European Journal of Pharmaceutical Sciences 179 (2022) 106294

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Drug delivery to the brain: *In situ* gelling formulation enhances carbamazepine diffusion through nasal mucosa models with mucin

Elisa Corazza [a], Massimiliano Pio di Cagno [b,*], Annette Bauer-Brandl [c], Angela Abruzzo [a], Teresa Cerchiara [a], Federica Bigucci [a], Barbara Luppi [a]

[a] Department of Pharmacy and Biotechnology, Alma Mater Studiorum, University of Bologna, Via San Donato 19/2, Bologna 40127, Italy
[b] Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Sem Sælands vei 3, Oslo 0371, Norway
[c] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, Odense 5230, Denmark

ARTICLE INFO

Keywords:
Carbamazepine
Nasal delivery
Thermosensitive polymers
In situ gelling
Mucoadhesion
Drug permeation

ABSTRACT

The objective of this work was to optimize a thermosensitive in situ gelling formulation to improve intranasal and nose-to-brain delivery of the antiepileptic drug carbamazepine (CBZ). A preliminary procedure of vehicles obtained just mixing different fractions of poloxamer 407 (P407) and poloxamer 188 (P188) revealed preparations with phase transition temperatures, times to gelation and pH values suitable for nasal delivery. Subsequently, the mucoadhesive properties of the most promising formulations were tuned by adding hydroxypropylmethylcellulose types of different viscosity grades, and the effect of the adhesive polymers was evaluated by testing *in vitro* time and strength of mucoadhesion on specimens of sheep nasal mucosa. The formulation that showed the greatest mucoadhesive potential *in vitro*, with a time and force of mucoadhesion equal to 1746,75 s and 3.66 × 10-4 N, respectively, was that composed of 22% P407, 5% P188 and 0.8% HPMC low-viscous and it was further investigated for its ability to increase drug solubility and to control the release of the drug. Lastly, the capability of the candidate vehicle to ensure drug permeation across the biomimetic membrane Permeapad®, an artificial phospholipid-based barrier with a stratified architecture, and the same barrier enriched with a mucin layer was verified. The final formulation was characterized by a pH value of 6.0, underwent gelation at 32.33°C in 37.85 s, thus showing all the features required by in situ gelling thermosensitive preparations designed for nasal delivery and, more notably, it conserved the ability to favor drug permeation in the presence of mucin. These findings suggest that the optimized gelling system could be a promising and easy to realize strategy to improve CBZ delivery to the brain exploiting both a direct and indirect pathway.

1. Introduction

The World Health Organization reported that about 50 million people worldwide are affected by a chronic and progressive brain disorder, named epilepsy (Beydoun et al., 2020; Khan et al., 2020; Liu et al., 2018; Martins et al., 2018). Patients suffering from this neurological disease show recurrent and unpredictable seizures along with brain alterations, which both arise from an abnormal neuronal activity (Khan et al., 2020; Liu et al., 2018). Currently available treatments mainly rely on administration of antiepileptic drugs that only affect the symptoms (Liu et al., 2018).

Since it was first marketed in the 1960's, carbamazepine (CBZ) has been the most frequently prescribed drug for epileptic seizures management in patients of different age, including pediatrics (Ana et al., 2019; Beydoun et al., 2020; Khan et al., 2020; Liu et al., 2018; Martins et al., 2018; Mawazi et al., 2019). This dibenzoazepine derivative is commonly administered orally and is available in multiple dosage forms, such as tablets, chewable tablets and oral suspensions (Beydoun et al., 2020), despite some limitations related to the pharmacokinetic properties of the drug and the route of administration. In fact, belonging to the class II of the Biopharmaceutics Classification System (BCS), CBZ shows a good permeability but a low water solubility, which makes the design of the formulation more complex and causes a slow and variable absorption of the active molecule (Ana et al., 2019; Khan et al., 2020). CBZ is also subjected to considerable hepatic metabolism, that results in extensive inter-individual variability of bioavailability and in the

* Corresponding author.
E-mail address: m.p.d.cagno@farmasi.uio.no (M.P. di Cagno).

https://doi.org/10.1016/j.ejps.2022.106294
Received 8 July 2022; Received in revised form 13 September 2022; Accepted 15 September 2022
Available online 15 September 2022

production of metabolites with relevant clinical toxicity (Ana et al., 2019; Beydoun et al., 2020; Gierbolini et al., 2016). Moreover, the therapeutic effectiveness of CBZ is also limited by the need to cross the blood-brain barrier (BBB), which is characterized by a reduced permeability towards both large and small molecules due to the protective function exerted by tight junctions and efflux transporters (Khan et al., 2020; Liu et al., 2018). CBZ is still representing the standard of care for epilepsy treatment and, even though a new generation of antiepileptic drugs with improved tolerability is available (Beydoun et al., 2020), new formulative strategies are needed to face the shortcomings associated with oral delivery. In fact, efforts have been made to rethink CBZ delivery through the oral route and some examples can be found in literature (Ana et al., 2019; Khan et al., 2020; Li et al., 2020). In an attempt of achieving a more effective strategy for the treatment of epilepsy, drug development for psychiatric disorders is moving towards nasal delivery as a potential alternative to the oral route (Cassano et al., 2021; Liu et al., 2018; Nguyen and Maeng, 2022).

Intranasal administration is an established strategy to deliver active pharmaceutical ingredients with either local or systemic effect, and it is raising great consideration because of its unique anatomical connection with the brain (Berillo et al., 2021; Cunha et al., 2021; Nguyen and Maeng, 2022; Pires et al., 2022). Indeed, the olfactory and trigeminal nerves, located in the olfactory and respiratory region respectively, allow for the nose-to-brain absorption of drugs aimed to act at the central nervous system (CNS) level (Cassano et al., 2021; Crowe and Hsu, 2022; Cunha et al., 2021; Nguyen and Maeng, 2022; Pires et al., 2022). Hence, the availability of a direct access to the brain makes it possible for drugs to both escape the firs-pass effect and avoid crossing the BBB, resulting in an improved bioavailability, increased accumulation in the CNS and faster onset of action, which is a key point in the management of acute seizure episodes (Berillo et al., 2021; Cassano et al., 2021; Cunha et al., 2021; Nguyen and Maeng, 2022; Pires et al., 2022). Anyway, thanks to the high vascularization of the nasal mucosa, an indirect pathway towards the brain through the systemic absorption is available. Moreover, the intranasal route is considered a valid alternative for patients for whom the oral administration is not suitable and, because it is easily accessible and painless, it is supposed to improve both the compliance and the adherence to the treatment (Berillo et al., 2021; Cunha et al., 2021; Pires et al., 2022). However, also nasal delivery presents some drawbacks. In fact, for drugs requiring high doses and characterized by low solubility, the first limit encountered is the small size of nostrils that reduces applicable volume of formulations (Nguyen and Maeng, 2022; Pires et al., 2022). Secondly, the nasal cavity is covered by a relatively thick mucus layer, which is subjected to a high rate of turnover due to regular cilia beating that causes mucous to move, a mechanism known as mucociliary clearance (MCC). To address the downsides related to nasal delivery, multiple strategies have been proposed, including the employment of "smart" polymers.

In situ gelling formulations are initially present in a liquid state, but undergo sol-gel transition once administered in the nasal cavity due to hydrophobic interactions within the gel components, which are triggered by various physical (temperature, pH and charge of the mucosal environment) or chemical factors (for instance, oxidative cross-linking) (Berillo et al., 2021; Crowe and Hsu, 2022; Cunha et al., 2021; Giuliano et al., 2018). In virtue of their behavior, they exhibit many advantages over other delivery vehicles for nasal application: uniformly conform to the mucosal tissue, increase drug retention and bioavailability by reducing post-nasal drip and MCC, possibly provide sustained drug release, hence improving patient adherence, and reducing both dosing frequency and systemic side effects (Cassano et al., 2021; Giuliano et al., 2018; Schilling et al., 2022). Hydrogels responsive to temperature variation are one of the most broadly investigated environment-sensitive drug delivery systems (Russo and Villa, 2019; Schilling et al., 2022; Yu et al., 2021) and particular, those based on poloxamers have been extensively studied to obtain *in situ* forming nasal gels (Giuliano et al., 2018). Poloxamers are water soluble tri-block non-ionic copolymers with amphiphilic and surface active properties, consisting of a central hydrophobic block of poly (propylene oxide) (PPO) and two hydrophilic terminal blocks of poly (ethylene oxide) (POE) (Abdeltawab et al., 2020; Giuliano et al., 2018). The presence of both polar and non-polar monomers enables the formation of ordered structures in solution, named micelles, which allow for the encapsulation of hydrophobic drugs (Russo and Villa, 2019). Further, heating the aqueous solution to the critical micelle temperature induces PPO chains to become less soluble resulting in micelle packing and entanglement followed by gelation (Zahir-Jouzdani et al., 2018). Lastly, poloxamers are FDA approved and listed in the United States and European Pharmacopoeia as they are neither toxic nor irritant, and have been widely exploited as potential excipients in pharmaceuticals with various applications, including targeting of the CNS and drug delivery (Abdeltawab et al., 2020; Giuliano et al., 2018; Russo and Villa, 2019; Schilling et al., 2022).

Some preliminary studies have already proved the feasibility of the nasal delivery for examples of drugs targeting the brain, such as antidepressant (Qi et al., 2020; Wang et al., 2020) and antiepileptic drugs (Khan et al., 2020; Liu et al., 2018); moreover, clinical trials demonstrated that benzodiazepine nasal delivery is as effective in preventing seizures as the same active molecule delivered using conventional approaches (Pires et al., 2022). Hence, to take advantage of the favorable features associated with intranasal administration, the purpose of the present work was to develop an *in situ* gelling thermosensitive formulation based on poloxamers for CBZ nasal delivery. Firstly, poloxamer 407 (P407) and poloxamer 188 (P188) were mixed at different fractions to obtain delivery vehicles with sol-gel transition temperature and time of gelation suitable for nasal administration. The attention was then shifted towards the mucoadhesive properties of the final product; thus high-viscous and low-viscous hydroxypropylmethylcellulose (HPMC) types were evaluated for their ability to improve the time and the strength of mucoadhesion of the gels. The ability of the poloxamers to enhance the solubility of CBZ was also investigated, as well as the influence of drug loading on formulation properties. The most suitable thermosensitive gels were further examined for CBZ release behavior together with the drug absorption profiles across *in vitro* models of the nasal mucosa, i.e. Permeapad® barrier an artificial phospholipid-based membrane with a stratified architecture, stressing the role of the gelling formulation in presence of a reconstituted mucin layer.

