IECP 2020

sciforum

Conference Proceedings Paper

Cyclodextrins in traditional and alternative drug formulations

Rita Ambrus*, Csilla Bartos, Gábor Katona, Tamás Kiss, Zoltán Aigner, Piroska Szabó-Révész

Institute of Pharmaceutical Technology and Regulatory Affairs, Faculty of Pharmacy, University of Szeged, Szeged Hungary, ambrus.rita@szte.hu (R.A.); bartoscsilla@pharm.u-szeged.hu (Cs. B.); katona.gabor@szte.hu (G. K.); tamas.kiss@pharm.u-szeged.hu (T. K.); aigner@pharm.u-szeged.hu (Z. A.),; revesz@pharm.u-szeged.hu (P. Sz.)

* Correspondence: ambrus.rita@szte.hu; Tel.: +36-208-232-139

Abstract: Our research interest focused on the development of preparation protocols using cyclodextrins (CDs) combined with other additives to produce suitable formulations (to reach local or systemic effect) to get effective therapies in different diseases. Niflumic acid, levodopa, and ciprofloxacin were used as a model active ingredients for preparation of samples which could be used by oral, intranasal and pulmonary applications. α-, β-, HPβCD and different kind of polymer as stabilizer (Polyvinyl alcohol, PVA; Polyvinylpyrrolidone, PVP) were also applied in order to change the unfavorable features and increase bioavailability of drugs. Samples were produced by kneading, solvent evaporation, co-grinding and spray drying technologies. The micrometric properties, structural characterization, *in vitro* drug release, permeability, aerodynamic tests and cytotoxicity studies were applied for characterization of products. The introduced case studies justified the role and efficacy of cyclodextrins in drug formulation.

Keywords: niflumic acid, levodopa, ciprofloxacin, cyclodextrins, technological protocols, alternative administration, physico-chemical characterization, *in vitro* tests, citotoxicity studies

1. Introduction

Efforts to innovate existing medication include the development of medicines with higher selectivity of action, less toxicity and side-effects, higher stability, a more favourable pharmacokinetic profile and improved patient compliance. Modern pharmaceutical technology is concentrated on new drug forms which are targeted to the exact site at the appropriate time, with maximum efficiency and with reduced side-effects. Cyclodextrins (CDs) have a wide range of applications in different field of drug formulation due to their complexation ability which could improve the solubility, stability, safety and bioavailability of drug molecules. The application of natural and chemically modified CDs could present inclusion complexes by different technological methods. CDs are able to extend the function of pharmaceutical additives therefore their application become effective and valuable tool for development of drug delivery systems during different administration routes [1].

Niflumic acid (NIF), an anthranilic acid derivative, is a frequently used anti-inflammatory drug, which also has a weak analgetic effect. It is primarily used to treat different forms of rheumatism, e.g. rheumatoid arthritis and arthrosis, and to decrease other inflammatory phenomena. It has some side-effects, such as nausea or vomiting. In cases of stomach ulcer, it may only be used under medical control. Therefore, our aim was to increase its solubility and dissolution rate by applying different formulation methods using CDs for per os application [2, 3].

Levodopa (LEVO) as the gold standard in the treatment of Parkinson's disease is usually administrated orally but its bioavailability is low. The oral administration of LEVO alone and in

combination are available in the market in immediate-release tablets and capsule dosage forms. Its alternative administration could offer a possibility of drug transport to the central nervous system (CNS), by passing the first-pass metabolism. Liquid formulations of this drug, such as nasal drops or sprays, cannot be prepared because of the fast degradation, therefore it is necessary to make a nasal powder. Some excipients can open the tight junctions between epithelial cells in the nasal cavity, e.g. PVA, PVP, α-,β-, γ- CDs and their derivatives. Besides, nasal powders have micronized drug particles with high cohesive property, therefore e.g. binary systems should be prepared with different excipients to decrease the cohesive and adhesive forces and to increase the flowability of the powder mixture. Beyond opening the tight junctions CD microspheres could be good mucoadhesive excipients for nasal delivery. Hydrophilic drugs like LEVO have low nasal absorption in humans, which can be increased with CD. The aim of our work was the development of a LEVO containing nasal powder form as a binary system by dry co-grinding process. In preformulation study the physical and chemical interactions between LEVO and the excipients (e.g. CD) were examined because of the high mechanical stress during grinding, and the dissolution extent of LEVO was also controlled [4, 5].

