Leibniz Association

Final Report
Leibniz Competition

Title: Development of a predictive solid-state tool for improved pharmaceuticals safety (PHARMSAF)
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Leibniz Institute in charge: Leibniz-Institut für Katalyse (LIKAT), Rostock

Project leader: Prof. Dr. Torsten Beweries

Executive Summary

Based on previous proof-of-concept studies we suggested developing an experimental method to predict long-term stability profiles and degradation pathways in pharmaceuticals (solid compounds, mixtures, and matrices). To realise this, an interdisciplinary approach that brings together specialists from photocatalysis, mechanochemistry, pharmaceutical analysis, kinetics, and organic synthesis was used. The main goal of this project was the implementation of a novel tool that can be used for a fast and reliable screening of different classes of APIs and formulations. Results obtained from this explorative project are expected to pave the way for a completely new interdisciplinary research field at the interface of heterogeneous catalysis, organic chemistry, and pharmaceutical chemistry.
In the first stage of the project, we have developed the methodologies for forced degradation studies in the solid state (mechanochemical, photochemical, oxidative) and in solution (oxidative), as well as the analytical tools for identification and quantification of the corresponding degradation products. To concentrate our efforts, all partners have first focused on the investigation of degradation of clopidogrel bisulphate and the results show the feasibility of our approach. We have extended this approach to other structurally related APIs and formulations thereof, demonstrating the generality of this concept.

1. Achievement of objectives and milestones

After setting up new equipment that was needed for the proposed studies and performing first benchmark studies on simple active pharmaceutical ingredients (APIs) and API-related structures such as 5-aminosalicyclic acid and ticlopidine, all project partners agreed on using clopidogrel bisulphate as the first API for forced degradation studies. This is different than initially proposed, however, to optimise the workflow within the project, this was considered important.
Along with this, some of the defined milestones and deliverable could not be achieved as expected/planned. Instead, some of the milestones and deliverables defined for a later stage of the project were achieved earlier than proposed (e.g., in WP2b “Acceleration of clopidogrel degradation by ball milling achieved”, defined for 12/2021).

2. Activities and obstacles

As mentioned above, all partners have agreed to use clopidogrel bisulphate (Figure 1) as the first API. To ensure comparability and reproducibility, a large batch of this compound was ordered and distributed among the project partners. Similarly, samples of other APIs such as ibuprofen and those from the sartan family (e.g., Losartan, Valsartan) were shared between the partners.

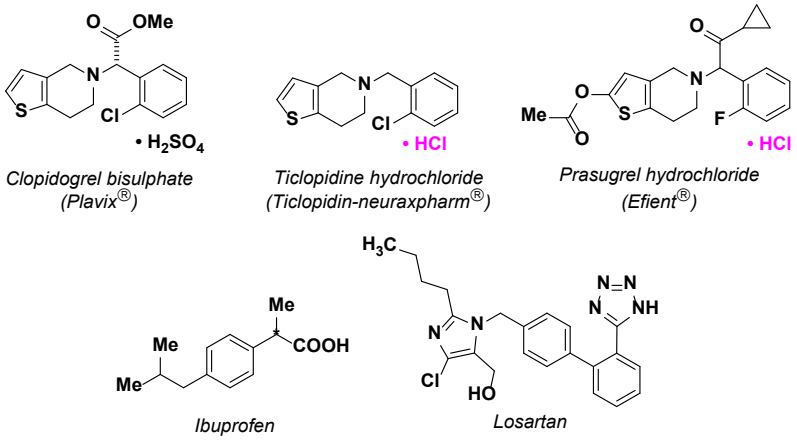

Figure 1. Selected APIs and drug products used in this project.

