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Life Cycle and Aquatic Biodegradation Assessments of Pharmaceutical Excipients: Development of a Selection Guide for Drug Formulations

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Zusammenfassung

Pharmazeutische Hilfsstoffe sind wesentliche Bestandteile von Arzneimitteln. Ihre Umweltauswirkungen wurden jedoch bisher weniger häufig untersucht als die von Wirkstoffen.

Eine wichtige Gruppe von Hilfsstoffen sind Polymere. Diese können wasserlöslich oder wasserunlöslich sein und werden als polymere Hilfsstoffe (engl. polymeric excipients, PEx) bezeichnet. Das Umweltverhalten von PEx wird maßgeblich durch ihre biologische Abbaubarkeit bestimmt. Die OECD-301-Screeningtests zur leichten biologischen Abbaubarkeit wurden jedoch ursprünglich nicht für Polymere entwickelt. Daher müssen diese Tests angepasst werden, um ihre Anwendbarkeit auf Polymere auszuweiten. Darüber hinaus müssen die Emissionen während des gesamten Lebenszyklus berücksichtigt werden, um ein umfassendes Bild ihrer Umweltauswirkungen zu erhalten. Diese Dissertation hat zum Ziel, diese Datenlücken zu schließen, und stellt dazu vier sich ergänzende Publikationen (Publikationen 1-4, P1-4) vor. In diesen Publikationen wurde die biologische Abbaubarkeit und die Auswirkungen von Hilfsstoffen auf den Lebenszyklus systematisch bewertet, wodurch eine Grundlage für das sichere und nachhaltige Design von PEx geschaffen wurde („Benign-by-Design“, BbD).

Zur systematischen Bewertung der biologischen Abbaubarkeit von PEx auf der Grundlage der OECD-Tests 301D und 301F wurde ein Ansatz mit stufenweiser Erhöhung der Inokulumdichte und -diversität verwendet (P1-3). Dabei wurden wesentliche Unterschiede in den biologischen Abbauraten zwischen den verschiedenen PEx und Testbedingungen beobachtet. So zeigten synthetische Polymere wie Polyvinylpyrrolidon und Polymethylmethacrylat keinen messbaren Abbau, da ihnen wahrscheinlich die für den mikrobiellen Abbau erforderlichen oxidierbaren oder hydrolysierbaren funktionellen Gruppen fehlen (P2). Ebenso wiesen Cellulose-basierte Hilfsstoffe mit hohem Substitutionsgrad und molarer Substitution keine biologische Abbaubarkeit auf. Dies deutet darauf hin, dass eine umfangreiche Derivatisierung eine Resistenz gegen enzymatische Hydrolyse zur Folge hat. Auch Poloxamer 188 (ein Blockcopolymer) wurde unter keiner der Testbedingungen biologisch abgebaut; das Verhältnis von Polyethylenglykol (PEG) zu Polypropylenglykol (PPG) scheint seine Abbaubarkeit zu beeinflussen (P3). Selbst bei einem stufenweisen Ansatz, der eine zunehmende mikrobielle Diversität und Dichte einbezog, ließ sich bei diesen nicht abbaubaren Verbindungen kein messbarer biologischer Abbau feststellen. Im Gegensatz dazu erwiesen sich andere PEx wie Polyethylenglykol (PEG) und Polyvinylalkohol (PVA) bei Tests mit ausreichender Inokulumdichte als leicht biologisch abbaubar. Die Ergebnisse verdeutlichen, dass nur bei

ausreichender mikrobieller Biomasse ein biologischer Abbau innerhalb der 28-tägigen Testperiode zuverlässig festgestellt werden kann.

Einige PEx waren teilweise biologisch abbaubar, mit Abbauwerten von 20 bis 59 %. Damit lagen sie unter dem OECD-301-Schwellenwert von 60 % für „leicht biologisch abbaubar“. So zeigte beispielsweise Polysorbat 80 einen Abbau im Bereich von 30 bis 45 % und führte zur Bildung von ethoxylierten Sorbitan-Strukturen, die als „Dead-End”-Transformationsprodukte identifiziert wurden (P3). Insgesamt zeigten P1-3, dass die Ergebnisse der biologischen Abbaubarkeit durch das komplexe Zusammenspiel von Molekülstruktur, mikrobieller Anpassung und Testbedingungen beeinflusst wurden. Darüber hinaus lieferten die Ergebnisse von P1-3 wertvolle empirische Daten, die das wissenschaftliche Verständnis und die regulatorische Debatte in zwei wichtigen Aspekten vorantrieben. Erstens konnten die OECD-301-Screening-Tests technisch auf polymere Substanzen angewendet werden, jedoch mussten ihre Limitierungen bedacht werden: Inokula aus Abwasser unterschätzten häufig den biologischen Abbau, während Belebtschlamm mit höherer mikrobieller Dichte und Vielfalt den Nachweis des biologischen Abbaus ermöglichte. Dies unterstrich die Bedeutung der Optimierung von Testprotokollen für PEx, um aussagekräftige und repräsentative Ergebnisse zu erzielen. Zweitens waren die binären „pass/fail“-Kriterien, die typischerweise in OECD-301-Tests verwendet wurden, unzureichend, um das gesamte Spektrum des biologischen Abbauverhaltens von PEx zu erfassen. Um diese Limitierung zu überwinden, wurde ein abgestuftes „Ampel“-Klassifizierungssystem entwickelt, das Stoffe als „non-biodegradable“, „slightly biodegradable“, „moderately biodegradable“ oder „readily biodegradable“ einstuft (P1-3). Diese Klassifizierung basiert auf Kurvenverläufen des biologischen Abbaus, auf Mineralisierungsraten und auf mechanistischen Erkenntnissen, die aus Struktur-Abbau-Beziehungen abgeleitet wurden. Dadurch ist eine genauere Interpretation der Ergebnisse auf Screening-Ebene möglich.

