas de HEC e HPMC proporcionaram uma permeabilidade superior, sugerindo uma janela de concentração eficaz entre 0.5% e 1.5%.

Estes resultados realçam o potencial dos derivados de celulose na otimização de micelas carregadas com vinpocetina para administração nasal. O estudo sugere que tais formulações podem ser desenvolvidas para melhorar a eficácia dos tratamentos em doenças neurodegenerativas.

Palavras-chave: administração nasal; derivados da celulose; micelas poliméricas; secagem por aspersão; vinpocetina.

Abstract:

This study aimed the development and optimization of a nasal drug delivery system using vinpocetine-loaded polymeric micelles, improving bioavailability and solubility. Vinpocetine, a drug utilized to enhance brain function and cerebral blood circulation, particularly for conditions like neurodegenerative diseases such as Alzheimer's Disease, dementia, faces challenges related to its poor water solubility and low bioavailability. The research investigates how different cellulose derivatives - specifically Hydroxypropyl-methylcellulose (HPMC), Carboxymethyl-cellulose (CMC), Methylcellulose (MC), and Hydroxyethyl-cellulose (HEC) - affect the properties of these polymeric micelles and their application in nasal drug delivery. These formulations were nano spray-dried, forming vinpocetine-loaded polymeric micelles, which were the subject of various tests.

The solid-state formulations were characterized, by determining the particle size and the drug content. The liquid state formulations were also analyzed and, in this case, the micelle size, micelle size distribution, zeta potential, encapsulation efficiency and thermodynamic solubility of each formulation were determined. *In vitro* nasal applicability studies were also done, with drug release and drug permeation studies. Solid-state characterization, including particle size determination via laser diffraction, showed that formulations with HEC and HPMC exhibited the most uniform particle size distributions. Encapsulation efficiency was highest with HEC at 0.5% w/v, demonstrating almost complete drug encapsulation. *In vitro* drug release studies indicated that all formulations showed rapid release within the first 15-20 minutes, crucial for nasal administration. Drug permeability tests further demonstrated that lower concentrations of HEC and HPMC provided optimal permeability, suggesting an effective concentration window between 0.5% and 1.5%.

These findings highlight the potential of cellulose derivatives in optimizing vinpocetine-loaded micelles for nasal delivery, supporting their viability for enhancing brain-targeted drug therapies. It was also crucial to determine the effect of these polymers on the nasal application, as they are commonly applied viscosity enhancers for liquid nasal dosage forms. The study suggests that such formulations could be further developed to improve the efficacy of treatments for neurodegenerative diseases.

Keywords: vinpocetine; nasal administration; polymeric micelles; cellulose derivatives; spray drying.

Abbreviations

APIs Active pharmaceutical ingredients
AD Alzheimer's disease
Aβ Amyloid-beta peptide’s
AVA Apovincamine acid
AUC Area under the curve
BCS Biopharmaceutics Classification System
BBB The blood-brain barrier
B-CSF-B Blood-cerebrospinal fluid barrier
Cmax Maximum concentration
CMC Carboxymethyl Cellulose
CNS Central Nervous System
CMC Critical micelle concentration
D-TRE D-trehalose-dihydrate
DLS Dynamic Light Scattering
EE Encapsulation efficiency
FDA Food and Drug Administration
HPLC High-performance liquid chromatography
HEC Hydroxyethyl Cellulose
DH Hydrodynamic diameter
HPMC Hydroxypropyl Methylcellulose
LOD Limit of detection
LOQ Limit of quantification
MC Methylcellulose
P188 Poloxamer 188
PBS Phosphate-buffered saline
PSD Particle size distribution
PDI Polydispersity index
PES Pore-sized polyether sulfone
R^2 Coefficient of linearity
SNES Simulated nasal electrolyte solution
SD Standard deviation
SP Soluplus®
SLS Statical Light Scattering
VP Vinpocetine

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1. Introduction

1.1 Aim of the project

This work was developed under the Erasmus+ Program, at the Institute of Pharmaceutical Technology and Regulatory Affairs, Faculty of Pharmacy, University of Szeged, in Szeged, Hungary, between the months of January and April of 2024. The aim of the project was to investigate the effect of cellulose derivatives on nasal application vinpocetine-loaded polymeric micelles formulations, developing nasal drug delivery systems using polymeric micelles to enhance vinpocetine's solubility and bioavailability and investigate the role of cellulose derivatives in improving the characteristics and effectiveness of these systems.

The project was already ongoing and as a result, some studies had already been carried out by the institute's research team. The base formulation for nasal administration had already been developed and was used to carry out my work. The full project was focused on the development of a nasal drug delivery system using polymeric micelles to increase the bioavailability of vinpocetine, a drug used to enhance brain function, particularly for conditions like neurodegenerative diseases (e.g., Alzheimer's disease). Vinpocetine (VP) was taken as a model drug (representative active substance) because of its well-understood pharmacological properties and due to its low bioavailability, low wettability, and low water solubility. The study aims to investigate how different cellulose derivatives— Hydroxypropyl Methylcellulose (HPMC), Carboxymethyl Cellulose (CMC), Methyl Cellulose (MC) - and Hydroxyethyl Cellulose (HEC) - affect the properties of these micelles and their application in nasal drug delivery. The focus was on using spray drying techniques to produce vinpocetine-loaded polymeric micelles that can efficiently deliver the drug through the nasal route, enhancing its absorption and bioavailability by bypassing the blood-brain barrier.

It was, therefore, necessary to investigate the spray-dried powder, the characteristics of the nanoparticles based on the different spray-dried products and nasal administration.

1.2 Neurodegenerative diseases

Neurodegenerative diseases are progressive conditions that impact the neurons in the central nervous system (CNS), leading to their damage and death. Neurodevelopmental disorders, such as intellectual disability, autism spectrum disorder, and attention-deficit/hyperactivity disorder, arise from disruptions in essential neurodevelopmental processes (1). Neurodevelopmental disorders show diverse patterns in acquiring motor, cognitive, linguistic, and socio-emotional

skills, impacting functioning across various contexts. The severity of these clinical manifestations varies, with different levels of cognitive and adaptive functioning disability.

The complexity of neurodegenerative diseases is evident in their diverse symptoms and variable rates of progression, compounded by the incomplete understanding of their underlying biochemical pathways, making their treatment challenging.

Alzheimer's disease (AD) stands out as the leading neurodegenerative condition, considered the main cause of global memory disorder. It can be defined as a slowly progressive neurodegenerative disease characterized by neurotic plaques and neurofibrillary as a result of amyloid-beta peptides (Aβ) and an abnormal form of protein tau accumulation in the brain structures (2). It causes memory loss, brain atrophy, and cerebrovascular changes. This neurodegenerative condition is associated with several risk factors, including aging, genetics, head injuries, vascular diseases, infections, and environmental factors. The exact cause of pathological changes in AD remains unknown, and currently, there is also no universally accepted theory to explain its pathogenesis (3).

The U.S. Food and Drug Administration (FDA) has approved five drugs for the treatment of AD - rivastigmine, galantamine, donepezil, memantine, and a combination of memantine with donepezil (3)However, none of these can effectively slow down or stop the process of neuron damage and loss characteristic of AD. Also, there are no drugs specifically approved by the FDA to treat behavioral and psychiatric symptoms that may develop in the moderate and severe stages of AD. When non-pharmacological therapies prove ineffective and these symptoms pose a risk of harm to the individual or others, physicians may resort to prescribing medications approved for similar symptoms in other conditions as hallucinations, aggression, and agitation. However, studies have indicated that certain antipsychotics are linked to a heightened risk of stroke and mortality in individuals with dementia.

The treatment of neurodegenerative and neurodevelopmental disorders faces challenges due to the blood-brain barrier and the blood-cerebrospinal fluid barrier. These barriers limit the effective passage of drugs from the systemic circulation into the central nervous system (4).

At present, over 46 million people worldwide are living with dementia, a number expected to rise to 131 million by 2050. AD is the most common cause of dementia, accounting for an estimated 60% to 80% of cases. Also, dementia imposes a substantial financial strain on society, with an estimated annual cost of 141 billion euros across Europe, and informal care accounts for 56% of these expenses (5).

The increasing prevalence of neurodegenerative diseases underscores the necessity for novel and innovative products on the market that may help in their efficient therapy. There is

an urgent need to gain a better understanding of the origins, progression, and potential treatment strategies for these conditions.

1.3 Vinpocetine

VP is a synthetic ethyl ester of apovincamine, an alkaloid obtained from the leaves of the Lesser Periwinkle (Vinca minor). It was discovered in the 1960s in Hungary and has been used since the 1980s in several countries including Germany, Poland, Russia, Japan, Portugal, and others. It was first commercialized in Hungary, as Cavinton® by Gedeon Richter Ltd., for the treatment of cerebrovascular and cognitive disorders (6). The effectiveness of VP on cognitive impairment, dementia, and especially AD has been reported in several studies. Only its oral administration form is available on the market and the recommended starting dose is 5 mg twice a day. Its chemical structure can be seen in Fig 1 (7).