2. Materials and methods

2.1. Materials

CBZ was provided from Sigma-Aldrich (Søborg, Denmark), whereas Lutrol® F68 (P188) and Lutrol® F127 (P407) were a kind gift from BASF SE (Ludwigshafen, Germany). HPMC high-viscous Benecel™ K100M Pharm (75000-140000 cps at 2% w/w) (K100M) and low-viscous Methocel™ E50LV Premium 5P (35-65 cps at 2% w/w) (E50LV) were supplied by Ashland Industries Europe GmbH (Schaffhausen, Switzerland) and Sigma-Aldrich (Milan, Italy), respectively. Green food coloring E-102 E-131 was provided from Candi Gestro srl (Siderno, Italy). Mucin type II from porcine stomach, all chemicals, and solvents were of analytical grade and purchased from Sigma-Aldrich (Milan, Italy), except for sodium chloride (NaCl) that was supplied by Carlo Erba (Milan, Italy). Phosphate buffer solution (PBS) at pH 7.4 was composed of 7.4 mM $Na_2HPO_4{\cdot}12H_2O$, 1.1 mM KH_2PO_4, and 136 mM NaCl. PBS at pH 5.5 was employed to simulate the pH of the nasal cavity and it was composed of 4.2 mM $Na_2HPO_4{\cdot}12H_2O$, 100 mM KH_2PO_4, 45.5 mM NaCl.

2.2. Preparation of thermosensitive in situ gelling formulations

Thermosensitive nasal gels were prepared following the cold method, that is the procedure to be selected when poloxamers (but also chitosan or carbopol) are used as gelling polymers (Singh et al., 2013).

Precisely, when formulations containing only the gelling polymers were prepared, different concentrations of P407 21-23% (w/v) and P188 3-5% (w/v) were solubilized in MilliQ ultrapure water (Millipore, Milford, MA, USA) at 4°C under gently stirring (magnetic stirrer, Velp Scientifica, Usmate Velate, Italy), then samples were stored at +2–8°C for 24h to obtain a clear and uniform solution. In contrast, when HPMC should be incorporated into the nasal gels, the mucoadhesive polymer was first dispersed in MilliQ ultrapure water at room temperature (RT equal to 25 ± 1°C) and the preparation was kept in the fridge overnight before adding poloxamers and stored for another night in the fridge. In the present work two different types of HPMC were employed in various fractions: high-viscous K100M 0.1-0.3% (w/v) and low-viscous E50LV 0.4-0.8% (w/v).

2.3. Characterization of in situ nasal gels

Initially, in order to identify the most suitable concentration ratio between the two poloxamers, plain formulations (without the mucoadhesive polymer) were prepared, and screened for three parameters: pH, sol-gel transition temperature, and time to gelation. The vehicles possessing the properties required from an *in situ* gelling nasal formulation, were then prepared with the addition of HPMC (mucoadhesive formulations), and the influence of the adhesive polymer on the performance of the gels was investigated by evaluating the same features.

2.3.1. pH

The pH of the developed formulations was determined by means of pH-indicator strips 0-14 (Whatman® Panpeha™, Sigma-Aldrich, Milan, Italy). The strip was completely immersed into the liquid-state sample and the evolving color compared to those depicted on the packaging.

2.3.2. Sol-gel transition temperature

The temperature at which the sol-gel transition ($T_{sol\text{-}gel}$) occurred was determined in accordance with the "magnetic stirring method", observing the interruption of a magnetic bar rotation as a result of the transition to the semi-solid state of a gelling formulation, as reported by Mura et al., but with slight modifications (Mura et al., 2018): 5 ml of each formulation was poured in a 15 mm jacketed cell with a flat-ground joint equipped with a V6A Stirrer (230V/50 Hz) (PermeGearInc., Hellertown, USA), containing a magnetic bar (5 × 12 mm). Cells were connected to a thermostated water pump (Julabo EH, JULABO Labortechnik GmbH, Seelbach, Germany) and they were gradually heated at a rate of 1°C/min from 25°C to 35°C. Since the temperature within the nasal cavity is reported to be in the range of 32 to 35°C (Serralheiro et al., 2014) and in previously published studies a temperature of 35°C was found adequate to reproduce the condition of the nasal cavity (Corazza et al., 2022; Wu et al., 2019), this value was chosen as the upper limit of the temperature interval. The temperature, at which the bar stopped moving, was read on the digital thermostat and recorded as the gelation temperature.

2.3.3. Time to gelation

The time required for the formulation to undergo sol-gel transition ($t_{sol\text{-}gel}$) was measured similarly to the $T_{sol\text{-}gel}$ (see Section 2.3.2). However, in this case, the jacketed cells were thermostated at 35°C, filled with 1 ml of each respective formulation and the time to gelation, that is the span required for the magnetic bar to stop rotating, was recorded using a stopwatch.

2.4. Mucoadhesive properties of in situ nasal gels

The mucoadhesive potential of the *in situ* gelling vehicles was investigated employing *ex-vivo* sheep nasal mucosa excised from nasal turbinates, due to its morphological similarity to the human one (Illum, 1996). The animal tissue was obtained from a local slaughterhouse (Sarsina, Italy) and handled as previously reported (Corazza et al., 2022). Briefly, the septum was removed, and the nasal turbinates were pulled out from the nasal cavity using forceps and scalpel. Finally, the mucosa was detached from the adhering cartilaginous tissue and washed with NaCl 0.9% (w/v). The biological specimens were stored in aluminum foils at -20°C until use.

2.4.1. Time of mucoadhesion

An *in vitro* evaluation of the time of mucoadhesion was carried out similarly to a previously described procedure (Mura et al., 2018). A section of sheep nasal mucosa (3 cm long and 1.5 cm wide) was positioned on a glass support and hydrated with a dispersion of dialyzed and lyophilized mucin (see Section 2.10) 0.05% (w/v) in PBS pH 5.5 for 2 min. 2 g of each formulation sample was stained with 1% (w/w) of a green food coloring and 200 µl of this solution was applied on nasal mucosa sections. The formulation was left to gel on the nasal mucosa at 35°C and subsequently placed on a heat mat thermostat (AIICIOO) already heated at the same temperature and positioned with an angle of inclination of 40°. Moreover, by means of a peristaltic pump (Gilson Miniplus2, Biolabo Intruments srl, Milan, Italy), the mucosa was subjected to a continuous flow (1 ml/min) of PBS pH 5.5 heated at 35°C, to mimic the physiological conditions of the nasal cavity. The time required for the complete removal of the gel from the mucosa, that is to say when the green color was no longer visible, was measured using a stopwatch.

2.4.2. Force of mucoadhesion

The mucoadhesive formulation exhibiting the longest time of adhesion and its respective plain counterpart (the vehicle obtained using the same fractions of P407 and P188, but without the mucoadhesive polymer) were characterized for their ability to interact with the mucosal tissue. For this purpose, the force of mucoadhesion was measured exploiting an adapted tensiometer (Krüss 132869; Hamburg, Germany) as reported by Abruzzo and co-workers, with some adjustments (Abruzzo et al., 2021). The nasal mucosa (0.1 g) was fixed to a circular support (diameter 0.90 cm, thickness 0.35 cm) with cyanoacrylate adhesive, hydrated with a dispersion of dialyzed and lyophilized mucin 0.05% (w/v) in PBS pH 5.5 for 5 min and suspended from the tensiometer spring. Further on, 3 ml of each sample were poured into a small beaker (diameter 3 cm), left to gel at 35°C and maintained at this temperature by means of a water bath. The mucosa was lowered until it reached the surface of the formulation and the two were kept in contact without applying any force for 2 min. Afterwards, the nasal mucosa was raised, and the force required for its detachment from the gel represented the adhesive bond strength between the mucosa and the nasal gel.

2.5. Viscosity measurement

The viscosity of the selected formulations was measured at three different temperatures: 20°C, at RT and at 35°C, that is the temperature of the nasal cavity.

To determine viscosities of formulations prior to gelation (20 and 25°C) the falling ball viscometer (HAAKE falling ball viscometer type C, Thermo Fisher Scientific, Milan, Italy) was employed. This tool enables the measurement of the sample viscosity by correlating it to the time required for a sphere to cover a distance of 100 mm through the examined fluid, that is placed inside a cylinder inclined of 10°, which can also be thermostated by means of an external jacket. A sample volume of approximately 40 ml was poured into the measuring tube together with a nickel iron alloy ball (ø = 15.595 mm; m = 16.1332 g; ρ = 8.124 g/cm^3; constant K = 0.10963 mPa × s × cm^3/g × s) suitable to measure viscosities comprised between 40 – 700 mPa × s. The viscometer was connected to a liquid circulator to control the temperature and the sample was subjected to a tempering time of 30 min prior to the analysis. The time required for the sphere to move from one side to the other of the testing tube was measured using a stopwatch and the

evaluation was repeated three times for each formulation replicate. The sample viscosity (mPa × s) was calculated according to the following equation:

$$h = t'(r_1 - r_2)'K$$

where K is the ball constant (mPa × s × cm^3/g × s), t is the falling time of the ball (s), ρ_1 and ρ_2 are the density (g/cm^3) of the ball and of the nasal gel, respectively.

Because of the phase transition of the *in situ* gelling formulations when they have reached $T_{sol\text{-}gel}$ at 35°C, a rotational viscometer (Visco Star-R, Fungilab S.A., Barcelona, Spain) was used. Approximately 20 ml of formulation was allowed to gel for 15 min inside the testing tube thermostated by a water jacket at 35°C. The measurement was conducted using the spindle TR 11 at a speed of 200 rpm.

2.6. Chromatographic conditions

HPLC analytical assay was performed using a Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV–vis detector set at 286 nm. Separation was obtained on a Phenomenex (Torrance, CA, USA) Sinergy™ 4 μm Hydro-RP 80Å LC column (150 × 4.60 mm) coupled to a Phenomenex Security Guard C18 guard cartridge (4 × 3.0 mm i.d., 5 μm). The mobile phase consisted of a mixture of MilliQ water/acetonitrile/methanol 50:25:25 (*v/v*) with the addiction of 0.1% of trifluoroacetic acid and it was flushed at a rate of 0.4 ml/min. Manual injections were made using a Rheodyne 7125 injector with a 20 μL sample loop and data analysis was carried out through the CromatoPlus software (Shimadzu Italia, Milan, Italy). Since release and permeation studies were conducted in slightly different conditions, it was necessary to obtain more than one calibration curve. The first, in PBS pH 5.5/ethanol (80:20 *v/v*) was characterized by a drug concentration range of 0.1 – 80.24 μg/ml, and a linearity coefficient (R^2) equal to 1. This curve was employed for the release study and limits of detection (LOD) and quantification (LOQ) were 0.43 μg/ml and 1.32 μg/ml, respectively. The calibration curve of CBZ in PBS pH 7.4/ ethanol (80:20 *v/v*), obtained with drug concentrations ranging from 0.05 μg/ml to 84 μg/ml, showed a good linearity ($R^2 = 1$) and it was used to evaluate the drug permeated during the *in vitro* diffusion studies. LOD and LOQ were 0.4 μg/ml and 1.21 μg/ml, respectively.