P4 zeigte deutlich, dass bei der Bewertung von Umweltrisiken von Hilfsstoffen nicht nur einzelne Aspekte wie die Abbaubarkeit betrachtet werden sollten, sondern eine ganzheitliche Umweltbilanzierung erforderlich ist, im Einklang mit dem „Safe and Sustainable by Design“ Konzept. Die Ergebnisse der Lebenszyklusanalyse zeigten, dass energiebedingte Emissionen den Hauptbeitrag zur Gesamtbelastung der meisten Hilfsstoffe verursachen. Dies ist auf den Strom- und Wärmeenergieverbrauch während der Herstellung zurückzuführen. Auch prozessbedingte Emissionen trugen erheblich zur Gesamtumweltbelastung bestimmter Hilfsstoffe bei, beispielsweise durch die Freisetzung des ozonabbauenden Methylchlorids aus chemisch methylierten Cellulosederivaten. Bei biobasierten Hilfsstoffen trugen sowohl der

Einsatz von Düngemitteln, die zur Eutrophierung führten, als auch der Wasserverbrauch für die Bewässerung erheblich zur Gesamtumweltbelastung bei. Aufgrund dieser Vielzahl an Emissionsquellen lässt sich ableiten, dass sich Umweltwirkungen nicht allein aus der Abbaubarkeit ableiten lassen. Die unterschiedlichen eingesetzten Hilfsstoffe können zu Zielkonflikten zwischen der Ökobilanz der Produktion und der Abbaubarkeit führen. Dies unterstreicht die Bedeutung ganzheitlicher Bewertungen für eine fundierte und nachhaltigere Entscheidungsfindung. Laktose zeigte beispielsweise eine hohe Abbaubarkeit, hatte jedoch einen signifikanten ökologischen Produktions-Fußabdruck. Im Gegensatz dazu wiesen Eudragit Derivate geringere Umweltauswirkungen bei der Produktion auf, waren jedoch nicht biologisch abbaubar. Ein zentrales Ergebnis dieser Forschung war die Entwicklung des „Excipient Selection Guide", der diese Erkenntnisse in praxisnahe Entscheidungshilfen überträgt. Dieses Tool fasst Abbaubarkeits- und Lebenszyklus-Daten zusammen, um die Auswahl von Hilfsstoffen zu erleichtern, die die Umwelt weniger belasten. Der „Excipient Selection Guide" unterstützt somit die Entwicklung sichererer und nachhaltigerer Arzneimittel im Einklang nach dem BbD Konzept.

P1-4 lieferten gemeinsam die erste systematische Bewertung pharmazeutischer Hilfsstoffe, in der Daten aus Abbaubarkeituntersuchungen mit denen aus Lebenszyklusanalysen integriert wurden. Der „Excipient Selection Guide" kann in den frühen Entwicklungsphasen von Arzneimitteln verwendet werden, um nachhaltigere Hilfsstoffe auszuwählen oder zu entwickeln. Da Hilfsstoffe universell in der pharmazeutischen Industrie eingesetzt werden, hat die Reduzierung ihrer Umweltbelastung durch diesen Guide erhebliche Auswirkungen auf den Sektor. Darüber hinaus kommen Hilfsstoffe in vielen anderen Branchen zum Einsatz, darunter in der Chemie-, Lebensmittel-, Kosmetik-, Körperpflege- und Haushaltsproduktindustrie. Dies erweitert die Anwendbarkeit des „Excipient Selection Guides" und sein Potenzial, die ökologische Nachhaltigkeit branchenübergreifend zu fördern.

Abstract

Pharmaceutical excipients are essential components of drug formulations, yet their environmental impacts have received less attention than those of active pharmaceutical ingredients (APIs). A major group of formulation excipients are polymers, which can be either water-soluble or water-insoluble and are referred to as polymeric excipients (PEx). The environmental fate of PEx is crucially determined by its biodegradability. However, standard OECD 301 ready biodegradability tests were not originally designed for polymers. Therefore, these tests must be adapted further to establish their applicability to polymers. In addition, emissions throughout their life cycle must be considered to obtain a comprehensive picture of their environmental impact. This dissertation aims to address these data gaps by presenting four complementary publications (publications 1-4, P1-4). These publications systematically evaluated the biodegradability and life-cycle impacts of excipients, providing a foundation for the safe and sustainable design of PEx ("Benign-by-Design", BbD).