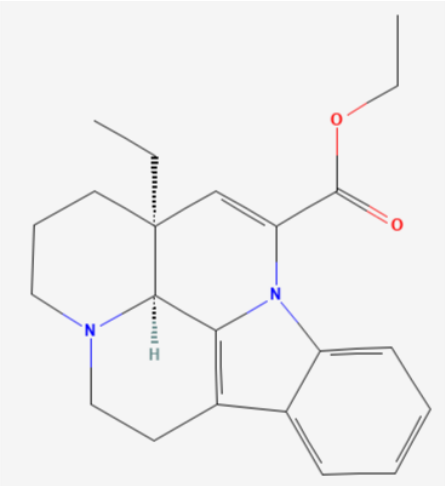

Figure 1: Chemical structure of vinpocetine. Extracted from (7).

VP has various pharmacological and biochemical actions such as [1] selective enhancement of the brain circulation and oxygen, [2] increased tolerance of the brain toward hypoxia and ischemia, [3] anticonvulsant activity, [4] inhibitory effect on phosphodiesterase enzyme and [5] inhibition of aggregation of thrombocytes. Since it has antioxidant, anticonvulsant, and anti-inflammatory properties, it is considered a neuroprotective agent. It promotes healthy brain function and improves brain blood flow, which enhances the delivery of oxygen and glucose, diminishes memory loss, and improves concentration (8).

Voltage-sensitive sodium channels are crucial for the regular function of the CNS as they play a key role in the initiation and conduction of neuronal action potentials. Many of the most widely used antiepileptic drugs suppress the abnormal neuronal excitability of ionic through

Na+ channels (9). So, vinpocetine is a potent inhibitor of the voltage-dependent Na^+ channels and the Ca^{2+}/calmodulin-dependent phosphodiesterase-1 which enhances cerebral blood flow by raising cGMP and cAMP levels, improving mitochondrial function, optimizing the utilization of glucose and oxygen by the brain (10).

The main active de-esterified metabolite of vinpocetine is apovincamine acid (AVA). The physical (11) and chemical properties of VP (12) are shown in Table 1.

Table 1: Physical and chemical properties of VP.

Chemical Formula	$C_{22}H_{26}N_2O_2$
ATC Code	N06BX18
Appearance	White or slightly yellow crystalline powder
Molecular Weight (Mr)	350.5 g/mol
Solubility	Practically insoluble in water, soluble in methylene chloride, slightly soluble in anhydrous ethanol
Melting Point	147-153 °C
logP	4.35
logS	-4.1
pKa (Strongest Basic)	7.31

VP, classified as a Biopharmaceutics Classification System (BCS) class-2 drug, with low solubility and high permeability, can benefit from a nanotechnological approach that enhances its water solubility. This improvement can lead to more efficient and rapid absorption (13)

1.3.1 Pharmacokinetics

VP is a poorly water-soluble base type, which shows a pH-dependent solubility profile. It is known that the aqueous solubility of base-type drugs, such as VP, is much higher at low pH values, whereas it is very poor at higher pH of the intestinal tract. Therefore, the release site from formulations of such drugs is restricted to the stomach or the upper part of the intestinal tract (14).

VP is rapidly absorbed when taken orally, reaching its maximum concentration (Cmax) within 1.5–2 hours. The elimination half-life is typically in the range of 1–2.5 hours. It is absorbed from the gastrointestinal tract and peak plasma levels are reached at about 1 hour after oral administration irrespective of dose and food intake (15). It is metabolized exclusively in

the liver, and widely distributed in the body systems including the CNS. When VP is taken orally, it has an elimination half-life of 1–2 hours, and the majority of it is eliminated from the body within 8 hours after administration (16). This suggests that there is no significant accumulation expected at the usual therapeutic dosage regimen (5–10 mg orally, 3 times a day). Notably, the area under the curve (AUC) values are practically equivalent on both the first and the seventh day of repeated oral VP administration.

The oral bioavailability of the compound ranges from 7% to 60%, due to a pronounced first-pass metabolism. It is greatly influenced by food intake, so the bioavailability of vinpocetine, Cmax, and AUC values were approximately 60–100% higher after food intake than under fasting conditions (9).

It is, therefore, necessary to find strategies to increase the bioavailability of VP by improving its administration, distribution, metabolization, and elimination characteristics.

1.4 Solutions for technological problems

1.4.1 Nasal administration

Nasal drug administration has been used as an alternative method to achieve systemic drug availability of drugs restricted to intravenous or oral administration (17). It was originally used for local drug delivery in the treatment of local allergic rhinitis, however, nowadays it is used to treat many diseases such as AD, epilepsy, brain tumors, and many others (1).

This route of administration is characterized by enhanced bioavailability, especially for drugs that easily cross the mucous membranes, due to the enriched vascular supply in the nasal cavity. It made many pharmaceutical companies and researchers investigate further about this, due to the rapid onset of the drug action potential, hepatic and gastrointestinal metabolism avoidance, noninvasiveness and ease of access, no sterilization requirements compared to parenteral, lower costs, non-irritative, and used for prolonged periods. Administrating via this route allows drugs to bypass common, intricate barriers and phenomena: blood-brain barrier (BBB), blood-cerebrospinal fluid barrier (B-CSF-B), and first-pass effect.

Despite these advantages, nasal delivery suffers from some restrictions such as poor drug permeability from nasal mucosa, mucociliary clearance, low drug retention time, and nasomucosal toxicity (18). Nasal drug administration presents numerous challenges due to the unique anatomical and physiological characteristics of the nasal cavity, as permeability barriers in the nasal mucosa include the mucus layer and the underlying epithelium. Also, the nasal cavity can hold 25 to 200 μL of liquid, however, the volume administered by the devices

currently on the market is 50 to 100 μL per dose. This small amount hinders drugs from being efficiently transported (19). The complex structure of the nasal cavity and the different absorption rates and transport pathways that drugs, particularly for high molecular weight drugs, have led to low clinical success rates in achieving systemic circulation or CNS delivery. Permeability is also crucial for the drug to enter the blood or brain.

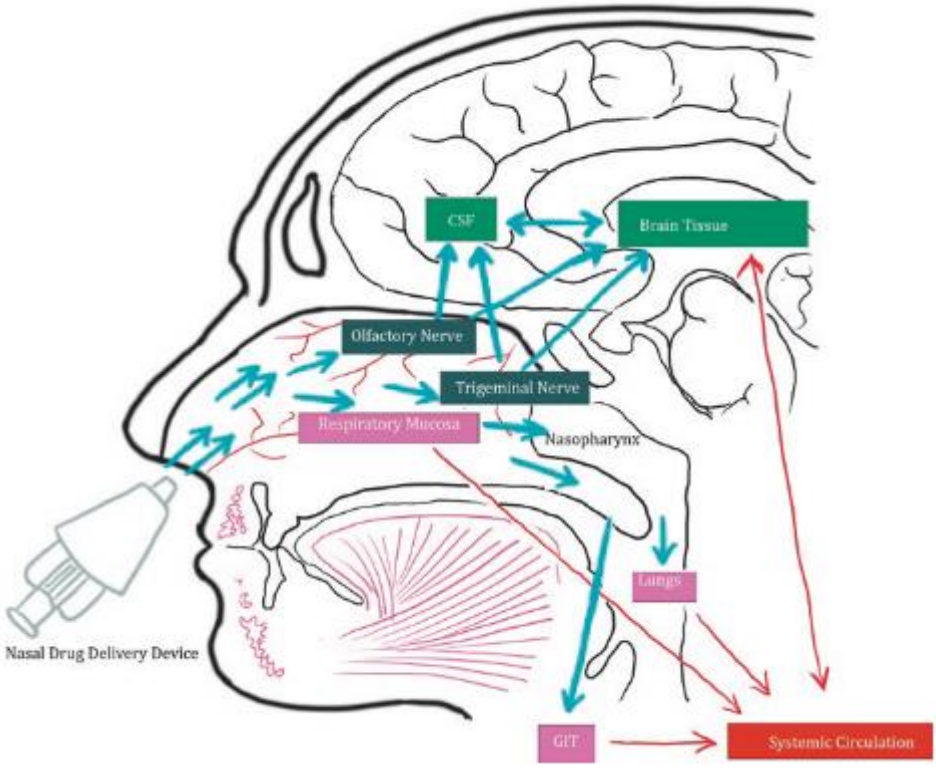

Figure 2: Nasal drug delivery system. Extracted from (17).

The olfactory region, regarded as a direct physiological link to the CNS, stands out as the most attractive part of this route of administration. It was described for the first time in 1989 and filed a patent on the intranasal delivery of peptides and other therapeutic molecules directly to the brain through an olfactory neural pathway (20).

