Stability testing of cyclodextrin-based meloxicam potassium containing nanospheres intended for nasal administration

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Abstract: Nasal delivery of drugs may substitute the parenteral or oral administration. Among the nasal drug delivery systems, powders have favourable properties such as their good adhesion to the mucosa or their improved stability due to their low moisture content. In this work, two cyclodextrin-based meloxicam potassium-containing nasal powder formulations were tested applying the accelerated stability test conditions according to the ICH guideline. Based on the results, the hydroxypropyl- β-cyclodextrin containing sample showed better stability, however, the experienced morphological change is a problem to be solved.

Keywords: *stability test, cyclodextrin, meloxicam potassium, nasal drug delivery, nasal powder*

1. Introduction

Nasal delivery of drugs may be a good alternative to the parenteral or oral administration [1] . It provides a non-invasive, painless way for the active pharmaceutical ingredients (APIs) to absorb rapidly to the systemic circulation – while avoiding the first pass hepatic metabolism – or the central nervous system [2,3] .

Most of the marketed nasal products are sprays or drops, however, these liquid formulations may be easily forwarded towards the throat by the mucociliary clearance [4,5] . This defence mechanism of the nose shortens the contact time of the API with the mucosa which results in lower drug absorption [6] . In contrast, powders can adhere better to the nasal mucosa potentially improving the bioavailability of the APIs. One of the most common preparation methods for powders is spray drying, which results in products with low moisture content and therefore, improved stability [7–9].

Stability tests are crucial parts of the development of new products by which we can get information about the quality changes under different environmental circumstances. These changes may affect the efficacy or the safety of the formulation, therefore monitoring them is important and necessary [10].

Previously, cyclodextrin-based meloxicam potassium containing nanospheres were studied [11], and the two most promising ones were chosen to be further investigated. The aim of our present work was to examine their six-month stability based on the ICH guideline and to study the effect of the possible changes on the nasal applicability of these formulations.

2. Materials and methods

2.1 Materials

Meloxicam potassium monohydrate (MELP) was from Egis Ltd. (Budapest, Hungary). (2-Hydroxy)-propyl-β-cyclodextrin (HPBCD) and α-cyclodextrin (ACD) were purchased from Cyclolab Ltd. (Budapest, Hungary), poly(vinylalcohol) (PVA) was from Sigma-Aldrich (Sigma-Aldrich Co. LLC, St. Louis, MO, USA).

2.2 Methods

2.2.1 Preparation of the MELP containing samples

The samples were produced by spray drying with BÜCHI Nano Spray Dryer B-90 HP (BÜCHI Labortechnik AG, Flawil, Switzerland) applying inlet air temperature of 80 °C, pump rate of 20%, aspirator capacity of 100% and compressed air flow of 130 $L \cdot h^{-1}$. The feeding solutions were prepared by dissolving 1:1 mol/mol ratio of MELP and cyclodextrin (HPBCD or ACD) in 0,1% (m/v) of aqueous PVA solution.

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2.2.2 Stability testing of the formulations

The accelerated stability test was carried out according to the ICH Q1A (R2) guideline. The samples were stored in HPMC (hydroxypropylmethyl cellulose) capsules without secondary packaging in a Binder KBF 240 (Binder GmbH Tuttlingen, Germany) constant-climate chamber at 40 ± 2 °C and 75 ± 5% RH. Samplings were carried out after one month, three months and six months.

2.2.3 Scanning electron microscope (SEM) measurements

The morphology and size of the prepared particles were examined by SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan) at 10kV. The samples were sputter-coated (Bio-Rad SC 502, VG Microtech, Uckfield, UK) with gold-palladium.

2.2.4 X-ray powder diffractometry (XRPD)

The crystalline state of the prepared samples was investigated by a Bruker D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) and VÅNTEC-1 detector was applied. The powders were irradiated by Cu $K_{\lambda 1}$ (λ=1.5406 Å) and were investigated at 40 kV voltage and 40 mA current in the range of 3°-40° 2θ at a step of 0.007°/0.1 sec.