The most common treatment for respiratory infection involves oral or parenteral administration of high doses of single or combined antibiotics which can show undesirable side effect because of high systemic bioavailability. Pulmonary dosage form of antibiotics can increase patient comfort and compliance, causing promoted treatment outcome. Dry powder inhalation systems (DPI) are formulated by micronized drug particles with aerodynamic particle sizes of less than 5μm using particle engineering.technologies. Ciprofloxacin has potent and effective activity against a wide range of Gram-positive bacteria and against most Gram-negative microorganisms and it is often used in the treatment of inhalation anthrax and other lung infections. Our goal was therefore to develop a carrier-free co-spray-dried DPI product containing the board spectrum antibiotic ciprofloxacin and excipients e.g. CD [6, 7].

2. Experiments

2.1. Materials

Niflumic acid (NIF) 2-[[3-(trifluoromethyl)phenyl]amino]-3-pyridinecarboxylic acid (G. Richter Ltd., Hungary). Polyvinylpyrrolidone (PVP): K-90, Mw: 1 300 000 (Pharmacopoeia Hungarica 7th Edition), Levodopa (LEVO), α-cyclodextrin (α-CD), were obtained from Sigma-Aldrich. Ciprofloxacin (CIP), a fluoroquinolone-type antibiotic was supplied by Teva Pharmaceutical Works Ltd. (Debrecen, Hungary). Polyvinyl alcohol 3-88 (PVA), a water-soluble synthetic polymer as a coating material was purchased from BASF (Cologne, Germany). The amino acid l-leucine (LEU) was obtained from Hungaropharma Ltd. (Budapest, Hungary). Hydroxypropyl beta-cyclodextrin (HPβCD), a chemically modified cyclic oligosaccharide was donated by Cyclolab Ltd. (Budapest, Hungary).

2.2. Sample preparation methods

Cyclodextrin binary and ternary complexes were prepared by using NIF, CD and PVP. The products were prepared in four different mole ratios (NIF:CD mole ratio = 2:1, 1:1, 1:2 and 1:3). Physical mixtures (PMs): The plain drug and CD were mixed in a mortar and sieved through a 100 μm sieve. Two types of solvent method were applied (kneading and ultrasonication). Kneaded products (KPs): PMs of the drug and HP-β-CD were mixed with the same mass quantity of a solvent mixture of ethanol + water (1:1). They were kneaded until the bulk of the solvent mixture had evaporated. The ultrasonicated systems (USs): the PMs were dissolved in 50 % ethanol, placed in the Grant ultrasonic bath XB2 (Keison, England) for 1 h, dried and pulverized. The three-component products were prepared in four different mole ratios (NIF:HP-β-CD mole ratio =

2:1, 1:1, 1:2 and 1:3), in all cases containing 15% (w/w) PVP K-90. Ternary physical mixtures (PMs+PVP): NIF, HP-β-CD and PVP were mixed in a mortar and sieved through a 100 μm sieve. Ternary kneaded products (KPs+PVP): PMs+PVP were mixed with the same quantity of a solvent mixture of ethanol + water (1:1), and were kneaded until the bulk of the solvent mixture had evaporated. After this, they were dried at room temperature and then at 105 °C, and were pulverized and sieved through a 100 μm sieve.

For the preparation of LEVO-CD binary mixtures, a planetary ball mill (PM 100 MA, Retsch GmbH, Haan, Germany) was used. Co-grinding was executed to reach the target range of particle size (5-40 μm). Two process parameters were changed: grinding time and the LEVO:excipient ratio, and their effect on the average particle size was investigated. Grinding was carried out for 60 min, at 400 rpm in a 50-mL milling chamber with ten stainless steel milling balls with a diameter of 8 mm. Sampling was performed in every 15 minutes to determine the optimal grinding time. Finaly the LEVO-excipient mass ratio was 30:70 as optimized formulation.

By the preparation of dry powder inhalers a small amount of ethanol (10%)in an aqueous solution is known to decrease the particle size because of its fast evaporation during spray drying (Büchi Mini Dryer B-191, BÜCHI Labortechnik, Flawil, Switzerland) could effect the final size. Therefore, the feed solution was prepared by dissolving 1 gram of CIP and different excipients at different concentrations in an aqueous solution containing 50 ml of 10% of ethanol. CIP_CD (1g_0.9g) and CIP_LEU_PVA_CD (1g_0.4g_0.2 g_0.9g) were the investigated samples.