Lipophilic drugs with small molecular sizes exhibit rapid and efficient absorption from the nasal cavity. When administered through the transnasal route, these drugs can lead to plasma concentration profiles comparable to those achieved through intravenous injection (100% bioavailability). For example, nanoparticles delivered via the nasal passageway reached the nasal bulb in under 5 minutes (21).

To optimize nasal administration bioadhesive hydrogels, bioadhesive microspheres, and liposomes have been studied. In addition, the incorporation of mucoadhesive excipients has been investigated, as they can extend the residence time of nanoformulations in the nasal

mucosa. This prolonged contact has been demonstrated to increase absorption, facilitate nose-to-brain transport, and enhance CNS bioavailability (22).

1.4.2 Mucoadhesive and gelling agents

Viscosity and mucoadhesivity are two vital parameters regarding nasal drug delivery. The formulation should have place in the nasal cavity for at least 15 min since the natural elimination mechanism, mucociliary clearance, can take place within this time (23).

Bioadhesives and mucoadhesives are drugs containing polymeric films with the ability to adhere to biological membranes after combining with moisture or mucus compounds. The mucoadhesive properties can be added to formulations, improving them, enhancing drug absorption, and facilitating controlled release of therapeutic agents.

Mucoadhesive polymers are crucial for increasing the bioavailability of active agents by improving the residence time, thereby extending absorption and improving the solubility and dissolution of drugs with poor solubility (24).

When it comes to mucoadhesive agents, several properties can differ based on the polymer, such as its charge, water solubility, and type of adhesive forces. Regardless of these variations, the polymers must be biocompatible, non-toxic, non-irritating, and should readily adhere to moist tissue (24). Also, the ability of the polymer to take up water from mucus and the pH of the target place are important factors determining the adhesive power of polymers.

There are multiple polymers with mucoadhesive properties, such as cellulose derivatives, which include HPMC and CMC, chitosan, alginates and pectin.

Polymers like HPMC and HEC are widely used in nasal drug delivery due to their mucoadhesive properties and ability to modulate drug release. At lower concentrations, these polymers facilitate permeability and drug absorption by creating a hydrated matrix that allows drug diffusion through the nasal mucosa (22). As the polymer concentration increases, the viscosity of the formulation rises, which can impede drug release by slowing down the diffusion process through the nasal membrane (25).

MC, a cellulose ether derivative with 27–32% hydroxyl groups substituted by methyl ether, is insoluble in most organic solvents and is used in compounding pharmacies for oral liquids and topical formulations. HEC serves as a thickening and suspending agent, commonly employed in topical gels and cosmetics. HPMC, also known as hypromellose, is a partly methylated and hydroxypropylated cellulose derivative used in nasal and topical formulations for thickening, emulsifying, and stabilizing purposes. CMC, available as calcium or sodium

salts, is widely applied in oral and topical formulations due to its viscosity-increasing properties (26).

Cellulose and its derivatives are commonly used as excipients in both pharmaceutical compounded and industrial products for various applications due to their numerous advantageous properties, including low cost, biocompatibility, and recyclability (26).

The commercially valuable properties of cellulose derivatives are influenced by their molecular weights, chemical structures, and the arrangement of substituent groups. These properties typically include solubility, solution viscosity, and resistance to biodegradation, heat, hydrolysis, and oxidation (27).

1.4.3 Polymeric micelles

Pharmaceutical nanocarriers are drug delivery vehicles of submicron size and high versatility. They include polymeric, lipidic and inorganic nanoparticles, liposomes, and many others. Nanocarriers play a key role in optimizing drug formulations by increasing the water solubility of hydrophobic active pharmaceutical ingredients (APIs) and stabilizing easily degradable compounds (28).

Polymeric micelles are nano-sized structures used for drug delivery, characterized by their core-shell structure originated from the self-assembly of amphi-philic block copolymers in aqueous solution (29). The hydrophobic section of the polymer forms the core, acting as a reservoir for solubilizing drugs with low water solubility, while the hydrophilic part forms the shell, protecting in limiting opsonin adsorption.

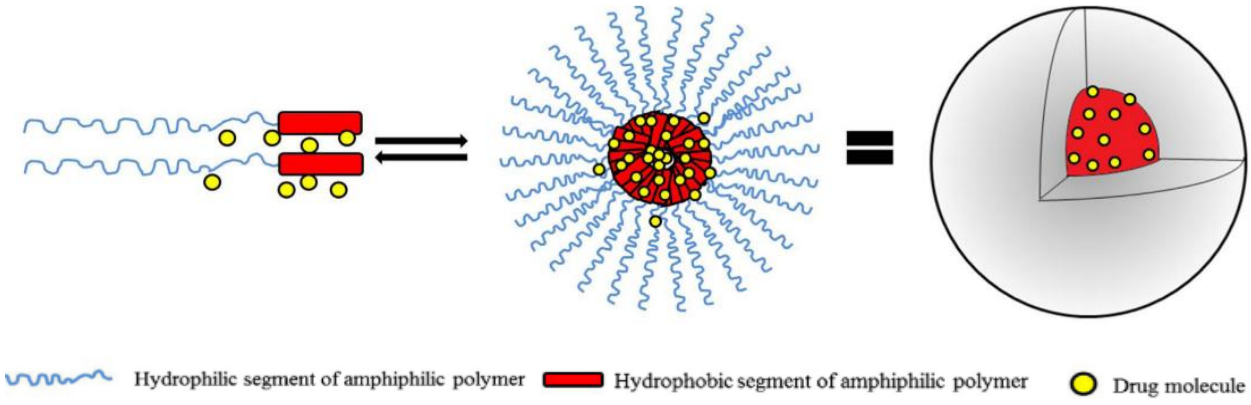

Figure 3: Structure of a polymeric micelle. Adapted from (29).

This unique structure offers some advantages as delivery vehicles: they provide longer circulation time, better physical stability, increased capacity for loading drugs, and improved water solubility. These structures are an effective delivery system for poorly soluble drugs since

they act as solubilizing agents (13). The amphiphilic macromolecules come together naturally to form nanostructures, which encapsulate drugs that do not dissolve in water. Therefore, one of the major challenges of the pharmaceutical industry is to improve the water solubility of drugs. This is especially crucial for drugs categorized under classes II and IV of the BCS, where the dissolution rate significantly affects absorption rates. The point at which individual amphiphilic polymer molecules begin to assemble into polymeric micelles is known as the critical micelle concentration (CMC). According to this, micelles are stable at concentrations of polymeric chains higher than the CMC while, below CMC, they present in solution as single molecules (29).

There are various polymeric micelles studied and available in the market, such as Soluplus® (SP) and Poloxamer 188 (P188). The mixed micelles system is a promising and effective carrier for significantly enhancing the solubility and transmembrane transport of hydrophobic drugs (30).

SP is a biodegradable co-polymer that has a low toxicity potential (LD50, oral > 5,000 mg/kg) and high solubilization capacity (31). Its amphiphilic nature makes it able to self-assemble into micelles once reaching the CMC (30). It has been widely used to increase the aqueous solubility and oral bioavailability of poorly soluble drugs. Among the various applications, SP has been proposed as a safe and versatile material to produce nano micelles in the pharmaceutical field, either alone or in combination with other polymers.

P188 is a bio-compatible and non-ionic linear copolymer, composed of two polyethylene oxide chains separated by a polypropylene oxide chain. It is among the most commonly used in this case for its good water solubility, solution clearness, optimal viscosity, and ocular tissue safety. The FDA has recognized P188 as a pharmaceutical excipient, and it is often used as a nanocarrier because of its safety, versatility, and commercial availability (30).

The small size, easy preparation process, and good solubilization properties make polymeric micelles interesting carriers for various administration routes. They have the potential to improve drug bioavailability and enable controlled, targeted drug release, which is beneficial for minimizing side effects (32).

1.4.4 Spray drying

Spray drying is a simple, fast, reproducible drying technology that can be easily scaled up. It is a continuous process to directly transform various liquids (e.g. solutions, emulsions, dispersions) into solid particles with adjustable size, distribution, shape, porosity, density, and

chemical composition (33). Spray drying is advantageous as it is an attractive technology to produce microparticles, allowing the encapsulation of nanoparticle-forming materials through a quick, single-step drying process (13).

The spray drying process involves four main steps: [1] heating of the drying gas, [2] droplet generation, [3] drying of the droplets, and [4] particle collection. First, the liquid feed is atomized in a nozzle. Within the drying chamber, the solvent in the sprayed droplets rapidly evaporates due to the continuous flow of hot drying gas. This results in the formation of dry particles, which are then separated from the gas stream and collected in a vessel. The final product is a fine powder that can be amorphous or crystalline (33).

A spray dried powder form provides higher stability, better protection from environmental factors such as oxidation, light, and temperature, and easier handling, storage, and redispersibility in aqueous solutions (34). The solubility of the final drug product is enhanced by spray-dried powder, attributed to the elevated surface-to-volume ratio of nanoparticles and the presence of more amorphous structures. This improved solubility results from the solvent being able to penetrate the particles more efficiently.