DIFFRACTPLUS EVA software was used for the manipulations: Kα_2-stripping, background removal and smoothing.

2.2.5 Thermal analysis

The thermal analysis was carried out with the Mettler Toledo DSC/TGA 821^e system (Mettler-Toledo GmbH, Greifensee, Switzerland). 3-5 mg of the samples were examined in sealed and pierced aluminium pans in the temperature range of 25-300 °C applying 10 °C·min^{-1} heating rate under a constant argon flow of 10 L·h^{-1}. STAR^e software (Mettler-Toledo GmbH, Greifensee, Switzerland) was used for the evaluation of the measurements.

2.2.6 High-performance liquid chromatography (HPLC)

The drug content change of the formulations throughout the stability test was studied using an Agilent 1260 HPLC system (Agilent Technologies, San Diego, United States). The mobile phase was composed of phosphate buffer (pH=2.8):MeOH=42:58 (v/v). A Kinetex® EVO 5 μm C18 100 *Å column (*150 x 4.6 mm, Phenomenex, Torrance, CA, USA) was used as the stationary phase. Powders containing theoretically 0.5 mg of MELP were dissolved in distilled water and filtered into vials through a syringe filter with a pore diameter of 0.22 μm. 10 μL of the prepared solutions was injected and separated with isocratic flow of 1 mL/min for 10 min at 30 °C. MELP was analysed at 364 nm with a diode array detector. Data were evaluated with the help of ChemStation B.04.03. Software (Agilent Technologies, Santa Clara, United States). The "before storage" state of the samples was considered as 100%.

The statistical analysis of the results was carried out using Minitab 17 Statistical Software (Minitab Ltd., Coventry, UK). The evaluation was conducted using analysis of variances (ANOVA), and the data were compared to the "before storage" state of the samples by applying Dunnett's post-hoc test. Results were considered significant when $p<0.05$.

2.2.7 *In vitro* permeation study

The *in vitro* diffusion of MELP under nasal conditions was tested using a modified horizontal diffusion cell. The donor phase was 9 ml of simulated nasal electrolyte solution (SNES), the acceptor phase was 9 ml of phosphate buffer solution (PBS; pH=7.4) and the two chambers were divided by an artificial membrane (WhatmanTM regenerated cellulose membrane filter with 0.45 *μm* pores) which was soaked in isopropyl myristate for 30 min before the investigation. 300 rpm stirring and 32 °C were provided for both of the phases during the experiment. The amount of MELP permeated into the acceptor phase was measured in real-time at 364 nm using an AvaLight DH-S-BAL spectrophotometer (AVANTES, Apeldoorn, The Netherlands).

3. Results and discussion

3.1 Morphology and particle size of the spray dried samples

The morphology and size of the prepared particles during the 6 month-period were investigated by SEM and ImageJ software. With nano spray drying, smooth surfaced spherical particles were prepared for both samples (ACD_MELP_PVA, HPBCD_MELP_PVA) with an average particle size of 0.83 and 0.89 μm, respectively (Table I). In the case of the ACD-based sample, after 6 months, irregularly shaped aggregated particles appeared suggesting the start of recrystallization, which caused the average particle size to increase to 1.59 μm. The

Table I *Average particle size*

Sample	Average particle size (μm)	
ACD_MELP_PVA	Before storage	0.83±0.36
	1 month	1.02±*0.34*
	3 months	1.47±*0.53*
	6 months	1.59±*0.61*
HPBCD_MELP_PVA	Before storage	0.89±*0.34*
	1 month	2.09±*0.81*
	3 months	n.m.
	6 months	n.m.

In the table n.m. means that the particle size was not measurable in those cases.