2.3. Investigations

The particle size distribution and morphology were determined with laser diffraction (using Malvern Mastersizer Scirocco 2000; Malvern Instruments Ltd., Worcestershire, UK) and scanning electron microscopy (Hitachi S4700; Hitachi Scientific Ltd., Tokyo, Japan). The wettability was investigated using contact angle system (Dataphysics OCA 20, Dataphysics Inc., GmbH, Germany). Physico-chemical properties were analyzed with differential scanning calorimetry (Mettler Toledo DSC 821e thermal analysis system with STAR^e thermal analysis program V6.0 (Mettler Inc., Schwarzenbach, Switzerland) and X-ray powder diffraction (Bruker D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany). *In vitro* drug release was carried out by modified paddle method in different dissolution media. Aerodynamic properties have been tested *in vitro* using Andersen Cascade Impactor (Copley, England). A modified horizontal diffusion cell was used for the *in vitro* penetration test. For cell culture A549 cells (ATCC, USA), a human immortalized alveolar type II like lung epithelial cell line, were cultured. A549 cells (passage number ⩽ 35) were grown in Dulbecco's modified Eagle medium supplemented with 10 % fetal bovine serum (FBS, Pan Biotech, Germany) and 50 μg/mL gentamicin, in a humidified incubator with 5% CO_2 at 37ºC. Kinetics of lung epithelial cell reaction to treatment was monitored by impedance measurement at 10 kHz (RTCA-SP instrument; ACEA Biosciences, San Diego, CA). Impedance measurement is a label-free, real time, noninvasive method, and correlates linearly with adherence, growth, number, and viability of cells.

3. Results

In the results part the most important results of the presented case studies are introduced.

3.1. Binary and ternary CD systems for per os application

The following preparation protocol was used and the products were compared with each other (Fig. 1). The solubility-increasing effects of the available CD derivatives were determined under uniform conditions. It was found, that the solubility of NIF was always increased by the CDs, and especially for NIF with HP-β-CD to 2.5-fold. The wettability study indicated that the products had a

much hydrophilic character comparing with NIF. Significantly lower wetting angles were measured for all samples, the decrease ranging was from 71º to 26º. There was a parallel result for the wettability relative to the saturation concentration in water. Different samples were examined as regards their dissolution with the rotating basket tester in simulated gastric media and simulated intestinal media (SIM). The dissolution was better in SIM for all samples, except the binary kneaded products.The intensity of the dissolution depended on the preparation method. The PMs and the USs always displayed prolonged dissolution profiles. The addition of PVP and the use of an organic solvent by kneading, such as ethanol led to rapid dissolution. It was also typical that the saturation concentration was reached in 15–20 min containing CDs. The dissolution rate increase was 1.5–2.5-fold for binary and ternary systems with CD.

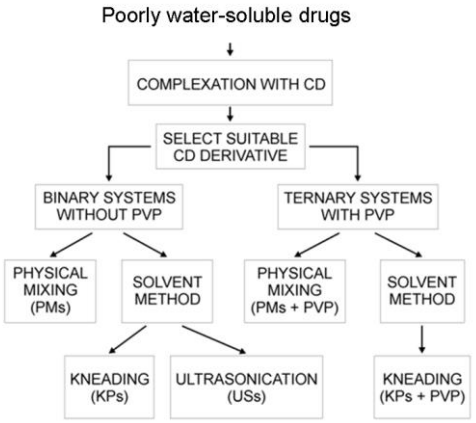


Figure 1. The schematic figure for the preparation of CD containing samples

3.2. Levo and CD containing system for nasal powder formulation

In this study different LEVO-CD binary mixtures were developed for nasal application using a planetary ball mill. Table 1. presents the optimized mass ratio and process parameters Different LEVO-excipient mass ratios were co-ground (10:90; 30:70; 50:50; 70:30; 90:10). It could be seen that micronization was achieved after 15 min grinding time.

Table 1. Characterization of LEVO-CD co-grinded products

LEVO:excipient	Optimized mass ratio	d(0.5) value of physical mixture (μm)	Properties of optimized co-ground product	
			Grinding time (min)	d(0.5) (μm)
LEVO:α-CD	70:30	27.29	15	6.71

It was found that the co-grinding process decreased the degree of crystallinity of LEVO in the ground products compared to the unground physical mixtures. This change can be related to the nature of the excipients. The α-CD had an intensive crystallinity degree reducing effect, as co-grinding agents. *In vitro* dissolution studies revealed that α-CD enhanced the dissolution of LEVO compared to LEVO powder (Table. 2). It can be explained with the good solubility of α-CD also. Hydrophilic drugs like LEVO have low nasal absorption in humans, which can be increased with CD.