A stepwise approach based on OECD 301D and 301F tests was used to systematically evaluate the biodegradability of PEx, incorporating progressively increasing levels of microbial density and diversity (P1-3). Substantial variability in degradation outcomes across different PEx and test conditions was observed. For instance, synthetic polymers such as polyvinyl pyrrolidone and polymethyl methacrylate showed no measurable degradation, most likely because they lack the oxidizable or hydrolysable groups necessary for microbial degradation (P2). Similarly, cellulose-based excipients with high degrees of substitution (DS) and molar substitution (MS) showed no biodegradation, suggesting that extensive derivatization hinders enzymatic accessibility (P1). Poloxamer 188 (a block copolymer) also showed no biodegradation under any of the test conditions; the ratio of polyethylene glycol (PEG) to polypropylene glycol (PPG) appears to influence its biodegradability (P3). Even when using a stepwise approach that incorporated increasing microbial diversity and density, there was still no measurable biodegradation of these non-biodegradable compounds. In contrast, other PEx, such as polyethylene glycol (PEG) and polyvinyl alcohol (PVA), were readily biodegradable when tested with inocula of sufficient microbial diversity and density. These results underscored the importance of adequate microbial biomass for effectively initiating and monitoring biodegradation within a 28-day timeframe. Some PEx displayed partial biodegradability, with degradation ranging from 20 to 59 %, which is below the OECD 301 "readily biodegradable" threshold of 60 %. For example, polysorbate 80 showed 30-45 % degradation, producing ethoxylated sorbitan residues that persisted as "dead-end" transformation products (P3).

Together, P1-3 revealed that the biodegradation outcomes are influenced by the complex interplay between molecular structure, microbial adaptation, and test conditions. In addition, findings of P1-3 provided valuable empirical data that advance scientific understanding and regulatory discourse in two key areas. First, OECD 301 screening tests can be technically applied to polymeric substances, however, their limitations must be recognized: Inocula derived from secondary effluent frequently underestimated biodegradation, whereas activated sludge, with higher microbial density and diversity, enabled detection of biodegradation. This emphasizes the importance of optimizing test protocols for PEx to ensure meaningful and representative results. Second, the binary pass/fail criteria typically employed in OECD 301 tests are inadequate for capturing the full range of biodegradation behaviors of PEx observed. To overcome this limitation, a graded "traffic-light" classification system has been developed that categorizes substances as non-biodegradable, slightly biodegradable, moderately biodegradable, or readily biodegradable (P1-3). This classification is based on biodegradation curve patterns, mineralization percentages and mechanistic insights derived from structure-biodegradability relationships, enabling a more accurate interpretation of screening-level results.

P4 emphasized the need to move away from single-parameter assessments of environmental risk, such as biodegradability, and adopt a systems-level approach in line with the “Safe and Sustainable by Design” framework. Life-cycle assessment results showed that energy-related emissions were the main contributor to the overall impact of most excipients, due to electricity and thermal energy use during manufacturing. Process-related emissions also made a significant contribution to the overall environmental impact of certain excipients, such as the release of ozone-depleting methyl chloride from chemically methylated cellulose derivatives. In case of bio-based excipients, both the use of fertilizers, which caused eutrophication, and water consumption for irrigation contributed significantly to the overall environmental impact. These multiple emission sources must be considered because they demonstrate that environmental impacts cannot be inferred from biodegradability alone. The different excipients involved can result in trade-offs between production impact and biodegradability. This highlights the importance of holistic assessments in sustainable decision-making. For instance, although lactose showed high biodegradability, it had a significant production footprint. In contrast, Eudragit derivatives exhibited lower production impacts but poor biodegradability. A key outcome of this research was the development of the Excipient Selection Guide, which translates these insights into practical guidance. This tool combines biodegradability and life-cycle data to enable the informed selection of excipients that have a lower overall environmental

impact. It supports the development of safer and more sustainable pharmaceuticals following the BbD concept.

Together, P1-P4 provided the first systematic evaluation of pharmaceutical excipients by integrating biodegradability with life-cycle considerations. The Excipient Selection Guide may be used in the early stages of pharmaceutical development to select or develop more sustainable excipients. As excipients are used universally across the pharmaceutical industry, reducing their environmental impact using this tool has significant implication for the sector. Furthermore, excipients are used in many other sectors, including chemistry, food, cosmetics, personal care and household products. This broadens the guide's applicability and its potential to enhance environmental sustainability across industries.