Characteristics of the nanospray dried drugs such as size, shape, surface charge, wall composition, molecular weight, etc. play a vital role in influencing the storage, stability, functional characteristics, release, and bioavailability of the ultimate pharmaceutical products. Understanding and characterizing these aspects are fundamental steps in ensuring the optimal performance and efficacy of the formulated drugs (33).

The analysis of nano spray-dried drugs is crucial for obtaining comprehensive insights into their quantitative and qualitative parameters. This information is essential for formulating nanocarriers tailored to different applications and administration routes. Properties such as particle size, size distribution, morphology, surface properties, and physicochemical attributes can be thoroughly examined in drugs encapsulated through nano spray drying (33).

The size analysis outcomes of nano spray-dried powder particles can be expressed through various parameters such as particle size distribution (PSD), polydispersity index (PDI), Sauter or Volume mean diameter, Span, and Z-average (based on intensity, number, or volume of particles) (33). Several analytical instruments are employed to measure the size of nano spray-dried particles, including Statical Light Scattering (SLS) and Dynamic Light Scattering (DLS).

2. Materials and Methods

2.1 Materials

As model active agent - VP, ethyl-apovincaminate- was utilized purchased from Sigma-Aldrich Co., Ltd. (Budapest, Hungary). As polymeric micelle forming agents, SP, poly(vinylcaprolactam)-poly(vinyl-acetate)-poly(ethylene glycol) graft co-polymer (PCL-PVAc-PEG)) was kindly gifted from BASF GmbH (Hannover, Germany) and P 188, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEG-PPG-PEG)) was acquired from Sigma-Aldrich Co., Ltd. D-trehalose-dihydrate (D-TRE), HPMC, MC, CMC HEC were also bought from Sigma-Aldrich Co. Ltd. For nasal administration studies, simulated nasal electrolyte solution (SNES) was applied with the following composition: 8.77 g/l of sodium chloride, 2.98 g/l of potassium chloride and 0.59 g/l of anhydrous calcium chloride in 1000 ml of purified water, adjusted to a pH of 5.6 via hydrochloric acid.

2.2. Methods

2.2.1. Quantitative analysis of vinpocetine

To determine the concentration of vinpocetine, high-performance liquid chromatography (HPLC) was applied using an Agilent 1260 Infinity (Agilent Technologies, Santa, Clara, CA, USA) instrument. 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: 1.54 % w/v ammoniuma-acetate solution (A) and acetonitrile (B) in a 40:60 ratio. The separation was performed via isocratic elution for 7 min at 40 °C with a flow rate of 1 ml/min. The injection volume was 10 µl. Chromatograms were detected at 280 ± 4 nm using a UV-Vis diode array detector. The evaluation was performed using ChemStation B.04.03. Software (Agilent technologies, Santa Clara, CA, USA). The determined retention time was 5.83 min, the limit of detection (LOD) and limit of quantification (LOQ) of VP were 6.31 and 19.11 ppm, respectively. Calibration was performed in the range of 20-100 µg/ml with a determined coefficient of linearity (R^2) of 0.9997.

2.2.2. Formulation of VP-loaded nano spray-dried polymeric micelles

At first, 25 ml of the ethanolic solution of VP was prepared with a concentration of 1 mg/ml, which was mixed with the 75 ml of aqueous solutions of 300 mg of SP and 250 mg of P 188.

The solutions were mixed, then, the system was incubated under constant stirring (750 rpm) at ambient temperature. Then, 5.0 g of D-TRE was dissolved in the mixture.

The batch was divided into five 20 ml portions where various cellulose derivatives were dissolved via hydration at room temperature in the following concentrations: 0.5–1.0–1.5–2.0 – 2.5% w/v.

A Büchi Nano Spray Dryer equipped with a small nebulizer (Büchi Nano Spray Dryer B90 HP, Büchi, Flawil, Switzerland) was used for further formulation. Based on prior experiment, the following settings were applied: inlet temperature – 100 °C, aspirator capacity – 100%, airflow rate – 115 ml/min and a pump rate of 20%.

2.2.3. Solid-state characterization of the optimized formulations

2.2.3.1. Determination of particle size via laser diffraction

The determination of particle size and particle size distribution of the nano spray-dried formulations, laser diffraction was used (Malvern Mastersizer Scirocco 2000, Malvern Instruments Ltd., Worcestershire, UK) with the dry dispersion unit. Approximately 0.1–0.2 g of products were loaded into the feeding tray. The dispersion air pressure was 3.0 bar and the vibration feed was at 75%. The following parameters were used for characterization: D[0.1] – 10% of the volume distribution is below this value; D[0.5] – the volume median diameter; D[0.9] – 90% of the volume distribution is below this value and the Span which represents the width of particle size distribution. All measurements were carried out in triplicate with individual batches (n =3). All results are expressed as average ± standard deviation (SD).

2.2.3.2. Determination of drug content

Samples were measured precisely, followed by the dispersion in 2 ml of methanol – purified water mixture in a 1:1 ratio (*v/v*). The samples were dissolved under constant stirring (30 min, 1200 rpm). Solutions were filtered via a 0.22 µm pore-sized polyether sulfone (PES) membrane. Concentration of VP was determined via HPLC. All measurements were carried out in triplicate with individual batches (n =3). All results are expressed as average ± SD.

2.2.4. Liquid state characterization of the formulations

2.2.4.1. Determination of micelle size, micelle size distribution and zeta potential

To determine micelle size, expresses as the average hydrodynamic diameter (D_H), the micelle size distribution (PdI, polydispersity index) and the zeta potential, dynamic light scattering measurements were applied using the Malvern Nano ZS Zetasizer (Malvern Instruments, Worcestershire, UK). The measurements took place in the target concentration of 250 μg/ml of VP. Samples were placed in folded capillary cells. The measurement was performed with a refractive index of 1.650 at 25 °C. All measurements were carried out in triplicate with individual batches (*n* =3). All results are expressed as average ± SD.

2.2.4.2. Determination of encapsulation efficiency

The indirect method was utilized to determine the encapsulation efficiency of the formulations. VP-loaded micelles were separated from the aqueous media via centrifugation using a Hermle Z323 K high-performance refrigerated centrifuge (Hermle AG, Gosheim, Germany) at 13,500 rpm at 4°C for 30 min. The clear supernatant was diluted 5-fold with methanol. The determination of VP was carried out via HPLC. All measurements were carried out in triplicate with individual batches (*n* =3). All results are expressed as average ± SD. The encapsulation efficiency (EE) was calculated via the following equation:

$$EE(\%) = \frac{initial\ VP\ (mg) - measured\ VP\ (mg)}{initial\ VP\ (mg)} x100$$

where EE represents encapsulation efficiency; initial VP as theoretical mass of VP, in milligrams; and measured VP as the real mass measured of VP.

2.2.4.3. Determination of thermodynamic solubility

0.5 ml of purified water was placed in vials and the formulations were dissolved until visible saturation. Covered with parafilm, the system was kept incubated for 72h at ambient temperature under a constant stirring of 750 rpm. After 72h, the suspensions were filtered through a 0.22 μm pore-sized PES membrane filter. The passed-through amount was measured via HPLC. All measurements were carried out in triplicate with individual batches (*n* =3). All results are expressed as average ± SD.

2.2.5. *In vitro* nasal applicability studies

2.2.5.1. *In vitro* drug release study

The *in vitro* drug release study was performed using the modified paddle method. The dispersed formulations with a concentration of 250 µg/ml of VP were placed in dialysis tubes (Spectra/Por® Dialysis Membrane with a 12–14 kD MWCO (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA)). Tubes were placed in 25 ml of SNES. The measurements took place at 36.5°C under 100 rpm of paddle rotation. 200 µl aliquots were taken up to 60 min and quantification was performed via HPLC. All measurements were carried out in triplicate with individual batches (n =3). All results are expressed as average ± SD.

2.2.5.2. *In vitro* drug permeation study

The nasal permeation study aimed to determine the passive diffusion tendencies of the formulations. The study was carried out in a modified Side-bi-Side® horizontal diffusion cell. As a membrane, a cellulose membrane impregnated via isopropyl myristate was used with a surface of 0.785 cm^2. The donor and acceptor cell volumes were 9.0 ml. The diffusion was investigated at 36.5 °C. The donor phase was 8 parts of SNES and 1 part of the corresponding formulation. The acceptor phase was a pH 7.4 PBS. Sampling was performed from the acceptor compartment and VP was measured via HPLC at predetermined time points. The flux, as the cumulative permeability up to a certain time point was calculated from the quantity of VP that permeated through the membrane divided by the surface of membrane and the duration of the experiment (µg/cm^2/h). All measurements were carried out in triplicate with individual batches (n =3). All results are expressed as average ± SD.