2.7. Solubility study and drug loading

CBZ does not show a pKa value in the physiological range (Huerta et al., 2013), thus its solubility was studied both in MilliQ water and in the selected formulations.

To assess drug solubility in water, an excess amount (10 mg in 10 ml) of CBZ was dispersed in ultrapure water and the dispersion was left under stirring for 72 h at RT. Further on, the sample was centrifuged at 5890 × g for 15 min (Microspin 12, Biosan, Riga, Latvia) and the supernatant filtered through syringe filters 0.22 μm cut-off (Cellulose acetate syringe filter, Sanford, FL, USA) to remove the fraction of undissolved drug. The sample was appropriately diluted and subjected to HPLC analytical assay, to quantify the maximum solubility of CBZ in the aqueous medium.

To investigate the solubilizing power of the thermosensitive vehicles, two different spectrophotometric approaches were employed: the turbidimetric analysis and the absorbance (ABS) measurement at 286 nm. In both cases, CBZ was dispersed in the selected mucoadhesive gelling system and in its respective plain counterpart at concentrations ranging from 0.25 mg/ml to 2 mg/ml. In contrast to what has been reported in literature (Singh et al., 2013), where methods suggest dissolving the drug in water and storing it at low temperature prior to polymer addition, CBZ was added directly to the final formulations at RT, with the aim to exploit the solubilization power of P407 and to avoid drug precipitation due to both its low water solubility and the reduced storage temperature. Moreover, to ensure drug solubilization, samples were kept under stirring at RT for 24h prior to analysis. The turbidity of the samples was measured at 650 nm through an UV–vis spectrophotometer (UV-1601 Shimadzu, Milan, Italy) and the baseline correction was made using the corresponding unloaded formulation. For the second analysis, samples were firstly centrifuged at 5890 × g for 15 min, then appropriately diluted and finally their ABS at 286 nm was measured.

2.8. Drug influence on formulation performance

To investigate whether CBZ loading would affect nasal gels performance, the selected mucoadhesive vehicle and its respective plain formulation were loaded with the drug at the concentration of 1 mg/ml (Serralheiro et al., 2014), following the procedure reported in Section 2.7. pH, $T_{sol\text{-}gel}$ and $t_{sol\text{-}gel}$ were thus evaluated (see Section 2.3) together with the force of mucoadhesion (see Section 2.4.2) and results obtained for the loaded formulations were compared to the unloaded ones.

2.9. In vitro release study

The release profiles of the selected mucoadhesive formulation and its respective plain nasal gel, prepared as reported in Section 2.7 (final drug concentration 1 mg/ml), were compared to that of a control sample, obtained dissolving CBZ in ultrapure water at a concentration equal to its maximum solubility in the aqueous medium. The study was conducted using Franz-type static glass vertical diffusion cells equipped with a V6A Stirrer (PermeGearInc., Hellertown, PA, USA), and a dialysis membrane cut-off 6000 – 8000 Da (Spectra/Por 1 dialysis membrane Spectrum Laboratories Inc., Rancho Dominguez, CA, USA), that was previously soaked in release medium for 30 min before being clamped between the donor and the receiving compartments. The release medium (12 ml) was composed of a mixture of PBS pH 5.5/ethanol 80:20 (v/v) thermostated at 35 ± 1°C and maintained under constant stirring, whereas the donor compartment was filled with 300 μl of the tested formulations. At predetermined time points, every 30 min for the first hour and every 60 min for the following 4 h, 200 μl were withdrawn from the acceptor chamber, replaced with fresh PBS pH 5.5/ethanol mixture, and finally analyzed by HPLC assay. The cumulative released drug was plotted as function of time.

2.10. In vitro permeation study

The most suitable thermosensitive formulation containing the mucoadhesive polymer and its respective plain counterpart were further compared to a CBZ solution, for their ability to enable drug passive diffusion across two different *in vitro* models of the nasal mucosa. Specifically, were employed Permeapad® barrier (InnoMe GmbH, Espelkamp, Germany) and Permeapad® functionalized with a mucin layer, which had already been exploited to predict *in vitro* absorption of active ingredients through the nasal mucosa, thanks to their capacity to mimic the cell membrane and the airway epithelium together with the mucus coating, respectively (Corazza et al., 2022; Wu et al., 2019).

Regarding the Permeapad® and mucin model, the reconstituted mucin layer was obtained as described by Corazza et al. (2022). Briefly, a mucin dispersion (50 mg/ml) in ultrapure water was dialyzed overnight using a standard RC tubing (molecular weight cut-off 6000–8000 Da), freeze dried and stored at +2–8°C until use. When diffusion studies were performed, the powder was reconstituted with PBS pH 5.5 (50 mg/ml) and 200 μl of purified mucin dispersion were placed on the top of the Permeapad® barrier and left to equilibrate for 5 min prior to sample addition in the donor compartment.

The permeation study was set out exactly as the release one, except for the composition of the receiving phase, that consisted of a PBS pH 7.4/ethanol mixture, the barrier system, that corresponded to the two Permeapad®-based models, and the time intervals for the samplings:

Table 1
Characterization of plain formulations. Data are expressed as means ± SD, n = 3.

P188 % (w/v)	P407 % (w/v)	pH	$T_{sol\text{-}gel}$ (°C)	$t_{sol\text{-}gel}$ (s)
3	21	5.5-6.0	> 35	No gel
	22	6.0	32.28 ± 1.04	33.98 ± 9.15
	23	6.0	28.95 ± 0.07	7.5 ± 0.71
4	21	5.5-6.0	> 35	No gel
	22	6.0	34.00 ± 0.00	59.47 ± 4.69
	23	6.0	30.00 ± 0.25	50.40 ± 1.45
5	21	5.5-6.0	> 35	No gel
	22	6.0	34.42 ± 0.42	62.56 ± 6.09
	23	6.0	31.85 ± 0.21	32.00 ± 2.83

every 15 min for the first 2 h and every 30 min for the following 3 h. Cumulative amounts of drug permeated per unit area (diffusion surface area = 1.77 cm^2) of the membrane (μg/cm^2) were plotted against time (minutes), and the flux (j) was calculated from the slope of the initial linear section of the curve.

2.11. Statistical analysis

All results are shown as mean ± standard deviation (SD) and SD was calculated from the values of three independent experiments. Data from all experiments were analyzed using a t-test, and differences were deemed significant for $p < 0.05$.

3. Results and discussion

3.1. Preparation and evaluation of thermosensitive nasal gels

Thermoresponsive nasal gels were developed using poloxamer P407 as the gelling polymer due to its advantageous features. In fact, it is characterized by good tolerability, low toxicity and it is almost not-irritant towards the mucosa, which make it a useful and safe excipient in nasal formulations (Mura et al., 2018). Moreover, P407 is compatible with numerous biomolecules and chemical excipients (Giuliano et al., 2018). Interestingly, the gelling ability of this polymer is concentration-dependent: at low concentration, P407 solution loses its gelling capacity, whereas at high concentration, its gelation temperature is lower than room temperature (Huang et al., 2016). Consequently, to tailor the temperature at which the sol-gel transition takes place, P407 is often mixed with other excipients, for instance P188, as in the present work. It is known that the gelation process is driven by the formation of spherical micelles, that result from the breakage of the hydrogen bonds between the aqueous solvent and the hydrophilic moieties of P407 at temperatures above the lower critical solution temperature of the polymer (Giuliano et al., 2018). However, the gelation temperature of poloxamer-based vehicles can be increased by adding the more hydrophilic P188 polymer. In fact, P188 causes a higher order of water molecules around the hydrophobic PPO units, making a further increase in temperature necessary to promote the hydrophobic interactions between the formed micelles (Fathalla et al., 2017).

To obtain *in situ* thermo-sensitive formulations, which can be administered into the nasal cavity and respond with sol–gel transition at nasal temperature, firstly it was necessary to identify the most suitable concentration ratio between P407 and P188. Considering that a minimum concentration of 15–20% of gelling polymer is essential to present the phase transition and to form thermo-sensitive hydrogels with adequate viscosity and partial rigidity (Yu et al., 2021), P407 and P188 were mixed at concentrations within the ranges of 21-23% (w/v) and 3-5% (w/v), respectively. The formulations obtained just using poloxamers (plain formulations) were characterized in terms of pH as well as concerning temperature and time of gelation, and results are summarized in Table 1. Regarding the pH, all the formulations prepared using the cold method showed pH values of approximately 6, which fits with the physiological pH of the nasal cavity that is reported between 5.5 and 6.5 (Cirri et al., 2021). This is of great relevance to avoid developing formulations that cause inflammation or toxicity of the nasal epithelium (Nguyen and Maeng, 2022; Pires et al., 2022). The thermoresponsive behavior of the poloxamer-based vehicles was investigated considering the $T_{sol\text{-}gel}$ and the $t_{sol\text{-}gel}$, which were measured through the magnetic stirring method. As it can be derived from the data reported, the formulations behaved as expected according to the mechanism formerly described and the gelation temperature trend was coherent with that reported by He and coworkers (He et al., 2011). Indeed, nasal gel temperatures of gelation decreased as the concentration of P407 was increased, whereas $T_{sol\text{-}gel}$ tended to raise when the concentration of P188 was increased. Among the screened formulations, three of them exhibited a temperature of phase transition comprised between 32 and 35°C, that is the physiological temperature within the nasal cavity (Serralheiro et al., 2014). This is a key point in the development of delivery strategies aimed to improve nasal administration of active ingredients. In fact, nasal *in situ* gelling formulations with a $T_{sol\text{-}gel}$ lower than 32°C would undergo sol-gel transition before being instilled in the nostril, thus hampering drug administration. Conversely, vehicles with a temperature of phase transition higher than 35°C would rapidly leak from the nasal cavity reducing drug absorption. To improve drug residence time on the airway epithelium and prevent its fast clearance, gelation should occur within 60 s (Cirri et al., 2021). Except for the preparations characterized by $T_{sol\text{-}gel}$ above 35°C, all tested plain formulations satisfied this last requirement. Overall, among the developed preparations, only nasal gels containing 22% (w/v) of P407 and from 3 to 5% (w/v) of P188 were found suitable for nasal delivery.

Despite their gelling properties, poloxamers also present some potential hurdles, including poor mucoadhesion. For this reason, they are frequently combined with mucoadhesive polymers in order to improve the bioadhesive properties of the nasal gel (Zahir-Jouzdani et al., 2018). Here, HPMC of different viscosities were employed in different amounts: high-viscous K100M at 0.1-0.3% (w/v), and low-viscous E50LV at 0.4-0.8% (w/v). In particular, they were added to the plain formulations that were selected during the previous screening procedure. Initially, the impact of HPMC addition on the gel basic properties like pH, $T_{sol\text{-}gel}$ and the $t_{sol\text{-}gel}$ was considered, and results referring to the mucoadhesive formulations (nasal gels containing the adhesive polymer) are reported in Table 2.