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List of Abbreviations

API	Active pharmaceutical ingredient
BbD	Benign-by-Design
CMC	Carboxymethyl cellulose
CA	Cellulose acetate
CFU	Colony forming unit
CSS	Chemicals Strategy for Sustainability
CBT	Closed bottle test
CTG	Cradle-to-gate
DP	Degree of polymerization
DS	Degree of substitution
EC	Ethyl cellulose
ERA	Environmental risk assessment
ESG	Excipient Selection Guide
GTG	Gate-to-gate
GWP	Global warming potential
HEC	Hydroxyethyl cellulose
HPC	Hydroxypropyl cellulose
HPMC	Hydroxypropyl methyl cellulose
HPMCAS	Hydroxypropyl methyl cellulose acetate succinate
LCA	Life cycle assessment
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
MRT	Manometric respirometry test
MC	Methyl cellulose
MCC	Microcrystalline cellulose
MgSt	Magnesium stearate
MS	Molar substitution
NREc	Natural resource energy combustion
NREm	Natural resource energy materials
NREt	Natural resource energy total
OECD	Organization for Economic Co-operation and Development
PAA	Polyacrylic acid
PEG	Polyethylene glycol
PEx	Polymeric excipients
POE	Polyoxyethylene
PPG	Polypropylene glycol
PS	Polysorbate
PVA	Polyvinyl alcohol
PVP	Polyvinyl pyrrolidone
P188	Poloxamer 188
P184	Poloxamer 184
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ	Research question
SLS	Sodium lauryl sulfate
SSbD	Safe and sustainable by design
SSF	Sodium stearyl fumarate
SSG	Sodium starch glycolate
WSP	Water-soluble polymers

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1. Introduction:

1.1 Regulatory Momentum toward Safer and More Sustainable Chemicals

Addressing environmental sustainability is becoming a core strategic imperative for the pharmaceutical industry due to its resource, waste and energy intensive nature and environmental impact profile, which must be balanced with the benefits of protecting individual and public health (Milanesi et al., 2020; Riikonen et al., 2024; Van Wilder et al., 2024). This imperative is reinforced by the European Green Deal (EC, 2019) and a series of European Commission initiatives aimed at promoting a more sustainable future. Among these, the EU has implemented the Zero Pollution Action Plan for Air, Water and Soil, which emphasizes the need for sustainability action across industries, including pharmacy (EC, 2021). The recent inclusion of the pharmaceutical sector in the EU Taxonomy, which sets out technical screening criteria to determine the environmental sustainability of economic activities, further increases the pressure to adopt environmental sustainability practices (EC, 2023a). The EU taxonomy outlines the preferred use of environmentally biodegradable ingredients, including both active and inactive ingredients ("excipients"), in medicines to reduce environmental impact. The term "excipients" refers to any component of a drug product other than the active ingredient. However, excipients have been studied little in terms of their environmental biodegradability potential, let alone their wider environmental and sustainability profile.

Polymeric excipients (PEx), a diverse group including both water-soluble polymers (WSP) and water-insoluble polymers, are becoming increasingly relevant in an environmental context. This is due to the European Commission’s concurrent efforts to develop standard information requirements for polymers that require registration under the revised REACH regulation. These efforts are driven by the Chemicals Strategy for Sustainability (CSS), which requires the registration of certain polymers to facilitate the transition to safer and more sustainable chemicals (EC, 2020).

1.2 PEx: Ubiquitous, Understudied, and Environmentally Relevant

PEx are generally considered to be pharmacologically inert, non-metabolizable and mostly excreted unchanged in the faeces. Their functions range from their use as binders, disintegrants, and diluents to coatings, among others (Debotton and Dahan, 2017). In addition to their use in the pharmaceutical industry, PEx are also used in various sectors such as personal care products, cosmetics, agrochemicals, household cleaning products and water treatment (Nigro et al., 2023). Despite their ubiquity, there is a considerable lack of quantitative analytical methods for

the detection of polymers, especially WSPs, in the environment (Huppertsberg et al., 2020). This analytical gap has resulted in limited data on their occurrence in aquatic environments. Available studies have reported varying concentrations of polyvinyl pyrrolidone (PVP) in wastewater treatment plant effluent, ranging from 0.9 mg L^{-1} to 7 mg L^{-1}, with concentrations of around 0.1 mg L^{-1} detected in river water (Antić et al., 2011). Similarly, polyoxyethylene (POE) has been found in wastewater up to 20 µg L^{-1}, with surface water samples showing concentrations above 1 µg L^{-1} (Pauelsen et al., 2023), raising concerns about the persistence of non-biodegradable polymers in the environment. Reports on PEx in the environment are concerning from an ecotoxicological perspective. For example, exposure to PVP has been linked to disruptions in host-parasite interactions, such as those between Gyrodactylus turnbulli and Poecilia reticulata, in freshwater ecosystems (Robison-Smith et al., 2024). Other studies have documented significant reproductive and behavioral effects in aquatic organisms exposed to polymers such as PVP, polyvinyl alcohol (PVA), polyethylene glycol (PEG) and polyacrylic acid (PAA). These polymers, at concentrations of 5-10 mg L^{-1}, exerted adverse effects on Daphnia magna (Mondellini et al., 2022). Zebrafish embryos showed toxicity from exposure to PVP, PVA and PAA at even lower concentrations (0.001, 0.5 and 1 mg L^{-1}, respectively), with further toxicity observed in fish and frogs exposed to PEG and PVA (Nigro et al., 2023; Zicarelli et al., 2024). Collectively, these studies highlight the potential risk caused by the presence of WSPs in aquatic ecosystems. Biodegradability is a critical property in reducing the environmental impact of chemicals, as it prevents their accumulation in ecosystems and limits exposure over time (Muir and Howard, 2006).