3. Results

3.1. Solid-state characterization of the optimized formulations

3.1.1. Particle size determination

The average particle size of the nano-sized formulations was measured via laser diffraction and the resulting particle size can be found in Table 2.

Table 2: D [0.1], D [0.5], and D [0.9] particle size values characterizing the nano-sprayed dried formulations of the applied concentration mucoadhesive excipients. Results are expressed as the average ± standard deviation (n=3).

		D [0.1]			D [0.5]			D [0.9]		
HEC	0.5%	1,114	±	0.294	1.257	±	0.391	2.055	±	0,474
	1%	1.108	±	0.187	1.208	±	0.472	2.317	±	0,667
	1.5%	1.239	±	0.351	1.627	±	0.387	2.306	±	0.298
	2%	1.2	±	0.301	1.706	±	0.495	2.448	±	0.621
	2.5%	1.305	±	0.277	1.688	±	0.424	2.405	±	0.533
MC	0.5%	1.554	±	0.39	2.054	±	0.371	4.85	±	0.589
	1%	1.463	±	0.063	2.305	±	0.489	9.701	±	0.414
	1.5%	1.619	±	0.072	2.311	±	0.401	8.112	±	0.279
	2%	2.003	±	0.34	2.284	±	0.363	9.307	±	0.193
	2.5%	2.183	±	0.343	2.397	±	0.496	5.258	±	0.638
CMC	0.5%	1.511	±	0.091	6.348	±	0.466	15.328	±	0.462
	1%	1.518	±	0.272	6.51	±	0.415	15.209	±	0.379
	1.5%	2.54	±	0.397	6.978	±	0.358	15.132	±	0.508
	2%	3.662	±	0.231	6.667	±	0.478	15.079	±	0.345
	2.5%	4.097	±	0.105	7.015	±	0.369	16.28	±	0.619
HPMC	0.5%	1.512	±	0.034	2.832	±	0.453	5.984	±	0.41
	1%	1.678	±	0.067	2.915	±	0.25	6.21	±	0.206
	1.5%	1.95	±	0.129	3.004	±	0.213	6.537	±	0.388
	2%	1.84	±	0.274	3.199	±	0.329	6.845	±	0.4
	2.5%	2.037	±	0.156	3.107	±	0.144	7.022	±	0.063

Formulations with different excipients (HEC, MC, CMC, and HPMC) show consistent results in average particle size, with small standard deviations. Also, for all the samples, we can see a direct relationship between the increase in excipient concentration and the increase in average particle size, suggesting that higher concentrations result in larger particles. The formulations with HEC and HPMC show the most homogeneous particle size distributions. This suggests that these formulations have the potential for more predictable and consistent performance, particularly in terms of bioavailability, which can be advantageous for faster and more uniform dissolution, as in the case of nasal administration.

Span and yield values were calculated using the D [0.1], D [0.5], and D [0.9] (table 3).

Table 3: Span and yield values characterizing the nano-sprayed dried formulations of the applied concentration mucoadhesive excipients.

		Span	**Yield (%)**
HEC	0.5%	1.001	73.15
	1%	1.124	66.48
	1.5%	1.097	78.22
	2%	1.263	61.79
	2.5%	1.184	72.33
MC	0.5%	1.457	64.5
	1%	1.406	75.87
	1.5%	1.412	67.92
	2%	1.423	69.14
	2.5%	1.467	77.63
CMC	0.5%	1.905	63.44
	1%	1.88	70.25
	1.5%	1.876	74.88
	2%	1.584	68.59
	2.5%	1.802	61.23
HPMC	0.5%	1.107	76.01
	1%	1.143	62.77
	1.5%	1.116	79.4
	2%	1.122	65.99
	2.5%	1.137	71.54

Based on the calculations, HEC and HPMC also have the lowest span values, suggesting that their formulations result in more uniform particles in terms of size, which may be beneficial for a more controlled distribution of the drug.

3.2. Drug content

The drug content of the solid-state sprayed dried formulations was determined. After dissolving the samples in purified water-methanol mixture, the quantification was carried out using HPLC. The theoretical and the measured drug content can be found in Table 4. The measured drug content is close to the theoretical one.

Table 4: The theoretical and measured vinpocetine content in 1000 mg of the nano-sprayed-dried powders. Results are expressed as average ± SD (n=3).

		Measured mass (mg/g)			Theoretical mass (mg/g)
HEC	0.5%	4.62	±	0.23	4.48
	1%	4.78	±	0.34	4.47
	1.5%	4.1	±	0.17	4.46
	2%	4.29	±	0.41	4.45
	2.5%	4.65	±	0.29	4.44
MC	0.5%	4.47	±	0.38	4.48
	1%	3.91	±	0.12	4.47
	1.5%	4.55	±	0.44	4.46
	2%	3.84	±	0.27	4.45
	2.5%	4.21	±	0.22	4.44
CMC	0.5%	3.71	±	0.36	4.48
	1%	4.33	±	0.31	4.47
	1.5%	4.12	±	0.19	4.46
	2%	4.73	±	0.4	4.45
	2.5%	3.99	±	0.13	4.44
HPMC	0.5%	4.01	±	0.42	4.48
	1%	3.58	±	0.3	4.47
	1.5%	4.69	±	0.15	4.46
	2%	3.77	±	0.26	4.45
	2.5%	4.25	±	0.33	4.44

3.3. Liquid-state characterization of the optimized formulations

3.3.1. Micelle size, size distribution, and zeta potential

Based on the results of the dynamic light scattering measurements (Table 5), all dispersed formulations had a micelle size corresponding to the average of 20 to 200 nm of polymeric micelles. Almost all PdI values of the various formulations had proper values, below 0.300, meaning a uniform size distribution. HEC and HPMC are the excipients with the best PdI values, with a more uniform distribution of micelle sizes at all concentrations. This suggests that these formulations tend to be more predictable and stable, which is beneficial for the effectiveness of drug release. In terms of charge, the positive charge is useful in the case of nasal administration, as the nasal mucosa can be characterized by a negative surface, meaning that stronger ionic interactions could also occur besides the physical ones. However, all have negative zeta potentials, and that can limit ionic interactions with the nasal mucosa.

Table 5: The average micelle size, polydispersity index, and zeta potential values of the nano-spray-dried formulations. These measurements were obtained via dynamic light scattering and zeta potential measurements. All data are presented as the mean ± SD from three individual batches.

		Micelle size (nm)	PdI	Zeta potential (mV)
HEC	**0.5%**	133.2 ± 5.62	0.247 ± 0.072	-28.3 ± 2.53
	1.0%	165.9 ± 7.48	0.196 ± 0.035	-28.1 ± 3.89
	1.5%	109.2 ± 6.14	0.23 ± 0.096	-25.3 ± 1.74
	2.0%	172.6 ± 4.98	0.233 ± 0.052	-30.4 ± 4.22
	2.5%	166.7 ± 8.01	0.178 ± 0.089	-37.5 ± 2.06
MC	**0.5%**	90.77 ± 6.87	0.253 ± 0.025	-24.3 ± 3.14
	1.0%	109.4 ± 4.55	0.324 ± 0.063	-29.9 ± 1.67
	1.5%	133 ± 7.23	0.388 ± 0.047	-24.5 ± 2.94
	2.0%	165.9 ± 5.89	0.241 ± 0.091	-30.5 ± 3.46
	2.5%	172.6 ± 4.31	0.244 ± 0.056	-24.3 ± 1.23
CMC	**0.5%**	90.03 ± 7.74	0.218 ± 0.034	-13.7 ± 4.03
	1.0%	146.4 ± 6.45	0.196 ± 0.087	-13.9 ± 2.78
	1.5%	150.5 ± 4.76	0.224 ± 0.039	-21.5 ± 1.95
	2.0%	138.4 ± 7.19	0.392 ± 0.074	-35.8 ± 3.61
	2.5%	167.3 ± 5.36	0.438 ± 0.045	-22.4 ± 2.39
HPMC	**0.5%**	110.4 ± 8.29	0.346 ± 0.092	-18.1 ± 4.12
	1.0%	115.9 ± 6.73	0.317 ± 0.059	-22.3 ± 1.48
	1.5%	93.95 ± 4.82	0.222 ± 0.081	-19.2 ± 3.72
	2.0%	91.58 ± 7.92	0.308 ± 0.032	-25.3 ± 2.67
	2.5%	90.11 ± 5.14	0.197 ± 0.066	-21.7 ± 1.89

3.3.2. Encapsulation efficiency and thermodynamic solubility measurement

The encapsulation efficiency reflects the amount of the API in a dissolved form compared to the whole system itself. The highest EE value was obtained in the case of HEC formulations, compared with the HPMC, MC, and CMC ones (figure 4). The formulation with HEC 0.5% had the highest encapsulation efficiency, almost reaching 100%, which means that almost all the drug has been encapsulated in the micelles. As the concentrations of the mucoadhesive agents increase, the encapsulation efficiency tends to decrease slightly.