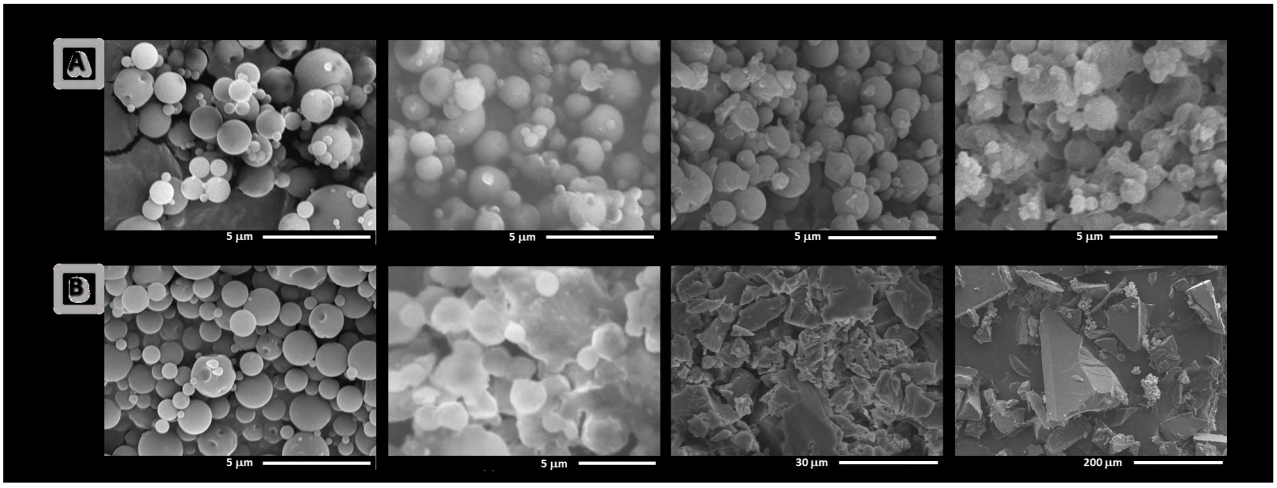

Figure 1 *SEM images of (A) ACD_MELP_PVA and (B) HPBCD_MELP_PVA samples*

HPBCD-based samples showed a non-negligible change in the morphology. After 6 months, no individual particles were detectable, amorphous, liquefied, glass-like blocks were formed suggesting that the formulation absorbed water from the highly humid environment. Because of the undefinable particles, the determination of the particle size was not possible from the 3rd month of the test.

3.2 Crystallinity of the samples

Figure 2 presents the diffractograms of the physical mixtures and the spray dried samples. In the physical mixture in Figure 2A, the characteristic peaks of MELP and ACD could be observed at 2θ of 6.1, 15.4, 30.9 ° and 12, 14.3, 21.8 °, respectively, confirming their crystalline state [12]. In Figure 2B, only the peaks of MELP were detected, because HPBCD and PVA were amorphous. Spray drying resulted in the amorphization of the samples in both cases, since, all the previously noticeable crystalline peaks disappeared. Considering ACD_MELP_PVA, recrystallization occurred after 6 months of storage, however, the appearing peaks were different from that of the physical mixture and were not identical to any of the known polymorphic forms of MELP, meloxicam or ACD according to the Cambridge Crystallographic Data Centre, so further investigations are needed. In the case of HPBCD_MELP_PVA, the amorphous state was kept through the hole examined period, presumably, the originally amorphous HPBCD had a stabilizing effect on the formulation.

3.3 Thermal properties

DSC and TG were used to examine the thermal properties of the samples, the thermograms can be seen in Figure 3. In the physical mixtures, the endothermic bands above 110 °C were due to water loss, and the endothermic peak at around 170 °C suggested the melting of crystalline MELP. The exothermic peaks at around 250 °C indicated the degradation of MELP. In the thermograms of the spray dried samples before storage, only the loss of water could be detected as an endothermic event – 3.4 % and 2.2% for ACD_MELP_PVA and HPBCD_MELP_PVA, respectively, according to the TG measurements (Table II). The peak as-