In work package 1 (**WP1**, Beweries) the technique of ball milling of heterogeneous systems was implemented. Model studies were performed on simple APIs. A set of heterogeneous matrix catalyst systems for forced degradation studies was prepared based on literature procedures. However, during the project it became evident that such rather complex catalyst structures are not optimal for obtaining reproducible results. Especially the varying water content of the matrix systems as well as the limited stability of some of the materials were found to be problematic. Thus, simple physical mixtures of the solid support, the reagent/oxidant/catalyst and the API were used for further forced degradation studies. Comparative simple kinetic studies of clopidogrel bisulphate degradation using KNO_3, $KMnO_4$, and peroxymonosulphate (trademark Oxone) showed that rapid degradation of the API occurs under moderate ball milling conditions. The nature of the degradation product (DP) strongly depends on the oxidant used. Most of the DPs were identified by LC-MS using a procedure that was developed in WP4 (vide infra). Finally, this approach was used for the mechanochemical oxidative degradation of a set of drug products containing thienopyridine API structures. We found that one-by-one addition of the excipient present in the respective drug product to the API does not alter the degradation profile. The excipients are thus inert. Model degradation studies can thus be performed using only the API structure and give degradation profiles that are identical to those of the final formulation. This finding has the potential to open new directions for the development and approval of pharmaceutical products.
In **WP2a** (Strunk) a methodological approach and a comprehensive protocol for testing the light stability of pharmaceutically active substances in the solid state was developed. The methodology was used to investigate the light stability of the pharmaceutically active model substance clopidogrel bisulphate under different artificial light sources, atmospheres and relative humidities. For short exposure tests, this thienopyridine was irradiated in a controlled sunlight simulator for a period between 8 and 144 hours (1500 W, Xe lamp). For comparative purposes, the long-term light stability of clopidogrel bisulphate was also investigated when irradiated with indoor light for a period of 249 days. The photolytic degradation products were analysed with two different HPLC systems (LC-UV; LC-UV-MS). Selected degradation products were separated by semi-preparative HPLC and their composition or structure was determined by high-resolution mass spectrometry (ESI-TOF-MS) and ^{1}H NMR techniques. The results obtained showed that clopidogrel bisulphate is approximately stable to both simulated sunlight and indoor light at low relative humidity (up to 21%). At higher relative humidities (between 52 and 100%), both the number of degradation products and the rate of degradation increase with increasing relative humidity under irradiation. The influence of oxygen on clopidogrel bisulphate degradation is relatively small and most degradation reactions also form in a humid argon atmosphere. Based on the results obtained, a light-induced degradation pathway for clopidogrel bisulphate in the solid state could be proposed.
In **WP2b** (Bolm) the focus of the first part of the project was on an intensive cooperation with the Holzgrabe group, in which we determined the degree of racemisation of dexibuprofen under mechanochemical conditions. For this purpose, samples of the compound were ball milled in the presence of KOH and $FeCl_3$ as well as Al_2O_3 as additives and the resulting mixture was then analysed by chiral capillary electrophoresis. Under optimised analytical conditions, dexibuprofen was shown to have high stereochemical stability and only the smallest amounts racemise. This means that tablets and other forms of administration with this active ingredient are at little risk and can be assessed as racemisation-free.
In another sub-project, the decomposition of clopidogrel bisulphate was studied with the cooperation partners in Rostock and Würzburg. The substance was ground in ball mills in the presence of oxidants such as Oxone, KNO_3 and $KMnO_4$, and the resulting products were analysed. As a result, decomposition pathways that would otherwise take a long time could be recreated in just a few minutes. These studies were later transferred to drug products containing thienopyridine based APIs. Before the end of the project, the mechanochemical decomposition studies of a whole series of sartan based APIs were started, again focusing on oxidative conditions. A large number of reactions carried out in Aachen resulted in a significant number of samples that are currently being studied in Würzburg.
In **WP3** (Baumann) the development of the project resulted in a shift of the focus from analytical development to chemical problems related to thienopyridine substance class. We started