A recent prioritization study by Brunning et al. (2025) identified several high-emitting polymer groups commonly found in wastewater and widely used in the pharmaceutical industry as PEx. This study categorized 339 substances across 26 polymer groups, with the highest emitting groups including polyol ethoxylate esters, alcohol alkoxylates, polycarboxylates, polyethers, copolymers, starch derivatives, silicones, polyquaterniums, PVA, and cellulose derivatives. Notably, among the 26 identified polymer groups, representatives of each group are also widely used in the pharmaceutical industry (Brunning et al., 2025).

Cellulose-based excipients are one of the most widely used classes of bio-based substances in pharmaceutical formulations (Klein, 2009). Despite the industrial relevance and high production volumes of these compounds, estimated to be in the hundreds of thousands of tonnes per year (Thielking and Schmidt, 2006), data on their environmental fate remain limited and fragmented. Cellulose derivatives are also among the top 10 polymer groups with the highest

emissions to the environment, with carboxymethyl cellulose (CMC) and hydroxyethyl cellulose (HEC) identified as the highest emitters within this category (Brunning et al., 2025). Previous studies have shown that some cellulose derivatives, such as microcrystalline cellulose (MCC), cellulose acetate (CA) and CMC, exhibit varying degrees of biodegradability (Komarek et al., 1993; Van Ginkel and Gayton, 1996; Menzies et al., 2023). However, a consistent comparative analysis of a wide range of pharmaceutical cellulose derivatives using harmonized test conditions has not been performed.

Another important group of pharmaceutical excipients are polymeric non-ionic surfactants, which are commonly used in oral, topical, and injectable drug formulations. The two predominant pharmaceutical surfactants, poloxamers and polysorbates (PS), belong to the following groups: poloxamers are classified as polyether copolymers, while PS are classified as polyol ethoxylate esters (Bollenbach et al., 2022; Roy et al., 2024). Despite their widespread use, reliable information on their environmental fate, particularly in aquatic systems, remains limited (Brunning et al., 2025). This is particularly concerning given that predicted environmental concentrations in surface water for PS 20 are approaching the predicted no-effect concentration, suggesting that these substances may pose ecological risks and warrant priority in environmental risk assessments (ERA) (Brunning et al., 2025; Straub et al., 2014). In addition, emerging evidence points to potential ecotoxicological effects of PS, such as adverse effects on grazer life history traits and planktonic ecosystem stability, highlighting the need for a more comprehensive understanding of their environmental biodegradation behavior (Yuan et al., 2024).

As part of the EU's policy for a toxic-free environment, there is an urgent need to provide standardized data on the biodegradability of PEx. However, a critical challenge in addressing these gaps is the complexity and diversity of polymeric materials, coupled with the lack of standardized test methods and inconsistent study designs. These factors hinder reliable cross-comparison and quantitative modelling of biodegradability (Kim et al., 2023; Kintzi et al., 2024; Lin and Zhang, 2025). There has therefore been a renewed focus on understanding the applicability and suitability of test methods for assessing polymer biodegradation (McDonough et al., 2023; Menzies et al., 2023; Kintzi et al., 2024). Importantly, the OECD 301 testing guidelines, which were designed to test biodegradability, are intended for use with well-defined, low-molecular-weight, mono-constituent, soluble or uniformly dispersed substances. Nevertheless, the validation of the test guidelines did not consider polymeric substances (Painter and King, 1985). While recent publications demonstrate the applicability of the OECD

301 tests to polymers (McDonough et al., 2017; Menzies et al., 2023), it is necessary to determine whether the test methods are fit for purpose or if modifications are required, such as extending the test duration or increasing the inoculum level.

1.3 Embracing Systems Thinking for Sustainable Pharmaceutical Innovation

The ongoing reform of EU pharmaceutical legislation provides an important opportunity to address these data limitations. The European Commission has stated that one of the five main objectives of this reform is to improve the environmental sustainability of medicines, indicating a shift towards a life-cycle-based regulatory approach (EC 2023b). This revised regulatory framework, which is still under development, could provide the basis for more robust and harmonized environmental requirements, including the extension of the ERA to a wider range of environmental stressors and product components such as excipients. In this context, it is important to note that the ERA currently required for human medicines at the time of marketing authorization in the EU remains narrow in scope and is largely limited to ecotoxicological risks associated with the excretion of the active substance (Gildemeister et al., 2023; Piët et al., 2024). This leaves significant upstream and system-related effects unaddressed, including those of excipients. To enable more sustainable pharmaceutical products, it is among other necessary to assess excipients across multiple environmental dimensions, including biodegradability. This broader perspective is essential to make more sustainable choices and avoid "regrettable substitutions" that may lead to unintended consequences (Fantke et al., 2020; Maertens et al., 2021). Sustainability is not simply a product of isolated factors, but results from the functioning of an entire system, not just isolated components (Zimek and Baumgartner, 2024). Therefore, systems thinking in both chemistry and pharmacy is crucial to assess whether "greener" excipients that meet biodegradability criteria are truly more sustainable alternatives (Constable, 2021; Mahaffy and Elgersma, 2022). In this context, life cycle assessment (LCA) provides a valuable method for assessing the broader environmental impacts of pharmaceutical excipients. Environmental LCA quantifies the environmental impacts associated with the entire life cycle of a product, from raw material extraction to the product’s disposal (Van Wilder et al., 2024). However, the application of LCA in the pharmaceutical industry remains fragmented, with most studies focusing on the environmental impacts of active pharmaceutical ingredients (APIs) only, particularly in terms of their synthesis and manufacturing processes (Jiménez-González and Overcash, 2014; Piffoux et al., 2024). In contrast, the environmental impact of pharmaceutical excipients has been largely overlooked, despite their essential role in drug formulations. Recent LCA studies have highlighted the growing importance of excipient