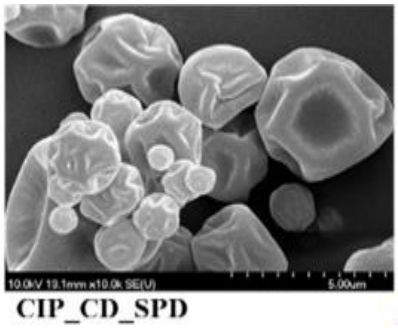
Table 2. Released concentration after 30 min

Products	t_{30min} (%)
Raw LEVO	46.91
LEVO:α-CD=70:30	69.58

163

164 *3.3 Dry powder inhalation sysems(DPIs) using CIP with CD and other additives*

165 This study has created the ability of development of DPI and spray-drying techniques to
166 produce microparticles containing CIP for pulmonary drug delivery. The final microparticles,
167 developed in green technology, ensures the respirable particle size range (3-5 μm), with spherical
168 morphology. The formulated microparticles as innovative product were tested for the stability in
169 stress and accelerated test in long term (6 months). Since the microparticles in the dry powder
170 system are amorphous and do not contain any stabilizer, the results of this test are very important.
171 The stable product may be considered suitable for scaled-up processes and pulmonary application.



172

173 **Figure 2.** The schematic figure for the preparation of CD containing samples

174

175 Following the physicochemical stability testing, it was shown that from five different types of
176 microparticles three presented acceptable stability (CIP_SPD, CIP_LEU_SPD, CIP_CD_SPD,
177 CIP_LEU_PVA_CD_SPD). From cytotoxicity view microparticles contain CIP without excipient and
178 CIP with LEU or combinations of excipients did not change the impedance of A549 lung epithelial
179 cell monolayers in the range of 1-300 μM concentrations, indicating no cellular toxicity. Fig. 3
180 illustrates kinetics of lung epithelial cell reaction treated with ciprofloxacin at 1, 10, 30, 100 and 300
181 μM alone or its formulations prepared with LEU, CD and PVA for 48 hours.

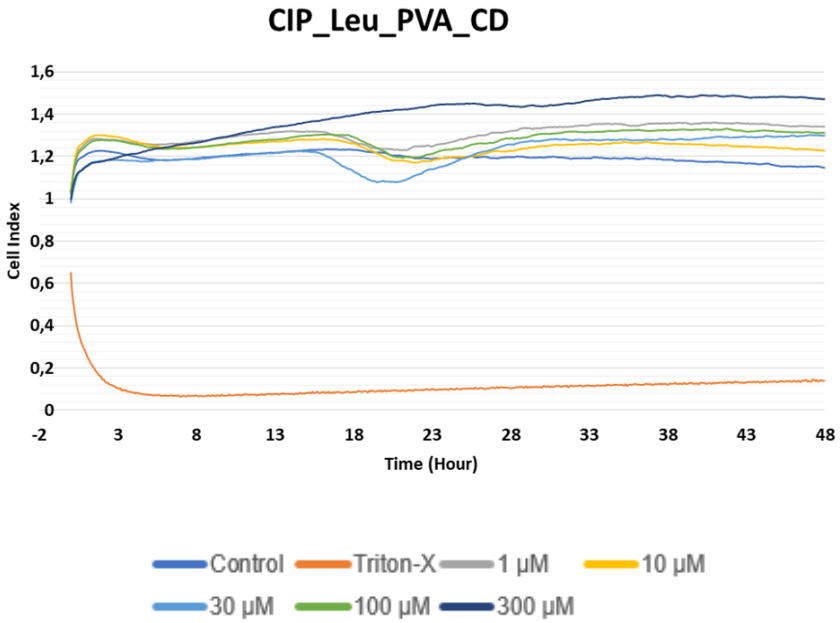


Figure 3. Kinetics of lung epithelial cell reaction to treatment with CIP

Before storage, the fine particle fraction (FPF) of microparticles containing CD was 58.54 ± 1.1 %, although after storage the FPF values of the samples decreased because of the fine particle aggregation. This is associated with the increased particle size and retaining the amorphous structure for 3 months. CIP_PVA_CD_LEU_SPD containing microparticles did not keep the FPF value, which could be explained by the microparticles losing their spherical morphology. In conclusion it was found that soluble CIP consisting of PVA and CDs physiochemical property decline during storage. Aggregation was minimized in formulations with LEU (Table 3).