developing characterisation methods by treating ticlopidine hydrochloride (a commercially available member of this class, chemically closely related to clopidogrel, the latter being unavailable at the start of the project) with various oxidants. With metal-based materials, characterisation of products is unfavourable because paramagnetic remainders stay in the mixtures. This prevents NMR analysis unless those residues are removed very carefully. Oxone proved similarly effective and did not cause such difficulties, it was chosen exclusively for further degradation experiments. Therefore, specific analytic techniques for solid oxidants were no longer needed. Another surprise emerged from these experiments, as the counter ion (chloride) participated in the degradation reaction. This is an aspect usually not considered in such studies before. This discovery prompted us to deviate from the planned pathway and to investigate these processes a bit further. It was possible to make this process useful for a synthetic application not only with ticlopidine and clopidogrel, but also with prasugrel where we needed it for the synthesis of a reference material: Forced degradation studies of “Efient”, a prasugrel-based pharmaceutical, in solid-state led to a hitherto unknown product which is generated by exactly this participation of a chloride counterion in the degradation process. Studies on the mechanism and the concurrent reactions of oxidation and halogenation were also performed and detailed investigation in the primary oxidation product of clopidogrel. These studies helped a lot when providing analytical support for work within other WP, in particular WP 2.

In the first project of **WP4** (Holzgrabe), the question was investigated whether it is possible to cause racemisation of an enantiomer by means of "ball milling". This was investigated using the example of dexibuprofen, the S-enantiomer of the non-steroidal analgesic ibuprofen. Using a chiral capillary selector porphoresis method with a cyclodextrin selector and magnesium acetate as electrolyte, isomerisation was observed after prolonged milling in a basic medium. The HPLC method for separating the degradation products of clopidogrel bisulphate created and validated by us was optimised by replacing formic acid with trifluoroacetic acid as an additive to the mobile phase. This reduced strong tailing of the main degradation product. XRPD measurements showed that the clopidogrel sample is polymorphic form I, which according to the literature can have an influence on the degradation profile of the drug. Prasugrel hydrochloride was investigated in the form of samples of the finished medicinal product Efient®. A mass-matched HPLC method for the separation of the degradation products was established. HRMS measurements were then performed on a SCIEX X500R qTOF mass spectrometer.

In another joint project, losartan samples were investigated with the addition of various copper salts. The degradation products were separated by HPLC and subsequently measured by mass spectrometry. Depending on the additive in the ball mill, very different degradation profiles were found. It was striking that the samples with the addition of $Cu(OTf)_2$ no longer contained losartan. Other sartan samples were ground in the ball mill with the addition of Oxone, $KMnO_4$ or KNO_3. In addition to losartan potassium, four other drugs were included in the study (olmesartan medoxomil, valsartan, irbesartan and telmisartan). HPLC methods for separating the degradation products were developed for the total of almost 120 samples. The samples were measured by HRMS in both positive and negative mode using a VTM Ion Source (ESI). The analysis of these data will be completed soon.

In **WP5** (Beller) new catalysts for the scission of highly inert C(sp3)–C(sp3) bonds were developed. We were interested in applying non-noble metals to facilitate the catalysis and to utilise air as a sustainable, nontoxic, and highly abundant oxidant. In detail, two copper catalysts – $Cu(OTf)_2$ and CuCl – were founded for the oxidative cleavage of a wide range of aliphatic and aromatic tertiary amines, in addition to pharmaceutically relevant piperazines and morpholines. The applied catalysts showed a higher efficiency in the presence of *N* ligands, particularly pyridine. Based on spectroscopic methods, including radical trapping experiments a reaction mechanism has been proposed. An alternative system using a combination of cobalt and manganese metal salts provided an enhanced reactivity for the oxidative cleavage of $C(sp^3)$–$C(sp^3)$ bonds in derivatised morpholines. The performance of the catalysis benefits greatly by the presence of both metals in this bimetallic system. Once again, *N*-ligands such *para*-methoxypyridine improved the effectiveness of the catalysis regarding product yields. In a third methodology a low cost, highly abundant and "biocompatible" iron catalyst was

developed for of C(sp^3)–C(sp^3) bonds reactions of *N*,*N*-diphenylpiperazine in good yields. The reactivity also extended to morpholine substrates to provide good, isolated yields of these coveted pharmaceutically-relevant cyclic motifs.
Finally, all catalytic protocols were applied to bond cleavage in target drug molecules. In case of the Fe system C-N bond cleavage in clopidogrel bisulphate was observed. The halogenated aromatic part was isolated in 37% yield, while the thiophene part was detection with GC-MS spectroscopy. No bond cleavage occurred with other sartane-type drugs using Co/Mn or Fe systems.