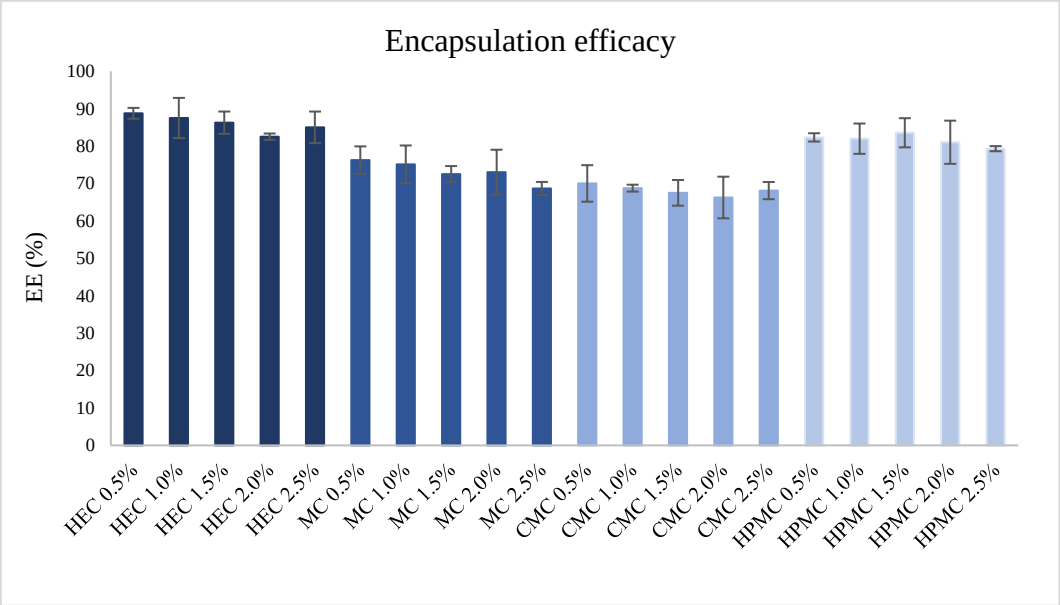

Figure 4: Encapsulation efficiency of VP at different polymer concentrations: HEC, MC, CMC and HPMC. Data are presented as average ± SD (n=3).

As for thermodynamic solubility, as the polymer concentration increases, solubility tends to decrease, with formulations with concentrations of 2% and 2.5% having lower solubilities (figure 5). The HPMC formulation has a more constant trend, with high solubility at lower concentrations, being more constant compared to other formulations such as HEC.

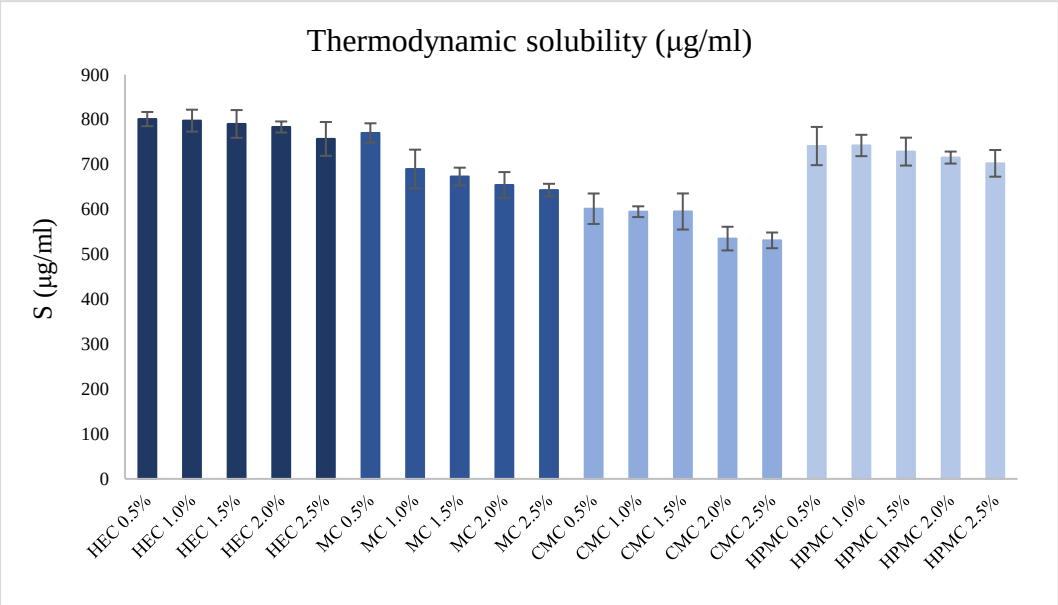

Figure 5: Thermodynamic solubility of VP at different polymer concentrations: HEC, MC, CMC and HPMC. Data are presented as average ± SD (n=3).

3.4. Nasal applicability studies

3.4.1. *In vitro* drug release study

An *in vitro* drug release study was performed under simulated nasal conditions. The criterion was that the fast release of the active substance must be ensured. It is visible on the drug-release-time curves (Figures 6 - 9) that all the formulations increased the drug release compared to it. The most critical time window is the first 15-20 min in all formulations, as this is how long the formulation resides on the nasal mucosa before mucociliary clearance would eliminate it. All four products, in their different concentrations, can be described with relatively rapid drug release and the burst effect can be seen, with a special effect on the HPMC. That means that a large amount of the API is released in a short period. The fastest and highest drug release was achieved via the HEC 0.5% formulation due to the highest solubilization and encapsulation efficiency experienced in prior measurements. It is also visible that the 0.5% concentration in all formulations with different mucoadhesive agents was the fastest and the most drug-release amount.

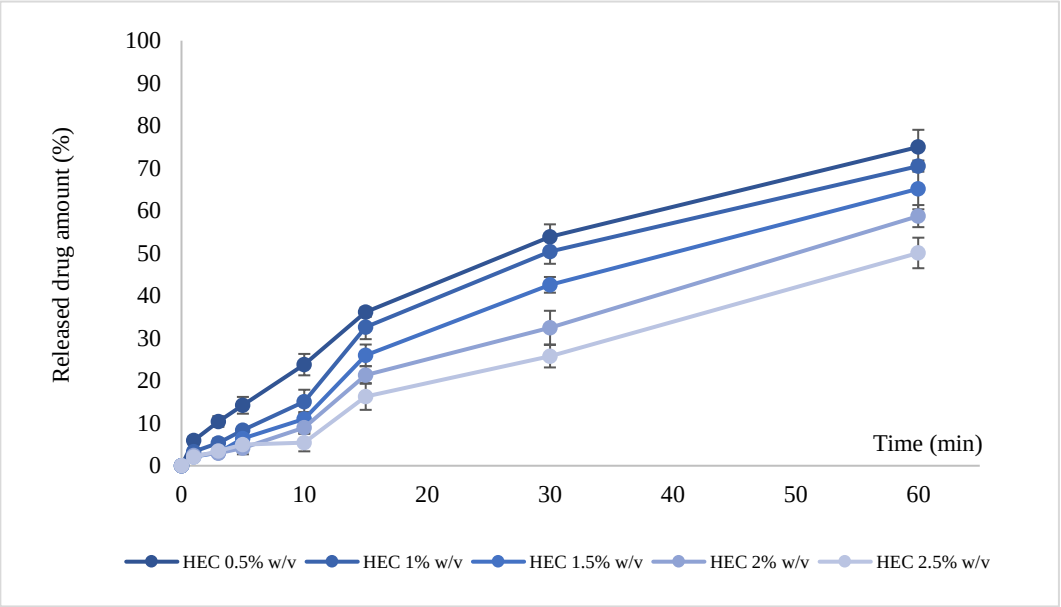

Figure 6: *In vitro* drug release curves for HEC, at simulated nasal conditions. Data are presented as average ± SD (n=3).