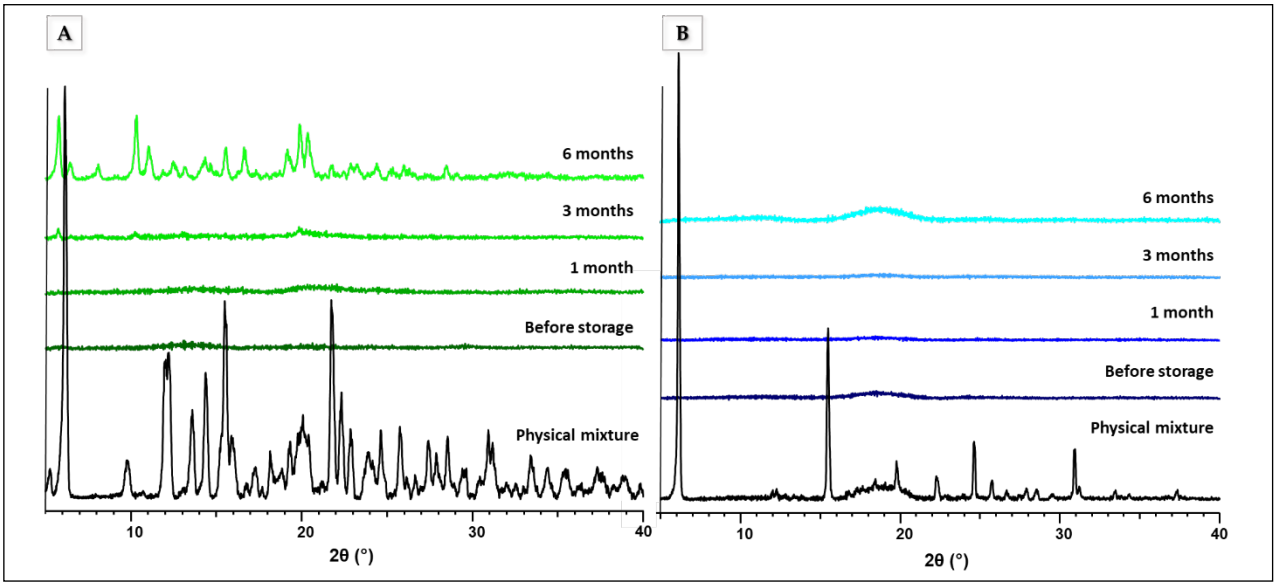

Figure 2 *Diffractograms of (A) ACD_MELP_PVA and (B) HPBCD_MELP_PVA*

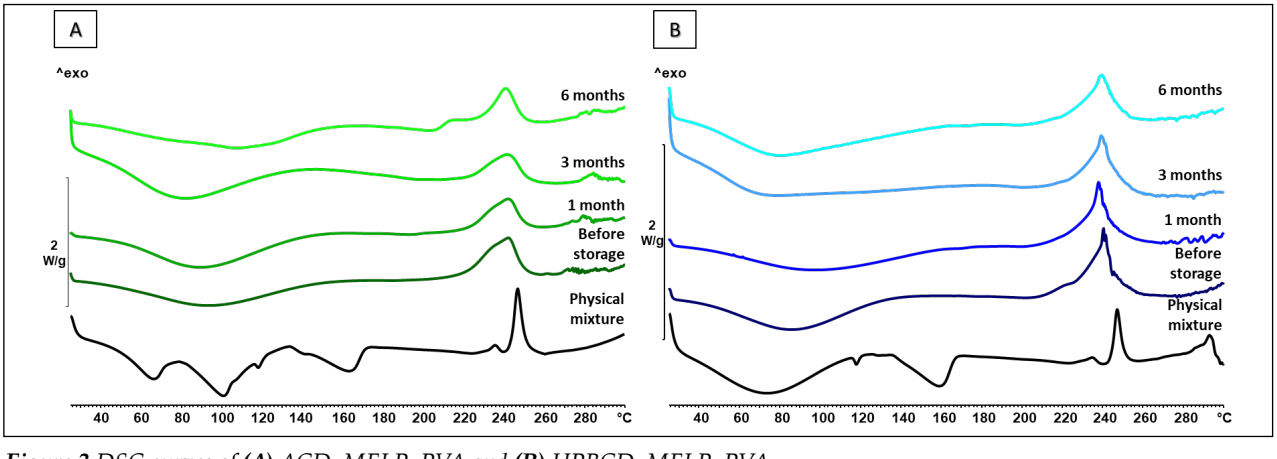

Figure 3 *DSC curves of* ***(A)*** *ACD_MELP_PVA and* ***(B)*** *HPBCD_MELP_PVA*

signed to the melting of MELP disappeared referring to its amorphization due to the preparation process. Moreover, the exothermic peaks that could be attributed to the decomposition of MELP widened and shifted compared to those detected in the physical mixtures suggesting the complexation of the drug. The TG measurements revealed, that after 6 months, the water loss was 6.2% for ACD_MELP_PVA, and 9.7% for HPBCD_MELP_PVA. The more than 4 times higher water content of the 6 month-HPBCD-based sample may provide an explanation for the morphological changes seen in the SEM images. Probably the formulation tends to absorb moisture, and it took up so much water from its environment, that the experienced amorphous, glassy appearance was developed. However, it is important to mention again, that there were no visible signs of recrystallization according to the XRPD and DSC results, the originally amorphous HPBCD could

Table II *Water loss of the samples based on the TG measurements*

Storage time	Water loss (%)	
	ACD_MELP_PVA	HPBCD_MELP_PVA
Before storage	3.4	2.2
1 month	4.9	3.2
3 months	5.3	6.0
6 months	6.2	9.7

Table III *Drug content of the samples*

Sample	Drug content (%)		Significance
ACD_MELP_PVA	1 month	98.44±1.80	n.s.
	3 months	93.89±3.02	*
	6 months	92.17±1.23	**
HPBCD_MELP_PVA	1 month	101.48±2.35	n.s.
	3 months	100.63±1.55	n.s.
	6 months	101.14±0.57	n.s.