selection in the environmental impact of drug formulations. For example, studies using the production of ibuprofen tablets as a model case have shown that the environmental impact of excipient formulations goes beyond the energy used in manufacturing. It also includes the environmental footprint of the raw materials and manufacturing processes associated with the excipients themselves (Wang et al., 2021; Hadinoto et al., 2022). These findings support the need to incorporate environmental criteria into formulation design decisions. Despite this growing recognition, there remains a significant gap in decision-support tools to help integrate environmental considerations into excipient selection in the early stages of drug formulation. A major hurdle in the development of such tools is the lack of comprehensive life cycle inventory (LCI) data for excipient manufacturing (De Soete et al., 2017; Parvatker and Eckelman, 2020). The literature on LCI data specific to excipient manufacturing is sparse, with only a few studies addressing the issue, and even then, without publishing the actual inventory data (Hadinoto et al., 2022; Tao et al., 2023). Furthermore, much of the existing research relies on aggregated LCI data from different databases, such as Ecoinvent, which are often non-transparent and inconsistent. This lack of clarity on whether gate-to-gate (GTG) or cradle-to-gate (CTG) inventories have been used makes it difficult to validate and ensure the quality of the data (Oberschelp et al., 2023). This data gap is critical: the European Commission estimates that more than 80 % of a product's environmental impact is determined during the design phase (Schenck et al., 2024). By incorporating systems-based tools in the early stages of chemical and pharmaceutical development, industry can prevent life cycle impacts and make informed sustainable design decisions (Matlin et al., 2022; Luu et al., 2022). The safe and sustainable by design (SSbD) framework developed by the European Commission (EC, 2022) aims to guide such developments but remains largely conceptual. Its practical implementation is hindered by insufficient life cycle and biodegradation data (Apel et al., 2024).

2. Overarching Research Questions and Objectives

This PhD thesis addresses critical data gaps in the environmental assessment of pharmaceutical excipients, providing a new basis for greener and more sustainable innovation in drug product development. For the first time, it evaluated excipients comprehensively across key environmental dimensions. A core element of this evaluation involved generating new environmental biodegradation data for a broad spectrum of polymeric substances used as PEx, using standardized OECD 301 testing methods. The thesis also critically examines the suitability and limitations of these methods, with a particular focus on polymeric and water-soluble substances that fell beyond the original scope of existing guidelines. To improve the environmental evaluation of these materials, the research proposes modifications to existing testing frameworks, such as extending testing periods, inoculum sources and concentrations, and modifying pass/fail criteria to align with the physicochemical properties of pharmaceutically applied polymers.

Alongside the biodegradation studies, the thesis adopted a life-cycle approach, recognizing that environmental sustainability must be evaluated at a systems level rather than through individual criteria. Integrating biodegradation testing with LCA allowed for a more comprehensive comparison of excipient options and helped to identify potential trade-offs between degradability and environmental impacts related to production.

The objectives of this work were approached through more detailed research questions. The research questions were addressed through four research publications (P1-P4; see list of publications in the appendix), which together form a comprehensive scientific database for the development of the "Excipient Selection Guide" (ESG). This guide is a key outcome of this work, providing practical decision support and a “Benign-by-Design” (BbD) tool for the pharmaceutical industry (Fig. 1). The overarching key research questions (RQs) and associated objectives connect P1-P4. Building on the research presented in the attached publications, the following five questions represent the general framework of this thesis.

2.1 RQ1: Biodegradation Assessment

How environmentally biodegradable are commonly used pharmaceutical excipients and what persistence risks do they pose to the environment?

Objective:

- Systematic study of the biodegradability of pharmaceutical excipients using OECD 301 standard tests.
- Development of a classification system based on biodegradation rates.

2.2 RQ2: Determinants of Biodegradation

What factors influence the biodegradation potential of different excipient classes and how can test methods be improved for better assessment?

Objective:

- Identify chemical, structural and environmental factors (e.g. molecular weight, chemical functionalities, microbial density) that influence biodegradation behavior.
- Evaluate the limitations of current test methods and propose refinements to improve their reliability and relevance for pharmaceutical excipients.

2.3 RQ3: LCA

LCA of pharmaceutical excipients: How do they compare in terms of environmental impact beyond biodegradability?

Objective:

- Quantify the broader environmental footprint of excipients using CTG LCA.
- Robust comparison of excipients across life cycle impact indicators.

2.4 RQ4: ESG

How can environmental assessment data support practical excipient selection through decision support tools?