K100M was firstly added to the formulation containing 22% (w/v) of P407 and 3% (w/v) of P188 at the three concentrations tested, observing a notable decrease in the temperature of gelation, that was far below the lower limit of the suitable range of temperatures. This was probably due to the thermal responsive gelation properties of HPMC itself (Joshi,

Table 2
Characterization of mucoadhesive formulations. Data are expressed as means ± SD, n = 3.

P188 % (w/v)	P407 % (w/v)	K100M % (w/v)	E50LV % (w/v)	pH	$T_{sol\text{-}gel}$ (°C)	$t_{sol\text{-}gel}$ (s)
3	22	0.1		6.0	30.30 ± 0.99	32.30 ± 1.25
		0.2		6.0	30.00 ± 0.20	32.67 ± 3.06
		0.3		6.0	29.00 ± 0.20	27.30 ± 6.11
4	22	0.1		6.0	32.60 ± 0.57	35.60 ± 6.02
		0.2		6.0	32.20 ± 0.20	42.85 ± 8.15
5	22	0.1		6.0	33.25 ± 0.35	52.93 ± 2.90
		0.2		6.0	32.50 ± 0.14	50.40 ± 1.25
			0.4	6.0	33.60 ± 0.57	60.20 ± 2.33
			0.8	6.0	32.33 ± 0.42	37.85 ± 6.52

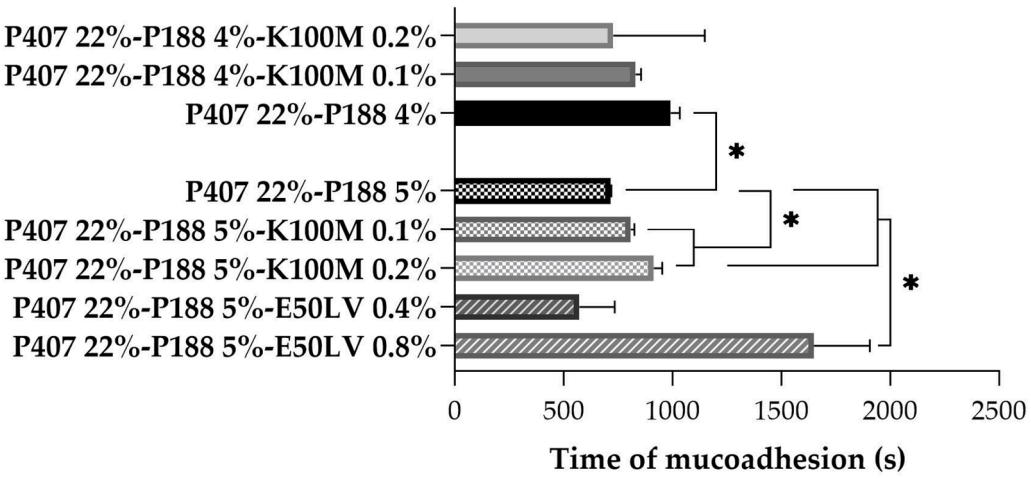

Fig. 1. Time of mucoadhesion measured for the selected mucoadhesive nasal formulations and the respective plain counterparts. Data are expressed as means ± SD, n = 3. Significance indicated by * = $p < 0.05$ between samples indicated by the brackets.

2011). Interestingly, the effect of K100M at 0.3% (w/v) on gel properties was already clear during the preparation steps, when an increase in sample viscosity was noted. These observations together with the higher poloxamer content of the following plain formulations, made it unnecessary to test the influence of K100M at the highest concentration considered. As expected, at both concentrations tested (0.1% and 0.2% w/v), HPMC high-viscous lowered the $T_{sol\text{-}gel}$ of the plain formulations containing 22% (w/v) of P407 and 4% or 5% (w/v) of P188, but in both cases the parameter was still between 32 and 35°C.

The increase in the $T_{sol\text{-}gel}$ in presence of growing concentrations of P407 and HPMC K100M was probably due to the gelling properties of both polymers, but it might also be the result of polymeric chains tendency to overlap because of their being in a greater quantity in the same dispersion volume, as pointed out by J.B. da Silva and coworkers (da Silva et al., 2020). Because of these observations, we considered HPMC low-viscous as a potential alternative to K100M. In fact, we expected that the presence of shorter polymer chains would reduce overlapping regions, thus allowing E50LV to be used at higher concentration than K100M without strongly altering the features of the vehicle.

E50LV was added exclusively to the plain formulation characterized by 22% (w/v) of P407 and 5% (w/v) of P188, because it was initially described by a higher temperature of gelation. In agreement with the former evidences, also low-viscous HPMC decreased the sol-gel transition temperature. Nevertheless, the resulting mucoadhesive formulation maintained the desired properties for a nasal *in situ* gelling vehicle.

Finally, as it can be observed, the inclusion of neither K100M nor E50LV affected the pH of the mucoadhesive gels, that remained unchanged compared to the respective plain formulations. Moreover, the inclusion of both HPMC types contributed to the decrease of the $t_{sol\text{-}gel}$, that was around or below 60 s.

3.2. Mucoadhesive properties of nasal gels

A major drawback of nasal delivery is the defense mechanism represented by the MCC: the mucous coating together with its high rate of turnover, due to cilia beating, create an obstacle towards xenobiotic entrance in the respiratory system. Unfortunately, intranasally administered formulations are subjected to the same clearance process, that strongly limits their retention time into the nasal cavity, thus reducing drug absorption. Nevertheless, drug residence time can be improved by including mucoadhesive agents, such as chitosan, cellulose derivatives, and polyacrylates in the delivery vehicles, exploiting their ability to both interact with the mucus layer and limit the MCC (Cassano et al., 2021; Crowe and Hsu, 2022). Based on these assumptions, the mucoadhesive formulations selected through the initial screening procedure were compared with the respective plain formulations for their ability to increase drug contact with the nasal mucosa.

Firstly, the influence of high and low-viscous HPMC on the time of mucoadhesion was investigated. Specifically, the *in vitro* evaluation was performed by recording the time required for the gelled system to be completely removed from a specimen of nasal mucosa, because of the continuous flush of buffer, that simulated the MCC phenomenon. As can be observed in Fig. 1, the plain formulation containing 4% (w/v) of P188 was retained on the mucosa for a significantly ($p < 0.05$) longer time (990.40 ± 41.86 s) compared to the one with 5% (w/v) of P188 (715.00 ± 7.07 s). This result was probably a consequence of the increased hydrophilicity of the second formulation, due to the higher concentration of P188. When K100M at the two concentrations tested, 0.1% and 0.2% (w/v), was added to the plain nasal gel P407 22%-P188 4%, no improvement in the time of mucoadhesion was detected. In contrast, when HPMC high-viscous was included in the plain formulation P407 22%-P188 5%, the *in vitro* retention time improved as the concentration of K100M increased. Regarding the impact of E50LV on the time of mucoadhesion of the plain formulation containing 5% (w/v) of P188, it was not significant ($p > 0.05$) when employed at the lower concentration (487.00 ± 105.43 s). Differently, the use of HPMC low-viscous at 0.8% (w/v) allowed for a notable increase in the retention time reaching 1746.75 ± 274.00 s, which was significantly higher ($p < 0.05$) compared to both the plain formulation and the one containing 0.2% (w/v) of K100M. Considering the results obtained so far, the nasal gel P407 22%-P188 5%-E50LV 0.8% was selected as the candidate delivery system that underwent further characterization steps.

The residence time of *in situ* gelling nasal formulations is also affected by the mucoadhesion strength. In fact, the stronger is the interaction between the vehicle and the mucosa, the higher is the probability that the system is longer retained at the absorption site (Mura et al., 2018). For this reason, the force required to detach the nasal mucosa from the gelling system with E50LV 0.8% (w/v) was measured and it was compared to that of the respective plain formulation. The graph in Fig. 4**c** (unloaded - CBZ) shows that the force necessary to separate the tissue from the gel notably increased from 2.51 ± 0.09 × 10^{-4} N in absence of the mucoadhesive polymer to 3.66 ± 0.32 × 10^{-4} N in the presence of HPMC low-viscous. This last result demonstrated the role of the mucoadhesive polymer in strengthening the interaction between the formulation and the target tissue once more.

3.3. Sol-gel transition influence on nasal gel viscosity

In situ gelling thermoresponsive formulations are so called in virtue of their ability to change from a liquid to a semisolid state, with the latter

Table 3
Viscosities of the selected mucoadhesive *in situ* nasal gel and the corresponding plain formulation at different temperatures. Data are expressed as means ± SD, n = 3.

			Viscosity (mPa × s)		
P188 % (w/v)	P407 % (w/v)	E50LV % (w/v)	20°C	25°C	35°C
5	22		77.75 ± 1.96	105.89 ± 2.28	4150 ± 200
5	22	0.8	118.85 ± 0.64	160.70 ± 1.00	4650 ± 200

being the form that comprise most of the interesting properties of such delivery system. However, these can be exploited provided that the formulation is properly instilled in the nostril. In this regard, if the formulation is to be administered by means of a nasal spray, the formulation must be characterized by a viscosity lower than 500 mPa × s at RT, otherwise the preparation would be difficult to handle, and the correct dosing would be prevented (Martin et al., 2022). Herein viscosity was measured at three different temperatures (Table 3), observing that both the selected mucoadhesive formulation and the respective plain one already showed a slight increase, around 35%, in viscosity when the temperature was raised from 20 to 25°C. Anyway, gel viscosities always were lower than the limit, suggesting that the candidate vehicle could be easily administered either if it is stored at RT or once outside the fridge. When samples were heated up to 35°C, the viscosity increase was even more noticeable, and it was the demonstration that the phase transition had occurred. Comprehensibly, the viscosities measured for the selected mucoadhesive formulation were higher compared to those of the respective plain gel.

3.4. Nasal gels solubilizing properties

Belonging to the BCS class II group of active ingredients, CBZ is featured by a low aqueous solubility, that can make its delivery through the nasal route rather challenging, since just a limited volume of formulation can be administered to the nasal cavity. Nevertheless, P407 is well known not only for its gelling behavior, but also for its ability to remarkably improve the apparent solubility of hydrophobic molecules, thanks to its surface-active properties (Giuliano et al., 2018). For example, Ban and co-workers observed that emodin solubility improved as the concentrations of both P407 and P188 increased, demonstrating that poloxamers were effective in enhancing the drug solubility (Ban et al., 2017). As a result, in the present study the possibility to exploit poloxamers as solubilizing agents was evaluated.

The analysis performed demonstrated that, in ultrapure water, CBZ reached a maximum solubility equal to 0.118 ± 0.69 mg/ml. This value is in good agreement with previously reported results, that indicated 0.113 mg/ml (Khan et al., 2020) and 0.126 mg/ml (Borisover et al., 2011) as the highest concentrations of CBZ that could be achieved in aqueous media.