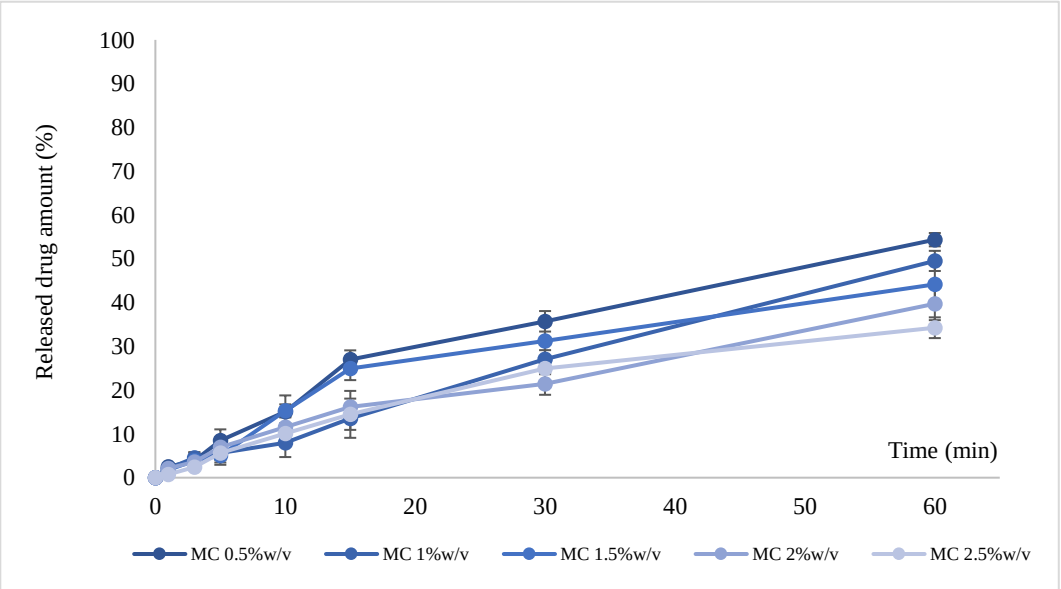

Figure 7: *In vitro* drug release curves for MC, at simulated nasal conditions. Data are presented as average ± SD (n=3).

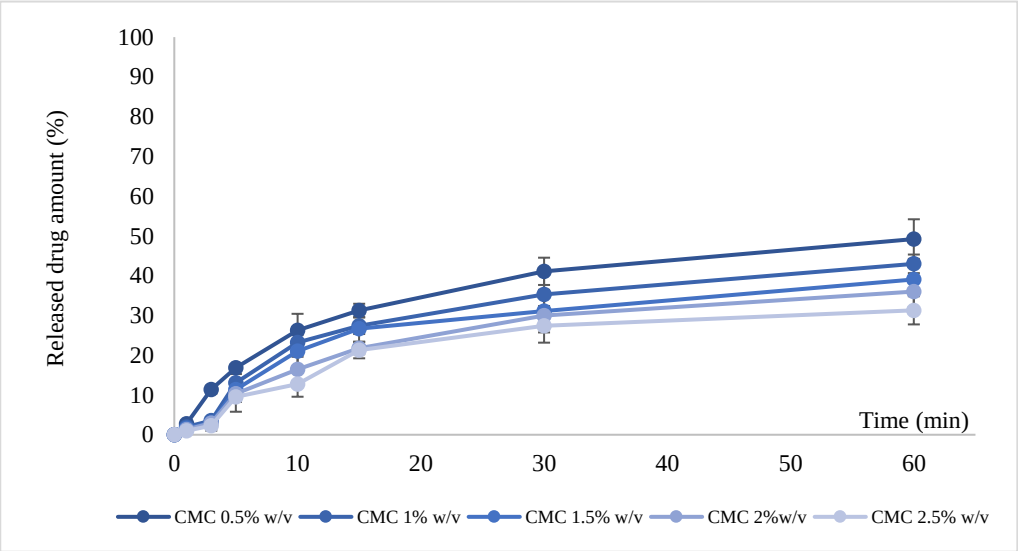

Figure 8: *In vitro* drug release curves for CMC, at simulated nasal conditions. Data are presented as average SD (n=3)

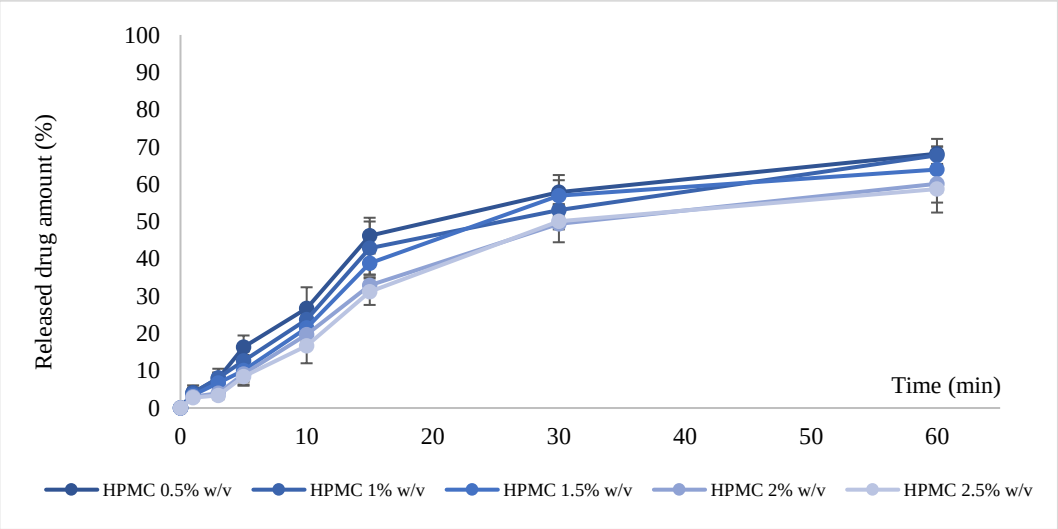

Figure 9: *In vitro* drug release curves for HPMC at simulated nasal conditions. Data are presented as average ± SD (n=3).

3.4.2. *In vitro* drug permeability study

In the *in vitro* measurements of nasal diffusion, passive diffusion was simulated under similar conditions to those in the nasal cavity. The cumulative-permeability-time curves show that the lowest polymer concentrations (around 0.5% to 1.5%) generally result in the highest cumulative permeability. As the polymer concentrations increase (especially 2% and 2.5%), the permeability tends to decrease, probably due to the formation of a denser barrier, which prevents the substance from passing through. The behavior of the different concentrations suggests that there is an ideal polymer concentration point where it facilitates permeation, but without obstructing the transport of the drug through the membrane.

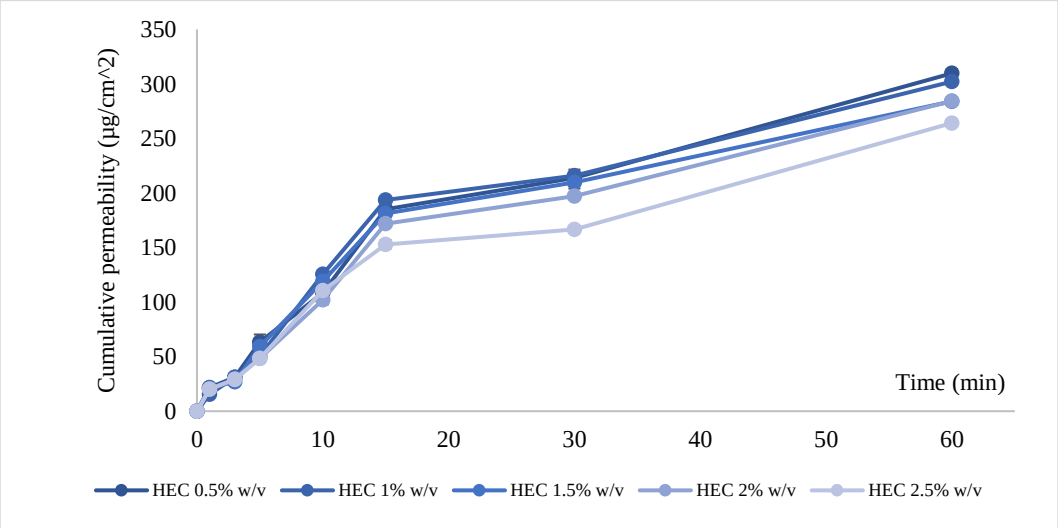

Figure 10: *In vitro* drug permeability curves for HEC at simulated nasal conditions. Data are presented as average ± SD (n=3).

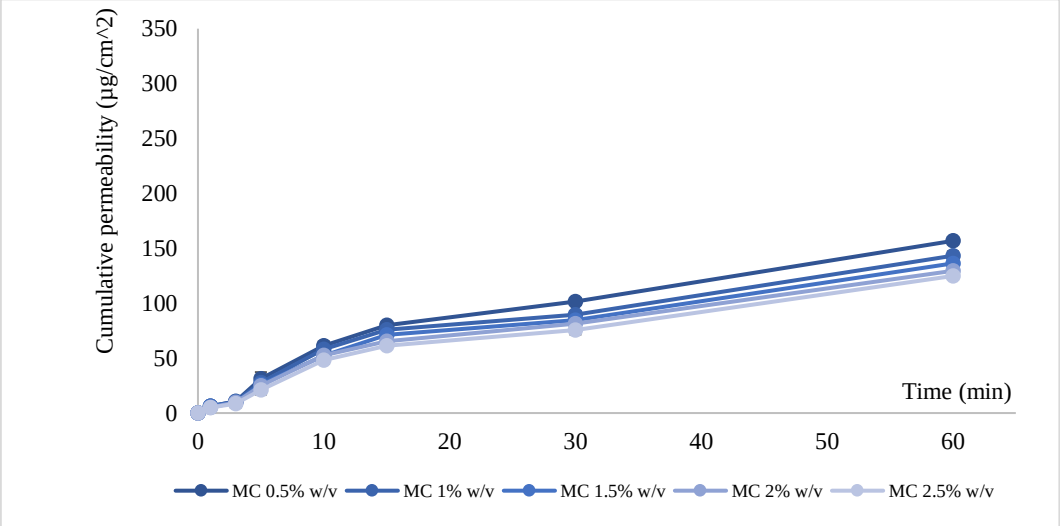

Figure 11: *In vitro* drug permeability curves for MC at simulated nasal conditions. Data are presented as average ± SD (n=3).