Objective:

- Develop a decision support tool (ESG) that integrates biodegradability and life cycle impact data.
- Visualization of environmental impact to facilitate selection of environmentally preferable excipients in pharmaceutical formulations.

2.5 RQ5: BbD Integration

How can biodegradability and lifecycle data guide the development of novel, environmentally friendly excipients, and how can this knowledge be incorporated into regulatory frameworks such as SSbD and be applied for already existing PEx?

Objective:

- BbD guidance: Propose chemical design principles that improve excipient biodegradability, along with recommendations for process improvements that reduce production-related environmental impacts and enhance overall sustainability.
- Environmental trade-off assessment: Evaluate potential trade-offs between biodegradability and production impacts.
- Regulatory integration: Explore ways to embed environmental performance criteria into regulatory frameworks such as SSbD, thereby encouraging the development and use of the next generation of sustainable excipients.

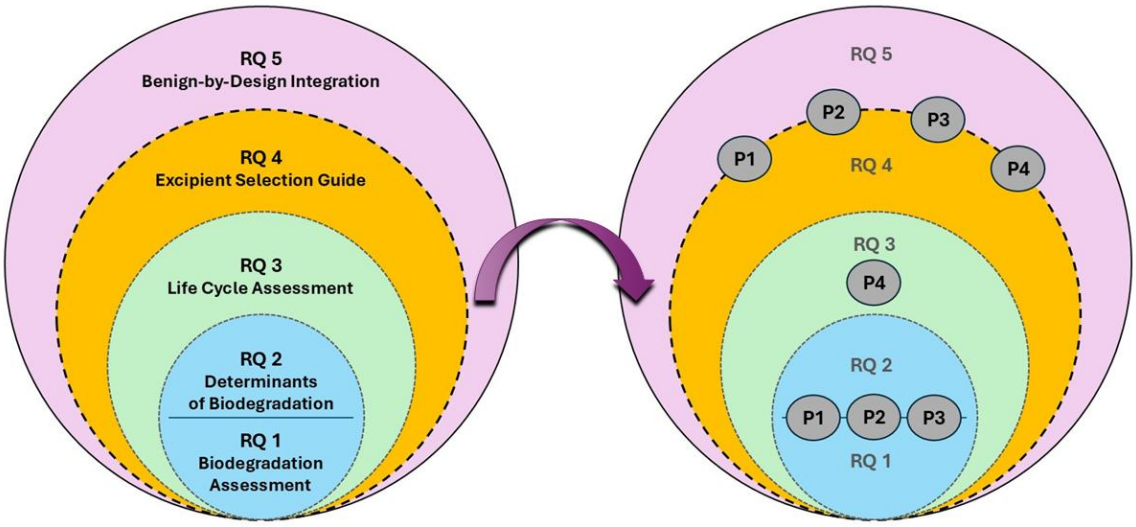

Fig. 1. Overview of the dissertation structure. The left diagram shows the five nested research questions (RQ1-RQ5), progressing from specific (biodegradation) to more holistic (BbD). The diagram on the right shows the corresponding publications (P1-P4) for each RQ. The research questions on the left are linked to their associated publications on the right by a consistent color scheme. Publications at the interface between RQ4 and RQ5 contribute to both the "ESG" and the overarching integration into BbD.

3. Methods

3.1.1 Biodegradation

For this research study, a stepwise approach was used with sequential screening biodegradation tests, each incorporating increasing levels of microbial density and diversity. Namely, it included standardized OECD 301D (Closed bottle test, CBT) and OECD 301F (Manometric respirometry test, MRT; two different versions) protocols. As shown in Fig. 2, the experimental approach progressed from Step 1 to Step 3, each designed to progressively increase the probability of biodegradation by increasing the density and hence the diversity of the microbial inoculum (for experimental details, see P1-P3). This design allowed the study to systematically evaluate how microbial diversity and density influenced biodegradation outcomes. The approach was not designed for regulatory approval, but rather to provide mechanistic insights into degradation behavior, i.e. tests being used as research tools, allowing meaningful comparisons between substances and test setups (RQ2, RQ5). In addition, substances that did not meet the ready biodegradability threshold in the 28-day MRT with activated sludge (Step 3) were tested in an extended 42-day MRT (Step 4) to assess the potential for delayed degradation.

The specific application of this stepwise approach varied between the three publications:

- In P1, only steps 1 to 3 were performed.
- In P2, the full stepwise approach was implemented, including the 42-day test extension (step 4).
- In P3, step 2 (OECD 301F with secondary effluent) was not performed. Based on the results of P1 and P2, it was concluded that higher inoculum densities are more appropriate for assessing biodegradation of polymeric substances. Nevertheless, for comparison, OECD 301D was conducted to gain mechanistic insight into microbial biodegradation adaptation potential to chemical compounds. This approach recognizes that biodegradation is influenced by both intrinsic substance properties and environmental conditions.

Microbial enumeration of the secondary effluent and activated sludge inocula was carried out to aid interpretation of the biodegradation results. Colony forming units (CFUs) were determined using the spread plate technique according to ISO EN 6222:1999. Inoculum samples were serially diluted, plated in triplicate (with water controls) and incubated at 22 °C for 68 ± 4 hours. After incubation, CFUs were counted manually.