** means p<0.05, ** means p<0.01 and n.s. means not significant*

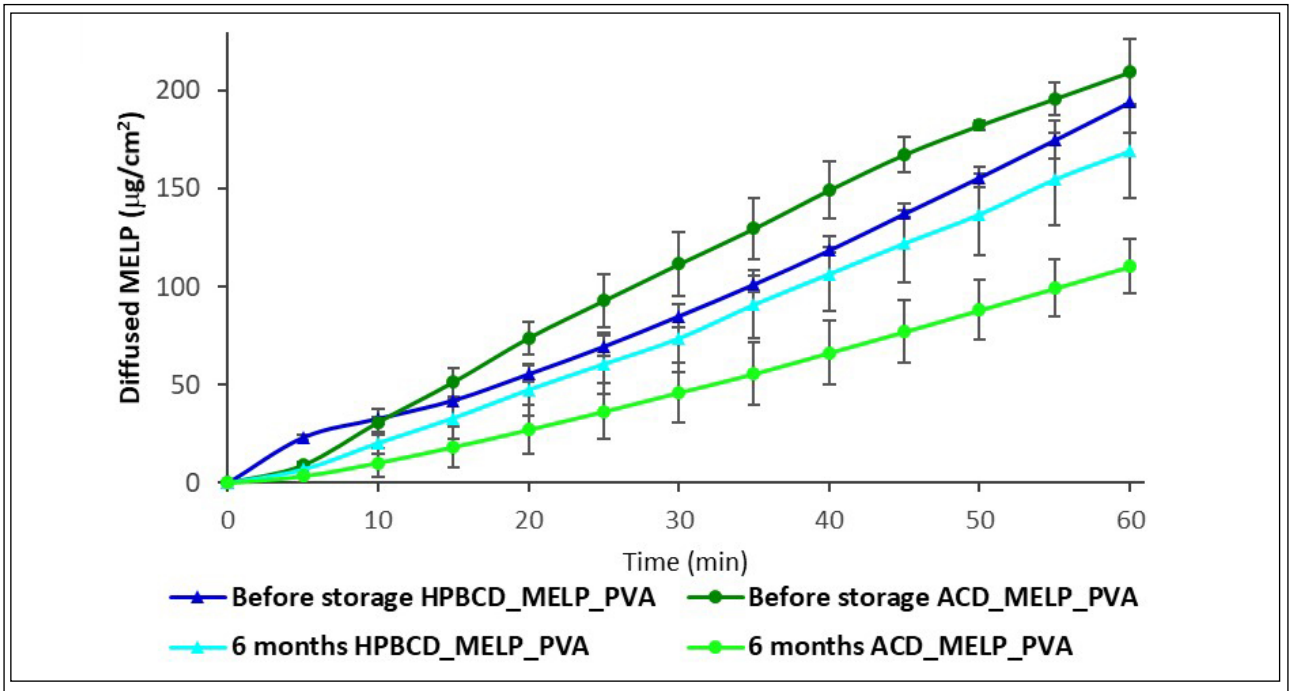

Figure 4 *Result of the in vitro drug permeation test*

stabilize the spray dried formulation in the amorphous state.

3.4 Drug content change throughout the stability test

The change in the drug content of the samples is demonstrated in Table III. Compared to the samples' "before storage" state, which was considered as 100%, regarding ACD_MELP_PVA, a significant decrease ($p<0.05$) was first detected after 3 months of storage under the forced stability test conditions. The MELP-content further decreased ($p<0.01$) under 95%, which is considered as an acceptance limit for the final products. The experienced reduction may have occurred due to the degradation of the API, however, the HPLC method used for the drug content studies was not suitable for the detection of the possible degradation products. In the case of HPBCD_MELP_PVA, no significant change in the API content was observed even after 6 months.

3.5 In vitro drug permeation

The *in vitro* drug permeation test was carried out before and after 6 months of storage to see if the changes in the physicochemical properties had any impact on the drug diffusion from the formulation under nasal conditions. In Figure 4, in the case of ACD_MELP_PVA, compared to the previously determined 209.4 µg/cm² permeation rate [11] , it decreased significantly to 110.4 µg/cm². This may have occurred due to the noticed recrystallization – which may have caused a change in the dissolution of the formulation – and the significant decrease in the drug content of the sample. Regarding HPBCD_MELP_PVA, no significant reduction was observed in the permeation of MELP after 6 months, which was 169.1 µg/cm² compared to the previously measured 194.2 µg/cm² [11] .

4. Conclusions

In this work, the stability testing of two different cyclodextrin-based formulation was carried out in order to reveal the effect of the accelerated stability test conditions (40 ± 2 °C and 75 ± 5% RH) on their physicochemical properties and *in vitro* drug diffusion.

The prepared ACD_MELP_PVA nanospheres were stable in terms of morphology and crystallinity up to 3 months, however, by the end of the stability test, probably the recrystallization detected by XRPD resulted in aggregated, irregularly shaped particles. The drug content decrease became significant after 3 months, which together with the recrystallization may have negatively affected the permeation of MELP.

HPBCD_MELP_PVA had an obvious change in the morphology of the particles presumably because of its more than four times higher moisture content compared to its before storage state. Its amorphous state did not change after 6 months, and the diffusion of MELP did not change significantly from it.

Based on our results, HPBCD_MELP_PVA is considered the more stable formulation, however, a non-permeable packaging would be necessary to maintain the morphology of the particles, which is crucial in the case of a nasal powder.