To perform the solubility study of CBZ in the gelling system, the drug was loaded at increasing concentrations from 0.25 to 2 mg/ml in the selected mucoadhesive formulation and in the corresponding plain formulation. Samples were then subjected to a turbidimetric analysis and results are reported in Fig. 2. For both the preparations, no turbidity raise was observed for CBZ concentrations up to 1 mg/ml. Differently, the addition of CBZ at higher concentrations led to a notable increment in the turbidity of the specimen, suggesting that part of the drug remained undissolved. Besides, the mucoadhesive gel seemed to have an increased solubilization power compared to its respective plain formulation. In fact, when CBZ was included at 1.5 and 2 mg/ml in the gelling vehicles, the ABS measured at 650 nm was significantly higher ($p <$

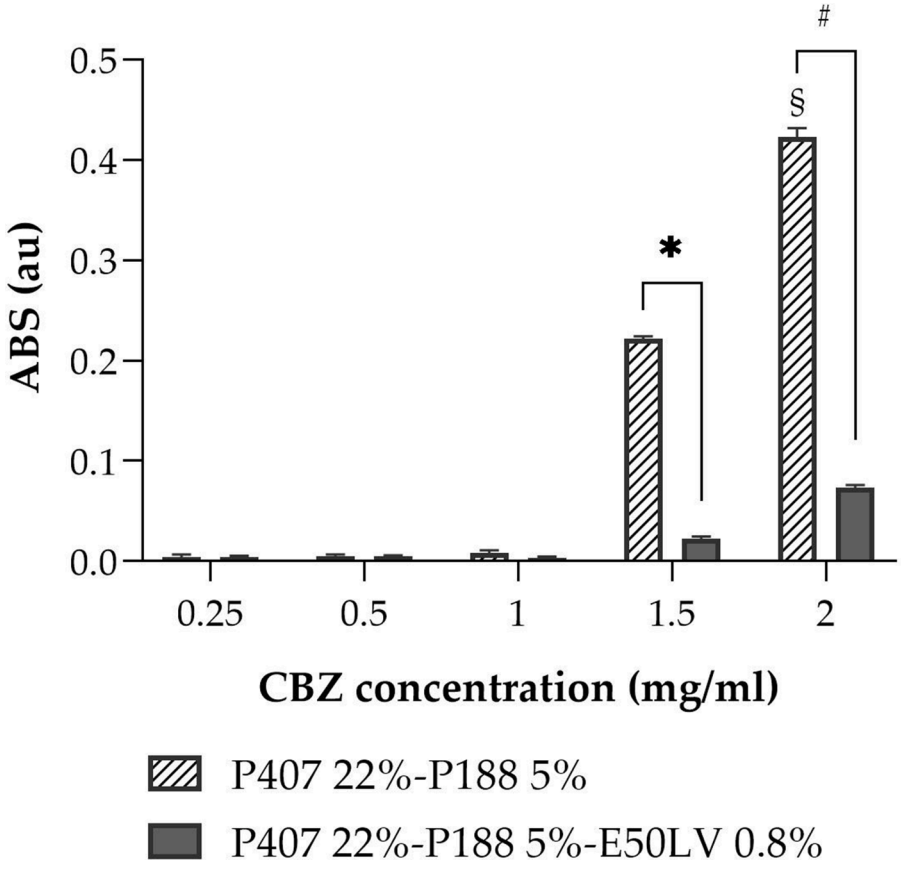

Fig. 2. Solubility study of CBZ in the selected mucoadhesive formulation and its respective plain counterpart using the turbidimetric method. Plotted absorbance at 650 nm for the formulations. Data are expressed as means ± SD, n = 3. Significance indicated by * = $p < 0.05$ between samples indicated by the brackets and the respective samples containing a lower concentration of CBZ; # = $p < 0.05$ between samples indicated by the brackets and the respective samples containing 1.5 mg/ml CBZ; by § = $p < 0.05$ compared to the mucoadhesive formulation containing 2 mg/ml of CBZ.

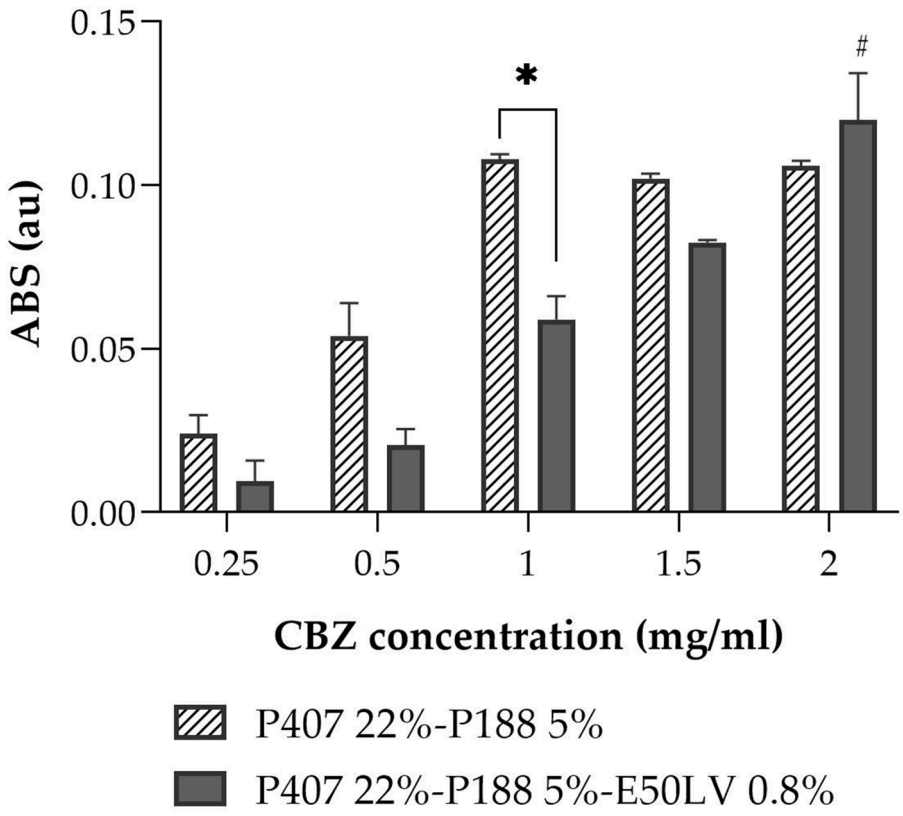

Fig. 3. Solubility study of CBZ in the selected mucoadhesive formulation and its respective plain counterpart by detection of absorbance at 286 nm. Data are expressed as means ± SD, n = 3. Significance indicated by * = $p < 0.05$ between samples indicated by the brackets and the respective samples containing a lower concentration of CBZ; # = $p < 0.05$ compared to the respective samples containing lower concentrations of CBZ.

0.05) for the plain formulation than for the one containing the mucoadhesive polymer. The latter result was consistent with data reported in a published research work, where HPMC low viscosity grade was successfully employed to increase the solubility of simvastatin, a poorly water soluble drug (Javeer et al., 2013).

To further confirm the findings of the turbidimetric analysis, samples were centrifuged to remove the fraction of undissolved drug and were evaluated for their ABS at 286 nm, that is the absorption peak of CBZ. This second evaluation clearly supported the previously reached conclusion (Fig. 3). For the plain formulation, the absorbance gradually increased in samples containing from 0.25 to 1 mg/ml of CBZ, then it remained constant, meaning that no more drug molecules could be solubilized. Differently, when CBZ was dispersed in the mucoadhesive formulation, a continuously growing trend was observed, which supported the hypothesis of the superior solubilizing properties of the gel containing the adhesive polymer. Overall, it can be stated that the poloxamer-based vehicles allowed for a significant ($p < 0.05$) increase in the drug solubility compared to CBZ solution and that CBZ at the concentration of 1 mg/ml was completely solubilized in both the tested formulations. On the basis of these results, and considering that previously reported *in vivo* preclinical studies were conducted by loading CBZ at the final concentration of 1 mg/ml in a thermoreversible nasal gel intended for intranasal administration of the antiepileptic drug (Serralheiro et al., 2014), 1 mg/ml was chosen as the drug loading concentration during the following experiments.

3.5. Evaluation of drug loaded thermoresponsive nasal gels

Before going ahead with the *in vitro* release and permeation studies, the influence of the drug loading on the performance of the nasal gel was investigated. The formulation P407 22%-P188 5%-E50LV 0.8% and the same without the mucoadhesive polymer were loaded with 1 mg/ml of CBZ and four parameters, pH, temperature and time of gelation together with the detachment force, were measured. The obtained results are graphically represented in Fig. 4a-c, which also shows the comparison with the data collected in the case of unloaded gels. Overall, none of the considered parameters, including pH (data not shown), was altered by the addition of CBZ and this was verified for both the formulation tested. This meant that, despite the presence of the drug, the candidate formulation maintained the desired features to be considered a suitable *in situ* thermosensitive vehicle for nasal and nose-to-brain delivery.

3.6. CBZ in vitro release from thermosensitive nasal gels

Along with solubility issues, CBZ effectiveness is also limited by its narrow therapeutic window, thus reduced blood concentrations may result in therapeutic failure, while high blood concentrations can increase the probability of toxicity occurrence (Li et al., 2020). Consequently, the use of formulations with a modified-release is preferred in order to reduce peak fluctuations, improve efficacy and tolerability and thus patient adherence to the pharmacological treatment (Beydoun et al., 2020; Gierbolini et al., 2016). *In vitro* release studies were performed to evaluate the ability of the thermoresponsive nasal formulations to allow for a controlled release of the loaded drug and their behavior was compared to that of a CBZ solution (control).