3. Results and successes

Until now, in total 22 manuscripts were published in peer-reviewed journals from authors involved in or funded through the PHARMSAF project.
Furthermore, four PhD projects were completed within the framework of the PHARMSAF
Project results were shared and discussed in internal biannual project meetings.

4. Equal opportunities

As a member of the Leibniz Association, LIKAT has committed itself to having its efforts to reconcile family and work certified and successfully applied for the TOTAL E-QUALITY award in 2011, which is valid for three years and certifies that the holder commits to equal opportunities for women and men at work (http://www.total-e-quality.de/). In 2020, LIKAT was awarded the Total E-Quality award for 2020-2022. Two out of five principal investigators (PIs) in the project are women (Prof. Ulrike Holzgrabe, Prof. Jennifer Strunk). Two of the PhD students and three postdocs affiliated with the project are international.

5. Structures and collaboration

The expertise coming together in this consortium allowed us to study and optimise all relevant scientific aspects of degradation and impurity profiling in pharmaceutical products. The studies could only be conducted by close collaboration of *all partners*. As part of this joint approach, API and DP samples as well as methodological knowledge were continuously transferred between the partners and the results were tested for reproducibility. As an example, analytical methods were developed and optimised by the Holzgrabe group (WP4) in close collaboration with partners active in WPs 1, 2a, 2b, and 3. This ensures that the developed methods are suitable to analyse not only model samples, but also those that are generated in forced-degradation studies under realistic conditions.
Dr. Helmut Buschmann and Dr. Norbert Handler (RD&C, Vienna, Austria) acted as external cooperation partners without financial participation in the project. Both are experts in the field of pharmaceutical drug development and contributed to the project by suggesting API structures of interest and bringing in expertise related to API stability and impurity profiling.
Results obtained from studies of the individual partners were continuously discussed between the partners, either in a bilateral fashion or in biannual internal project meetings.

6. Quality assurance

PD Dr. Baumann (WP3) is LIKATs elected ombudsperson, who ensures compliance with the rules of good scientific practice. He acts as an advisor in the exercise of that function. LIKAT has its own binding rules in force, directed at ensuring good scientific practice, in addition to procedures for how to deal with any scientific malpractice at LIKAT. The team at LIKAT makes the principles of good scientific practice an everyday reality. Violations of these rules are not tolerated. To ensure this in practice, results are frequently discussed and questioned, not only internally, but also presented to the partners in biannual project meetings.
All raw data collected in the frame of this project are saved on servers of LIKAT for documentation. The results obtained within the project have been published in peer-reviewed

journal (see section 3). Thus, their quality has been indirectly approved by independent reviewers.

7. Additional in-kind resources

All PIs and technical and analytical staff involved in the project are funded through the basic funding of LIKAT and the external partners RWTH Aachen and University of Würzburg.
Most of the technical equipment used in this project is part of the institute's basic equipment and/or was purchased using basic funding or funding related to other, previous projects. Exceptions are ball mills used in WPs 1 and 3.

8. Outlook

Clopidogrel bisulphate was found to be an excellent candidate for forced degradation studies, allowing for the use of different approaches (mechanochemical, wet-chemical, photochemical) and comparison of the data. The results obtained from these studies were published in peer-reviewed journals and thus made available for the public. Furthermore, the transfer and extension of the knowledge to more complex APIs was possible. Studies on sartan-based APIs and drug products are ongoing. For all studies the development of reproducible and scalable degradation protocols and analytical methods was essential. Our work highlights the potential of the mechanochemical approach for the targeted degradation of specific structural motifs that could not only be relevant for the pharmaceutical sciences, but also for organic synthesis in general. In future work, it is desirable to transfer this mechanochemical approach to other drug families and evaluate the role of other stimuli such as light or temperature for the forced degradation process. Our approach has the potential to significantly simplify the acquisition of stability data and degradation profiles that is required for approval of new drugs.