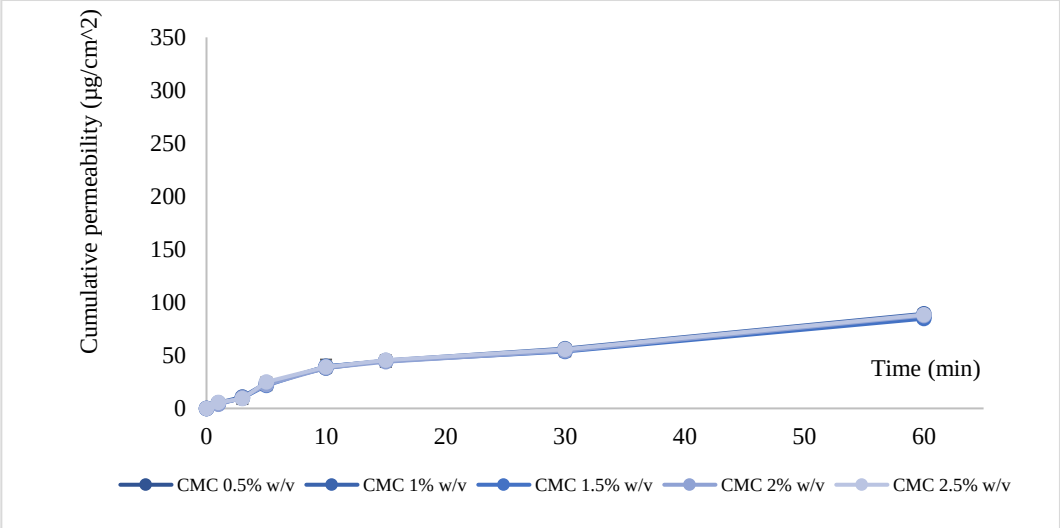

Figure 12: *In vitro* drug permeability curves for CMC at simulated nasal conditions. Data are presented as average ± SD (n=3).

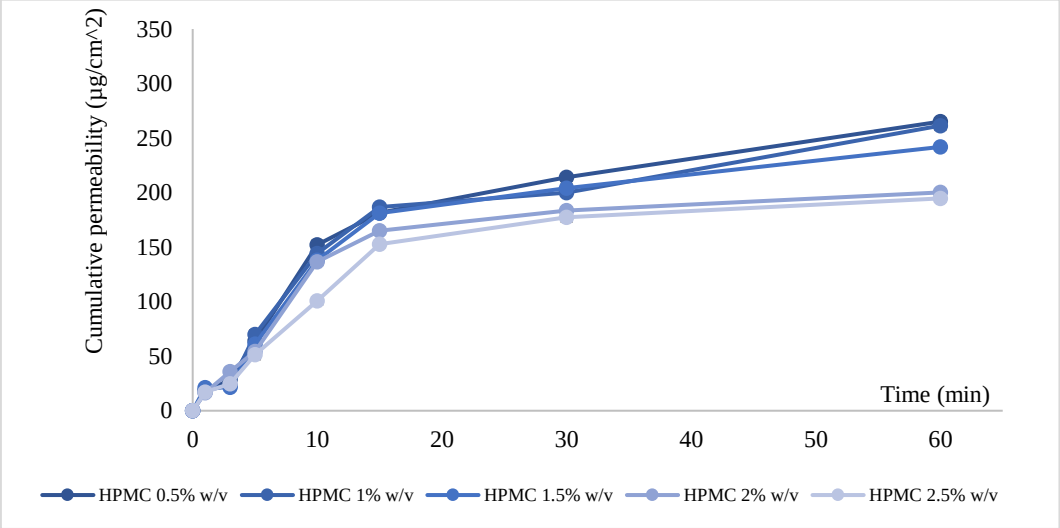

Figure 13: *In vitro* drug permeability curves for HPMC at simulated nasal conditions. Data are presented as average ± SD (n=3).

4. Discussion

In the initial phase of this research, polymeric micelle formulations were developed, incorporating the molecule being studied, VP. Cellulose derivatives were introduced into each formulation at concentrations of 0.5%, 1%, 1.5%, 2%, and 2.5%. These formulations were then subjected to a spray-drying process, converting them into nanoparticles, which became the focus of several tests.

First, the spray-dried powder underwent characterization through a laser diffraction analysis and a drug content assessment. Subsequently, the characteristics of the nanoparticles were examined based on the different spray-dried products, using dynamic light scattering studies, encapsulation efficiency, and thermodynamic solubility tests. Finally, nasal delivery was explored through *in vitro* drug release and drug permeation studies.

Regarding average particle size and particle size distribution, the study results revealed that formulations with HEC and HPMC exhibited the most homogeneous particle size distributions. Smaller and more uniform particles are critical in nasal delivery as they enhance absorption through nasal mucosa and optimize bioavailability of the encapsulated drug, by showing better interaction with biological barriers, such as the nasal epithelium (29). In contrast, CMC's larger particle size may result in reduced permeability and slower release, making it less ideal for rapid drug action via nasal administration. The low span and high yield values for these polymers also indicate that the spray-drying process is efficient with these excipients.

The measured drug content for all formulations was close to theoretical value. This consistency confirms the robustness of the formulation and preparation process, ensuring that the desired drug dosage is effectively loaded into the particles, which is crucial for achieving therapeutic efficacy.

DLS measurements showed that the micelles were within the desirable size range of 20-200 nm. The PdI values were acceptable, with HEC and HPMC again showing the most uniform size distribution. Zeta potential values for all formulations were negative, which could limit the interaction with the negatively charged nasal mucosa. However, as nasal mucosa can allow mucopenetration mechanisms, the formulations could still be effective in crossing the mucosal barrier. These mucoadhesive agents are called mucopenetration micelles, where the mucus layer allows carrier-intact transcytosis or paracellular transport (35)

Encapsulation efficiency was highest for HEC at 0.5%, while the efficiency tended to decrease with increasing polymer concentrations. Similarly, thermodynamic solubility decreased with higher polymer concentrations, with HPMC showing a more stable solubility

trend. High encapsulation efficiency, as formulations with lower concentrations of HEC, supports the role of these polymers in stabilizing hydrophobic drugs such as vinpocetine. These polymers enhance encapsulation due to their amphiphilic nature, which allows for efficient entrapment of lipophilic drugs within their micellar structures.

Nasal administration was studied by *in vitro* tests for drug release and drug permeability. The *in vitro* drug release studies revealed a rapid release of vinpocetine within the first 15-20 minutes for all formulations, with the burst effect being particularly pronounced for the HPMC formulations. Rapid drug release is beneficial for nasal administration, where a quick onset of action is desired. The fastest release was seen with HEC at 0.5%, indicating superior solubilization and encapsulation efficiency and being the optimal formulation for achieving fast release, making it a promising candidate for nasal delivery of API. The *in vitro* permeability study showed that lower polymer concentrations resulted in higher cumulative permeability - HEC and HPMC formulations showed relatively high permeability at lower concentrations (0.5% to 1.5%), which indicates that these polymers are more conducive to promoting VP passage through the membrane. There appears to be an optimal concentration window (likely between 0.5% and 1.5%) where vinpocetine is released efficiently, and permeability is maximized. Exceeding this threshold with higher concentrations (2% and above) diminishes permeability, likely due to micelle densification and increased viscosity.

5. Conclusion

The results obtained throughout the study provide a comprehensive analysis of the impact of cellulose derivatives on the characteristics of VP-loaded polymeric micelles and how different cellulose derivatives impact the properties of these formulations.

The use of cellulose derivatives like HPMC and HEC proved effective for the formulation of VP-loaded polymeric micelles. These excipients were shown to significantly enhance encapsulation efficiency, particle size control, and drug release. So, we can say that cellulose derivatives play a crucial role in optimizing both the mucoadhesion and viscosity of the nasal formulations, impacting drug retention and absorption positively.

These findings support the viability of VP-loaded polymeric micelles as a nasal drug delivery system, with significant potential for enhancing the delivery of CNS-targeted drugs. The formulations not only improve drug stability and bioavailability but also demonstrate practical advantages for ease of use and scalability in production. It can be stated that similar approaches could be applied to other poorly soluble drugs requiring rapid and efficient brain delivery, especially for the treatment of neurodegenerative diseases like Alzheimer’s and Parkinson’s diseases.

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