The mean cumulative percentage of CBZ released from the tested formulations over the considered time period is shown in Fig. 5. Since the control sample was prepared by dissolving CBZ as a saturated solution in ultrapure water, the drug was immediately available. In fact, 99.70 ± 0.50% of the drug was found released within 180 min. Differently, both the mucoadhesive formulation and the corresponding plain thermosensitive gel showed a slower and prolonged release: after 5 h, only approximately half of the drug was released (49.58 ± 1.79% and 52.00 ± 2.74%, respectively). The release profiles of the two

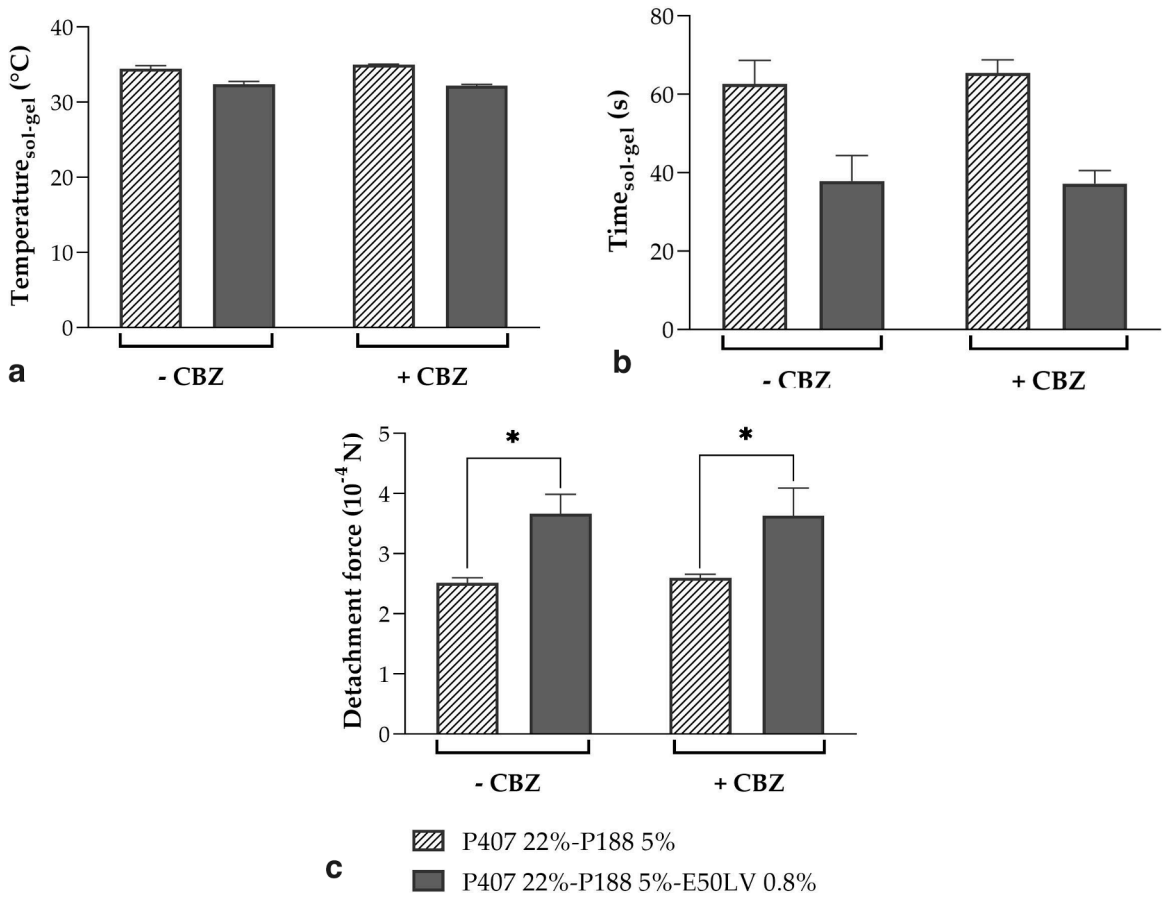

Fig. 4. Drug loading (1mg/mL) influence on a) $T_{sol-gel}$; b) $t_{sol-gel}$ and c) detachment force. For each considered parameter, the selected mucoadhesive formulation and the respective plain one were compared both in absence (- CBZ) and presence (+ CBZ) of 1 mg/ml of CBZ. Data are expressed as means ± SD, n = 3. Significance indicated by * = $p < 0.05$ between samples indicated by the brackets.

thermosensitive nasal gels underlined their ability to provide a controlled and sustained released of the loaded drug. This behavior may be hypothesized to be connected to the viscous nature of the *in situ* gelling preparations, however, the curves were found to overlap, despite the different viscosities of the vehicles (see Section 3.3). Other mechanisms apart from diffusion of free molecules may be involved, such as drug partitioning into poloxamers micelles and polymers chains relaxation. This hypothesis is inspired by a research study, in which poloxamers and different mucoadhesive polymers (HPMC included) were employed to develop *in situ* gelling vehicles for intranasal delivery of rivastigmine (RV) (Abouhussein et al., 2018). The authors obtained non-fickian mechanism of drug release for most of their formulations, and they proposed that it might indicate that RV release was controlled by an erosion-diffusion mechanism, thus fickian diffusion occurred together with the relaxation of the polymer matrix.

3.7. CBZ in vitro permeation

The absorption of drugs, the targets of which are located in the brain, can occur through the intranasal route by means of three different pathways: trigeminal, olfactory and systemic. Despite the advantage of alternative ways of drug delivery to the CNS, the absorption is hampered by multiple barriers. In fact, the active molecule can reach the systemic circulation provided that it crosses both the pseudostratified columnar epithelium and the mucus layer that lies on it. These obstacles partially remain also if the drug has to be absorbed by the neuronal pathway, because neither the trigeminal neuronal endings nor the olfactory neural cells are directly exposed to the nasal cavity (Cassano et al., 2021). As a result, to prove that the selected *in situ* gelling formulation containing poloxamers and low-viscous HPMC could be a promising vehicle for intranasal and nose-to-brain delivery of CBZ, permeation studies were assessed. The latter were performed across two different *in vitro* diffusion barriers, which had already been demonstrated to be promising models of the nasal mucosa (Corazza et al., 2022).

The first was the recently developed Permeapad® barrier that, being composed of phospholipids (soybean phosphatidylcholine S-100) between two regenerated cellulose support sheets, it is known as a biomimetic and cell-free *in vitro* model useful for testing drug passive diffusion. In fact, it is assumed that the dry lipids, which correspond to the middle layer of the stratified architecture, spontaneously form a tightly packed vesicular structure upon contact with aqueous media, resembling the cell membrane. Moreover, in virtue of the barrier's structure, the formed phospholipid vesicles remain in close proximity between each other, thus mimicking the tissue morphology (Berben et al., 2018). As a result of its mechanism of action, the PermeaPad® barrier was employed as a useful *in vitro* model to predict drug permeation across some biological barriers, such as the buccal (Bibi et al., 2016), intestinal (Ilie et al., 2020) and nasal mucosa (Wu et al., 2019). Fig. 6a displays the cumulative amount of CBZ permeated through the barrier plotted against time, when the drug was dissolved in ultrapure water (control sample) or loaded in either the mucoadhesive formulation or in the respective plain gel. The permeation profile of the control sample shows a good linearity for up to 90 min, during which the rapid diffusion was triggered by the fact that the drug was already available for absorption (as demonstrated by the *in vitro* release study, see Section 3.6). Thereafter, the diffusion rate gradually decreased until it reached zero (after 300 min, more than 90% of the drug was found in the receptor phase). This behavior was due to the gradual loss of

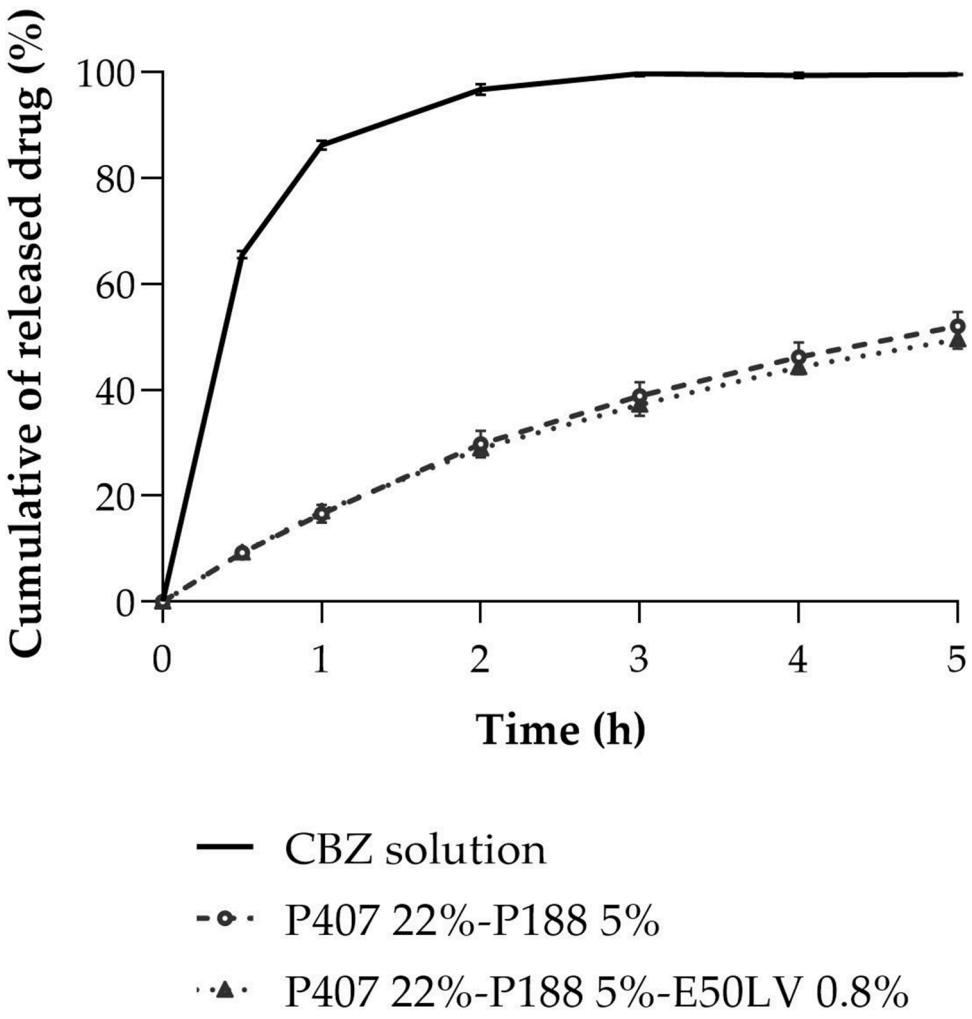

Fig. 5. Release profile of CBZ from the selected mucoadhesive formulation and its respective plain vehicle in comparison to a CBZ solution. Data are expressed as means ± SD, n = 3.