Table 3. Fine particle fraction values predict the lung deposition

Samples	**FPF (%) 0. day**
CIP_SPD	31.68±1.4
CIP_CD_SPD	45.93±1.4
CIP_PVA_CD_LEU_SPD	58.54±1.1

4. Conclusions

In consequence of the poor water-solubility of the pharmaceutical ingredient, NIF, our aim was to increase its solubility and dissolution rate by applying several technological methods. This work involved a preformulation study to introduce the technological possibilities e.g. in the field of a supergeneric formulation. The solubility-increasing effects of the available CD derivatives were determined under uniform conditions. It was found, that the solubility of NIF was always increased by the CDs, and especially for NIF with HP-β-CD to 2.5-fold. Different preparative mole ratios and three methods (PMs-physical mixtures, KPs-kneaded products and USs-ultrasonicated products) were applied to form complexes, and 15 m/m% PVP K-90 was used to prepare ternary systems

(PMs+PVP and KPs+PVP) to improve the solubility. Using CD, we suggested the ultrasonicated binary and the kneaded ternary 1:3 products to prepare per os or semisolid dosage forms for relief of different pain and treatment rheumatism. In these cases the permeability and wettability properties of the drug are very useful.

The optimized co-grinding process parameters (LEVO:excipient ratio and grinding time) resulted in the desired particle size range (5-40 μm) of nasal powder formulation. Chemical degradation of LEVO in the products was not detected even after the accelerated stability test. The dissolution rate of LEVO was increased with α-CD. This study can be a starting-point for development of an innovative nasal formulation of LEVO for treatment Parkinson's disease.

The formulated DPI using CD and CIP illustrated a novel possibility in treatment of respiratory tract infection and the innovative technology and product present to be of great potential in pulmonary drug delivery systems. We concluded that in the presence of CD, a stable, non-toxic formulation could be reached with high lung deposition.

Acknowledgments: This work was supported by the Ministry of Human Capacities, Hungary grant (20391-3/2018/FEKUSTRAT) and GINOP 2.2.1-15-2016-00007 Project.

Author Contributions: R. A., Z. A. and P. R. conceived and designed the experiments; Cs. B. and T. K. and G. K. performed the experiments; R. A., Cs. B., G. K. and T. K. analyzed the data; R. A. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Arun, R.; Ashok, K C. K.; Sravanthi, V. V. N. S. S. ; Cyclodextrins as Drug Carrier Molecule: A Review. *Sci Pharm.* **2008**, 76: 567–598.
2. Kata, M.; Ambrus, R; Aigner, Z. Preparation and investigation of inclusion complexes containing nifluminic acid and cyclodextrins. *J. Incl. Phenom*. **2002**, 44: 123–126.
3. Ambrus, R; Aigner, Z., Catenacci, L.; Bettinetti, G.; Szabó-Révész, P.; Sorrenti, M. Physico-chemical characterization and dissolution properties of nifluminic acid-cyclodextrin-PVP ternary systems. *J Therm Anal Calorim.* **2011**, 104: 291-297.
4. Bartos, C.; Pallagi, E.; Szabó-Révész, P.; Ambrus, R.; Katona, G.;, Kiss, T.; Mernaz, R.; Csóka, I. Formulation of levodopa containing dry powder for nasal delivery applying the quality-by-design approach. *Eur J Pharm Sci* . **2018**, 123:475-483.
5. Ugwoke, M. I.; Agu, R. U.; Verbeke, N.; Kinget, R. Nasal mucoadhesive drug delivery: background, applications, trends and future perspectives. *Adv Drug Deliv Rev*. **2005**, 57(11):1640-1665.
6. Boraey, M. A.; Hoe, S.; Sharif, H.; Miller, D. P.; Lechuga-Ballesteros, D.; Vehring, R. Improvement of the dispersibility of spray-dried budesonide powders using leucine in an ethanol-water cosolvent system. *Powder Technology* **2013**, 236: 171–178.
7. Pitha, J.; Milecki, J.; Fales, H.; Pannell, L.; Uekama, K. Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs. *Int J Pharm.* **1986**, 29: 73-82.