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References

1. Laffleur F, Bauer B. Progress in nasal drug delivery systems. International Journal of Pharmaceutics [Internet]. 2021 Sep [cited 2023 Aug 30];607:120994. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378517321008000 https://doi.org/10.1016/j.ijpharm.2021.120994
2. Forbes B, Bommer R, Goole J, Hellfritzsch M, De Kruijf W, Lambert P, et al. A consensus research agenda for optimising nasal drug delivery. Expert Opinion on Drug Delivery [Internet]. 2020 Feb 1 [cited 2023 Aug 30];17(2):127-32. Available from: https://www.tandfonline.com/doi/full/10.1080/17425247.2020.1714589 https://doi.org/10.1080/17425247.2020.1714589
3. Khafagy ES, Morishita M, Isowa K, Imai J, Takayama K. Effect of cell-penetrating peptides on the nasal absorption of insulin. Journal of Controlled Release [Internet]. 2009 Jan [cited 2023 Aug 30];133(2):103-8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365908005300 https://doi.org/10.1016/j.jconrel.2008.09.076
4. Maeng J, Lee K. Systemic and brain delivery of antidiabetic peptides through nasal administration using cell-penetrating peptides. Front Pharmacol [Internet]. 2022 Nov 14 [cited 2023 Aug 30];13:1068495. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2022.1068495/full https://doi.org/10.3389/fphar.2022.1068495
5. Ozsoy Y, Gungor S, Cevher E. Nasal Delivery of High Molecular Weight Drugs. Molecules [Internet]. 2009 Sep 23 [cited 2023 Aug 30];14(9):3754-79. Available from: http://www.mdpi.com/1420-3049/14/9/3754 https://doi.org/10.3390/molecules14093754
6. Inoue D, Tanaka A, Kimura S, Kiriyama A, Katsumi H, Yamamoto A, et al. The relationship between in vivo nasal drug clearance and in vitro nasal mucociliary clearance: Application to the prediction of nasal drug absorption. European Journal of Pharmaceutical Sciences [Internet]. 2018 May [cited 2023 Aug 30];117:21-6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0928098718300393 https://doi.org/10.1016/j.ejps.2018.01.032
7. Tepper SJ, Johnstone MR. Breath-powered sumatriptan dry nasal powder: an intranasal medication delivery system for acute treatment of migraine. MDER [Internet]. 2018 May [cited 2023 Aug 30];Volume 11:147-56. Available from: https://www.dovepress.com/breath-powered-sumatriptan-dry-nasal-powder-an-intranasal-medication-d-peer-reviewed-article-MDER https://doi.org/10.2147/MDER.S130900
8. Tiozzo Fasiolo L, Manniello MD, Tratta E, Buttini F, Rossi A, Sonvico F, et al. Opportunity and challenges of nasal powders: Drug formulation and delivery. European Journal of Pharmaceutical Sciences [Internet]. 2018 Feb [cited 2023 Aug 30];113:2-17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0928098717305195 https://doi.org/10.1016/j.ejps.2017.09.027
9. Henriques P, Fortuna A, Doktorovová S. Spray dried powders for nasal delivery: Process and formulation considerations. European Journal of Pharmaceutics and Biopharmaceutics [Internet]. 2022 Jul [cited 2023 Aug 30];176:1-20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S093964112200090X https://doi.org/10.1016/j.ejpb.2022.05.002
10. González-González O, Ramirez IO, Ramirez BI, O'Connell P, Ballesteros MP, Torrado JJ, et al. Drug Stability: ICH versus Accelerated Predictive Stability Studies. Pharmaceutics [Internet]. 2022 Oct 28 [cited 2023 Aug 30];14(11):2324. Available from: https://www.mdpi.com/1999-4923/14/11/2324 https://doi.org/10.3390/pharmaceutics14112324
11. Varga P, Ambrus R, Szabó-Révész P, Kókai D, Burián K, Bella Z, et al. Physico-Chemical, In Vitro and Ex Vivo Characterization of Meloxicam Potassium-Cyclodextrin Nanospheres. Pharmaceutics [Internet]. 2021 Nov 6 [cited 2023 Aug 30];13(11):1883. Available from: https://www.mdpi.com/1999-4923/13/11/1883 https://doi.org/10.3390/pharmaceutics13111883
12. Chen M, Chen X, Yam K. Encapsulation complex of chlorine dioxide in α-cyclodextrin: Structure characterization and release property. Food Control [Internet]. 2020 Jan [cited 2023 Aug 30];107:106783. Available from: https://linkinghub.elsevier.com/retrieve/pii/S095671351930372X https://doi.org/10.1016/j.foodcont.2019.106783