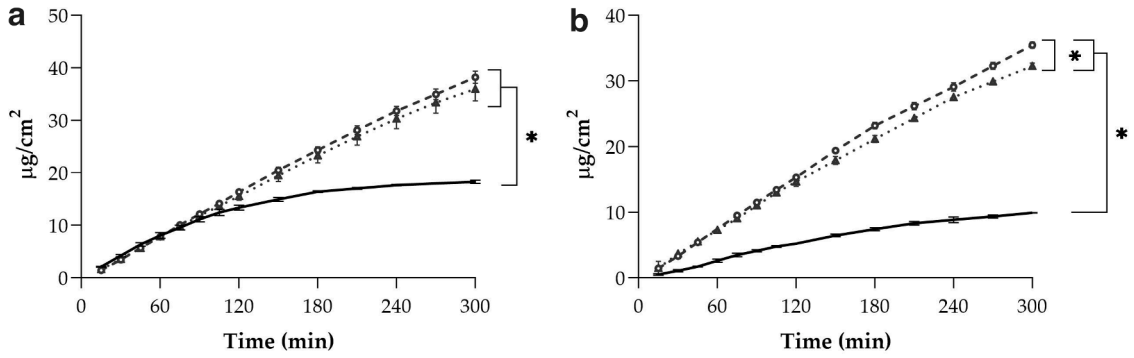

Fig. 6. Permeation profile of CBZ across a) the Permeapad® barrier, b) the Permeapad® barrier functionalized with a mucin layer. Data are expressed as means ± SD, n = 3. Significance indicated by * = $p < 0.05$ between samples indicated by the brackets.

concentration gradient. Differently, when CBZ was loaded in the thermosensitive gels, the drug permeation profile maintained its linearity for almost 240 min, as a result of the controlled release mechanism of CBZ from the gelling formulations. In fact, the graph indicates that the *in situ* gelling vehicles acted as drug depots. As the slope of the curves is almost identical in the first 70 min, this indicates that the concentration gradient (regarding the molecularly dissolved drug) should also be very similar between the three formulations. However, after this time point, the control sample profile decreases reaching a plateau, whereas the two formulations sustain the drug permeation. After 300 min, the mass permeated across the surface unit was 18.29 ± 0.33 μg/cm^2 for the CBZ solution, whereas for the nasal gels it was significantly ($p < 0.05$) higher 38.19 ± 1.19 μg/cm^2 for the P407 22%-P188 5% formulation and 35.92 ± 2.26 μg/cm^2 for the P407 22%-P188 5%-E50LV 0.8% formulation.

Since the trigeminal neuronal endings are located within the lower regions of the epithelia (Crowe and Hsu, 2022) and that the olfactory bipolar neurons extend their dendritic processes into the mucus layer (Cassano et al., 2021), drug passive diffusion was also investigated using

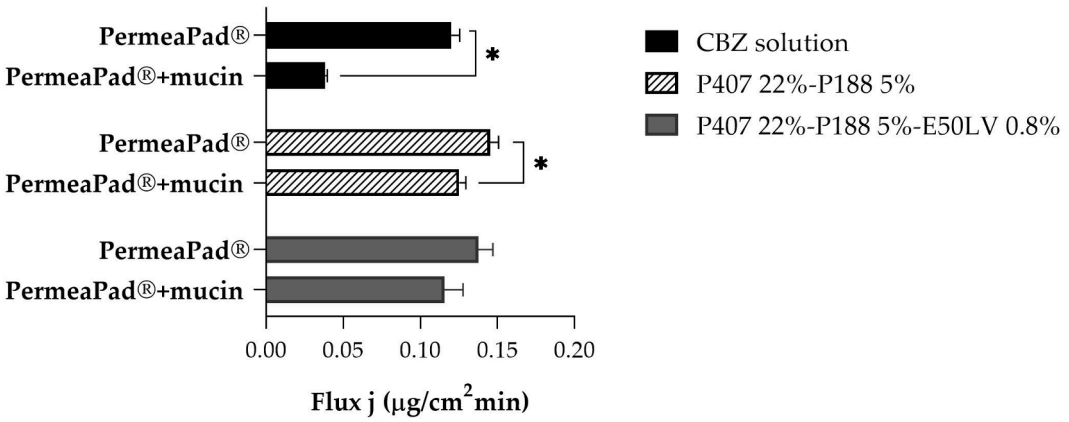

Fig. 7. Drug flux across the Permeapad® barrier and the Permeapad® barrier functionalized with a mucin layer. Data are expressed as means ± SD, n = 3. Significance indicated by * = $p < 0.05$ between samples indicated by the brackets.

a Permeapad® barrier functionalized with a mucin layer. Fig. 6b depicts the permeation profile of the drug across this enriched membrane. Similarly to the previous diffusion study, the permeation profiles of the nasal gels were characterized by a more extended period of linearity compared to the control, even though their permeation rate started to slightly decrease after 180 min. However, in this case, the slope of the initial part of the profile is greatly higher for CBZ in the two formulations as compared to the control solution. The result could be due to the interaction between the polymer network and mucin, that probably altered drug availability to permeation. Also in this case, the gelling formulation allowed for a greater mass diffusion after 300 min with respect to the CBZ solution (9.93 ± 0.06 μg/cm^2), and a small but significant ($p < 0.05$) difference was noted between the two thermosensitive vehicles. In fact, the plain formulation allowed for the diffusion of 35.44 ± 0.48 μg/cm^2 compared to the mucoadhesive gel, that reached 32.25 ± 0.46 μg/cm^2. This observation could be owed to the higher viscosity of the P407 22%-P188 5%-E50LV 0.8% compared to the plain gel, which contributed to an increased resistance of the polymer matrix, resulting in the slowdown of the diffusion process. It needs to be noted that the presence of the mucus left the amount of permeation widely unchanged for the two formulations as compared to the plain Permeapad® barrier.

The permeation profiles were also used to determine the drug flux in the different tested conditions, and data are summarized in Fig. 7. We decided to report the fluxes instead of the apparent permeability as the concentration of the molecularly dissolved drug within the gelling systems was not determined by a direct analytical method. Results clearly pointed out the negative influence of the mucin layer on drug diffusion rate when CBZ was solubilized in ultrapure water: the drug flux notably ($p < 0.05$) decreased from 0.12 ± 0.005 μg/cm^2 min in absence of mucin to 0.04 ± 0.001 μg/cm^2 min in presence of the mucous layer. This was an expected outcome since it is reported that the mucous coating mostly affects the diffusion of lipophilic and charged hydrophilic molecules rather than that of uncharged hydrophilic ones (Crowe and Hsu, 2022). Moreover, it was in agreement with our previous findings regarding the effect of mucin on hydrocortisone absorption through the nasal mucosa (Corazza et al., 2022). Concerning the nasal gels, an interesting result was obtained. When CBZ was loaded in the plain formulation, the drug flux across the Permeapad® barrier functionalized with mucin was decreased with respect to standard Permeapad® model, even though j was reduced to a lower extent compared to the control sample. Conversely, any remarkable differences were noted in the drug flux across the different models of the nasal mucosa when the thermoresponsive formulation containing 0.8% (w/v) of E50LV was employed. This effect might be consequence of the improved ability of the P407 22%-P188 5%-E50LV 0.8% formulation to interact with the mucous layer (see Section 3.2) that, as we had already observed for other molecules with bioadhesive properties (Corazza et al., 2022), favored drug diffusion.

4. Conclusions

The present work allowed for the development of a thermosensitive formulation capable of undergoing sol-gel transition once in the nasal cavity and intended for nasal and nose-to-brain delivery of CBZ. The candidate vehicle, which is composed of 22% P407, 5% P188 and 0.8% of E50LV, was obtained by means of a very straightforward procedure and using materials characterized by a good safety profile. The low viscosity of the formulation makes it suitable for an easy ad comfortable instillation as a nasal spray. Moreover, it shows a $T_{sol\text{-}gel}$ comprised between 32-35°C, undergoes gelation in less than 60 s and is featured by a pH value of 6, thus it presents all the properties required by an *in situ* gelling vehicle for nasal drug delivery. In addition to this, the developed formulation exhibits remarkable mucoadhesive properties, because it can strengthen the interaction with the mucous layer and the *in vitro* studies demonstrated that it is retained on the nasal mucosa for nearly 30 min. The possibility to exploit this optimized formulation as a platform for the delivery of CBZ is further supported by its ability to increase the solubility of the hydrophobic substance, release approximately 50% of the loaded drug in a controlled and sustained manner, as well as to favor CBZ permeation across both Permeapad® based models of the nasal mucosa as compared to a drug solution. Lastly, the outstanding result obtained in this work is that the drug flux across the Permeapad® barrier functionalized with the reconstituted mucin layer is not decreased when the selected gelling system is used. This strongly highlights the importance of bioadhesive agents when tuning the properties of these emerging smart delivery systems to improve drug absorption across mucosal tissues.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Elisa Corazza: Investigation, Data curation, Formal analysis, Writing – original draft. **Massimiliano Pio di Cagno:** Conceptualization, Supervision, Writing – review & editing. **Annette Bauer-Brandl:** Writing – review & editing. **Angela Abruzzo:** Supervision, Writing – review & editing. **Teresa Cerchiara:** Supervision, Writing – review & editing. **Federica Bigucci:** Supervision, Writing – review & editing. **Barbara Luppi:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank PHABIOC GmbH for providing the Permeapad® barriers and BASF SE for suppling Lutrol® polymers, and also Stephanie Yen Tran and Anna Semplici for their technical contribution to this work.

References

Abdeltawab, H., Svirskis, D., Sharma, M., 2020. Formulation strategies to modulate drug release from poloxamer based *in situ* gelling systems. Expert Opin. Drug Deliv. 17, 495–509. https://doi.org/10.1080/17425247.2020.1731469.

Abouhussein, D.M.N., Khattab, A., Bayoumi, N.A., Mahmoud, A.F., Sakr, T.M., 2018. Brain targeted rivastigmine mucoadhesive thermosensitive *in situ* gel: Optimization, *in vitro* evaluation, radiolabeling, *in vivo* pharmacokinetics and biodistribution. J. Drug Deliv. Sci. Technol. 43, 129–140. https://doi.org/10.1016/j.jddst.2017.09.021.

Abruzzo, A., Crispini, A., Prata, C., Adduci, R., Nicoletta, F.P., Dalena, F., Cerchiara, T., Luppi, B., Bigucci, F., 2021. Freeze-dried matrices for buccal administration of propranolol in children: physico-chemical and functional characterization. J. Pharm. Sci. 110, 1676–1686. https://doi.org/10.1016/j.xphs.2020.10.033.

Ana, R., Mendes, M., Sousa, J., Pais, A., Falcão, A., Fortuna, A., Vitorino, C., 2019. Rethinking carbamazepine oral delivery using polymer-lipid hybrid nanoparticles. Int. J. Pharm. 554, 352–365. https://doi.org/10.1016/j.ijpharm.2018.11.028.

Ban, E., Park, M., Jeong, S., Kwon, T., Kim, E.-H., Jung, K., Kim, A., 2017. Poloxamer-based thermoreversible gel for topical delivery of emodin: influence of p407 and p188 on solubility of emodin and its application in cellular activity screening. Molecules 22, 246. https://doi.org/10.3390/molecules22020246.

Berben, P., Bauer-Brandl, A., Brandl, M., Faller, B., Flaten, G.E., Jacobsen, A.-C., Brouwers, J., Augustijns, P., 2018. Drug permeability profiling using cell-free permeation tools: overview and applications. Eur. J. Pharm. Sci. 119, 219–233. https://doi.org/10.1016/j.ejps.2018.04.016.

Berillo, D., Zharkinbekov, Z., Kim, Y., Raziyeva, K., Temirkhanova, K., Saparov, A., 2021. Stimuli-responsive polymers for transdermal, transmucosal and ocular drug delivery. Pharmaceutics 13, 2050. https://doi.org/10.3390/pharmaceutics13122050.

Beydoun, A., DuPont, S., Zhou, D., Matta, M., Nagire, V., Lagae, L., 2020. Current role of carbamazepine and oxcarbazepine in the management of epilepsy. Seizure 83, 251–263. https://doi.org/10.1016/j.seizure.2020.10.018.

Bibi, H.A., Holm, R., Bauer-Brandl, A., 2016. Use of Permeapad® for prediction of buccal absorption: a comparison to *in vitro, ex vivo* and *in vivo* method. Eur. J. Pharm. Sci. 93, 399–404. https://doi.org/10.1016/j.ejps.2016.08.041.

Borisover, M., Sela, M., Chefetz, B., 2011. Enhancement effect of water associated with natural organic matter (NOM) on organic compound–NOM interactions: a case study with carbamazepine. Chemosphere 82, 1454–1460. https://doi.org/10.1016/j.chemosphere.2010.11.035.

Cassano, R., Servidio, C., Trombino, S., 2021. Biomaterials for drugs nose–brain transport: a new therapeutic approach for neurological diseases. Materials 14, 1802. https://doi.org/10.3390/ma14071802.

Cirri, M., Maestrelli, F., Nerli, G., Mennini, N., D'Ambrosio, M., Luceri, C., Mura, P.A., 2021. Development of a cyclodextrin-based mucoadhesive-thermosensitive *in situ* gel for clonazepam intranasal delivery. Pharmaceutics 13, 969. https://doi.org/10.3390/pharmaceutics13070969.

Corazza, E., Abruzzo, A., Giordani, B., Cerchiara, T., Bigucci, F., Vitali, B., di Cagno, M. P., Luppi, B., 2022. Human Lactobacillus biosurfactants as natural excipients for nasal drug delivery of hydrocortisone. Pharmaceutics 14, 524. https://doi.org/10.3390/pharmaceutics14030524.

Crowe, T.P., Hsu, W.H., 2022. Evaluation of recent intranasal drug delivery systems to the central nervous system. Pharmaceutics 14, 629. https://doi.org/10.3390/pharmaceutics14030629.

Cunha, S., Forbes, B., Sousa Lobo, J.M., Silva, A.C., 2021. Improving Drug Delivery for Alzheimer's disease through nose-to-brain delivery using nanoemulsions, nanostructured lipid carriers (NLC) and *in situ* hydrogels. Int. J. Nanomed. Vol. 16, 4373–4390. https://doi.org/10.2147/IJN.S305851.

da Silva, J.B., Cook, M.T., Bruschi, M.L., 2020. Thermoresponsive systems composed of poloxamer 407 and HPMC or NaCMC: mechanical, rheological and sol-gel transition analysis. Carbohydr. Polym. 240, 116268 https://doi.org/10.1016/j.carbpol.2020.116268.

Gierbolini, J., Giarratano, M., Benbadis, S.R., 2016. Carbamazepine-related antiepileptic drugs for the treatment of epilepsy - a comparative review. Expert Opin. Pharmacother. 17, 885–888. https://doi.org/10.1517/14656566.2016.1168399.

Giuliano, E., Paolino, D., Fresta, M., Cosco, D., 2018. Mucosal applications of poloxamer 407-based hydrogels: an overview. Pharmaceutics 10, 159. https://doi.org/10.3390/pharmaceutics10030159.

He, Z., Wang, Z., Zhang, H., Pan, X., Su, W., Liang, D., Wu, C., 2011. Doxycycline and hydroxypropyl-β-cyclodextrin complex in poloxamer thermal sensitive hydrogel for ophthalmic delivery. Acta Pharm. Sin. B 1, 254–260. https://doi.org/10.1016/j.apsb.2011.10.004.

Huang, W., Zhang, N., Hua, H., Liu, T., Tang, Y., Fu, L., Yang, Y., Ma, X., Zhao, Y., 2016. Preparation, pharmacokinetics and pharmacodynamics of ophthalmic thermosensitive *in situ* hydrogel of betaxolol hydrochloride. Biomed. Pharmacother. 83, 107–113. https://doi.org/10.1016/j.biopha.2016.06.024.

Huerta, B., Jakimska, A., Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2013. Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1288, 63–72. https://doi.org/10.1016/j.chroma.2013.03.001.

Ilie, A.-R., Griffin, B.T., Brandl, M., Bauer-Brandl, A., Jacobsen, A.-C., Vertzoni, M., Kuentz, M., Kolakovic, R., Holm, R., 2020. Exploring impact of supersaturated lipid-based drug delivery systems of celecoxib on *in vitro* permeation across Permeapad® membrane and *in vivo* absorption. Eur. J. Pharm. Sci. 152, 105452 https://doi.org/10.1016/j.ejps.2020.105452.

Illum, L., 1996. Nasal delivery. the use of animal models to predict performance in man. J. Drug Target. 3, 427–442. https://doi.org/10.3109/10611869609015963.

Javeer, S.D., Patole, R., Amin, P., 2013. Enhanced solubility and dissolution of simvastatin by HPMC-based solid dispersions prepared by hot melt extrusion and spray-drying method. J. Pharm. Investig. 43, 471–480. https://doi.org/10.1007/s40005-013-0092-1.

Joshi, S.C., 2011. Sol-gel behavior of hydroxypropyl methylcellulose (HPMC) in ionic media including drug release. Materials 4, 1861–1905. https://doi.org/10.3390/ma4101861.

Khan, N., Shah, F.A., Rana, I., Ansari, M.M., Din, F.ud, Rizvi, S.Z.H., Aman, W., Lee, G.-Y., Lee, E.-S., Kim, J.-K., Zeb, A., 2020. Nanostructured lipid carriers-mediated brain delivery of carbamazepine for improved *in vivo* anticonvulsant and anxiolytic activity. Int. J. Pharm. 577, 119033 https://doi.org/10.1016/j.ijpharm.2020.119033.

Li, H., Zhang, M., Xiong, L., Feng, W., Williams, R.O., 2020. Bioavailability improvement of carbamazepine via oral administration of modified-release amorphous solid dispersions in rats. Pharmaceutics 12, 1023. https://doi.org/10.3390/pharmaceutics12111023.

Liu, S., Yang, S., Ho, P.C., 2018. Intranasal administration of carbamazepine-loaded carboxymethyl chitosan nanoparticles for drug delivery to the brain. Asian J. Pharm. Sci. 13, 72–81. https://doi.org/10.1016/j.ajps.2017.09.001.

Fathalla, M.A., Vangala, Z., Longman, A., Khaled, M., Hussein, K.A., El-Garhy, A.K., Alany, O.H., 2017. Poloxamer-based thermoresponsive ketorolac tromethamine *in situ* gel preparations: Design, characterisation, toxicity and transcorneal permeation studies. Eur. J. Pharm. Biopharm. 114, 119–134. https://doi.org/10.1016/j.ejpb.2017.01.008.

Martins, I.L., Nunes, J., Charneira, C., Morello, J., Pereira, S.A., Telo, J.P., Marques, M. M., Antunes, A.M.M., 2018. The first-line antiepileptic drug carbamazepine: Reaction with biologically relevant free radicals. Free Radic. Biol. Med. 129, 559–568. https://doi.org/10.1016/j.freeradbiomed.2018.10.408.

Mawazi, Al-Mahmood, Chatterjee, Hadi, Doolaanea, 2019. Carbamazepine gel formulation as a sustained release epilepsy medication for pediatric use. Pharmaceutics 11, 488. https://doi.org/10.3390/pharmaceutics11100488.

Mura, P., Mennini, N., Nativi, C., Richichi, B., 2018. *In situ* mucoadhesive-thermosensitive liposomal gel as a novel vehicle for nasal extended delivery of opiorphin. Eur. J. Pharm. Biopharm. 122, 54–61. https://doi.org/10.1016/j.ejpb.2017.10.008.

Nguyen, T.-T.-L., Maeng, H.-J., 2022. Pharmacokinetics and pharmacodynamics of intranasal solid lipid nanoparticles and nanostructured lipid carriers for nose-to-brain delivery. Pharmaceutics 14, 572. https://doi.org/10.3390/pharmaceutics14030572.

Pires, P.C., Rodrigues, M., Alves, G., Santos, A.O., 2022. Strategies to improve drug strength in nasal preparations for brain delivery of low aqueous solubility drugs. Pharmaceutics 14, 588. https://doi.org/10.3390/pharmaceutics14030588.

Qi, X.-J., Liu, X.-Y., Tang, L.-M.-Y., Li, P.-F., Qiu, F., Yang, A.-H., 2020. Anti-depressant effect of curcumin-loaded guanidine-chitosan thermo-sensitive hydrogel by nasal delivery. Pharm. Dev. Technol. 25, 316–325. https://doi.org/10.1080/10837450.2019.1686524.

Russo, E., Villa, C., 2019. Poloxamer hydrogels for biomedical applications. Pharmaceutics 11, 671. https://doi.org/10.3390/pharmaceutics11120671.

Schilling, A.L., Cannon, E., Lee, S.E., Wang, E.W., Little, S.R., 2022. Advances in controlled drug delivery to the sinonasal mucosa. Biomaterials 282, 121430. https://doi.org/10.1016/j.biomaterials.2022.121430.

Serralheiro, A., Alves, G., Fortuna, A., Falcão, A., 2014. Intranasal administration of carbamazepine to mice: a direct delivery pathway for brain targeting. Eur. J. Pharm. Sci. 60, 32–39. https://doi.org/10.1016/j.ejps.2014.04.019.

Singh, R.M., Kumar, A., Pathak, K., 2013. Mucoadhesive *in situ* nasal gelling drug delivery systems for modulated drug delivery. Expert Opin. Drug Deliv. 10, 115–130. https://doi.org/10.1517/17425247.2013.746659.

Wang, Q.-S., Li, K., Gao, L.-N., Zhang, Y., Lin, K.-M., Cui, Y.-L., 2020. Intranasal delivery of berberine *via in situ* thermoresponsive hydrogels with non-invasive therapy exhibits better antidepressant-like effects. Biomater. Sci. 8, 2853–2865. https://doi.org/10.1039/C9BM02006C.

Wu, I.Y., Bala, S., Škalko-Basnet, N., di Cagno, M.P., 2019. Interpreting non-linear drug diffusion data: utilizing korsmeyer-peppas model to study drug release from

liposomes. Eur. J. Pharm. Sci. 138, 105026 https://doi.org/10.1016/j.ejps.2019.105026.

Yu, Y., Cheng, Y., Tong, J., Zhang, L., Wei, Y., Tian, M., 2021. Recent advances in thermo-sensitive hydrogels for drug delivery. J. Mater. Chem. B 9, 2979–2992. https://doi.org/10.1039/D0TB02877K.

Zahir-Jouzdani, F., Wolf, J.D., Atyabi, F., Bernkop-Schnürch, A., 2018. *In situ* gelling and mucoadhesive polymers: why do they need each other? Expert Opin. Drug Deliv. 15, 1007–1019. https://doi.org/10.1080/17425247.2018.1517741.