These microbial enumeration experiments were carried out after the publication of P1 and P2 are only presented in P3. However, they provide valuable context for the interpretation of the biodegradation results in both P1 and P2.

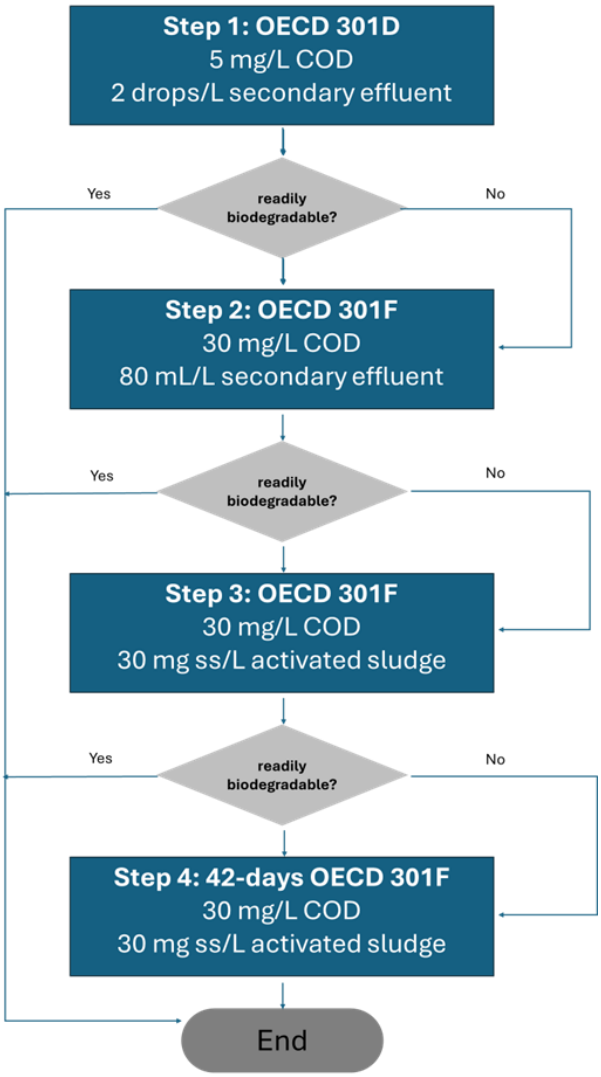

Fig. 2. Workflow of the biodegradability testing.

3.1.2 Classification of Biodegradation

Based on the comprehensive data collected in these studies, the PEx were categorized according to their degradation levels into the following subgroups, as previously outlined: "readily" in line with the OECD test guidelines (≥ 60 %), "moderately" (20-59 %), "slightly/weakly" (10-19 %), and "non-biodegradable" (<10 %). In P1, the initial threshold for defining non-biodegradability was set at 5 %. However, this was revised to 10 % in P2 to more accurately reflect observed biodegradation behavior. The detailed classification system described in P1

and P2 accounts for differences in biodegradation curves across test durations and conditions. This offers a more accurate and informative representation of environmental biodegradability than a binary classification alone.

3.1.3 Assessment of Transformation Products of PS 80

The transformation products of PS 80 were monitored using a UHPLC system (Vanquish, Thermo Scientific, Dreieich, Germany) coupled to an Orbitrap Exploris 240 mass spectrometer (Thermo Scientific, Dreieich, Germany) equipped with a heated electrospray ionization source, operating in full scan and positive ionization mode. MS^2 spectra were used for the analysis. Detailed methodological information can be found in P3.

3.2 LCA

A unit process-based LCA approach to quantify the environmental impacts of pharmaceutical excipients. Manufacturing process flow diagrams and engineering design techniques were used to collect detailed GTG inventory data, including process inputs, emissions, energy use and water consumption, following established methods (Jiménez-González et al., 2000, 2001). These GTG inventories were combined into CTG LCIs, covering raw material extraction, energy consumption, transport and excipient manufacturing. The functional unit for all analyses was set at 1000 kg of excipient. Further methodological details and results are presented in Paper 4.

Environmental impacts were assessed using the TRACI 2.1 framework (US EPA, 2013), focusing on the following seven key life cycle impact categories:

- Total Natural resource energy (NREt=NREc+NREm; NREt: Total extraction of fossil resources.), [MJ HHV]; the total cumulative energy of all fossil fuels used to produce each of the seven process energies listed above. NREt is the sum of Natural Resource Energy Combustion (NREc) and Natural Resource Energy for Materials (NREm), reflecting the total energy value of fossil fuels extracted from the ground. It is akin to the non-renewable fossil component of the Cumulative Energy Demand metric that is widely used in LCA.
- Global warming potential, [kg of CO_2 equivalents]
- Blue water consumption, [kg of blue water]; blue water is measured as all water that is removed from the supply chain, including water lost to evaporation and water incorporated into the product.
- Acidification [kg of SO_2 equivalents]

- Eutrophication [kg of N equivalents]
- Stratospheric ozone depletion [kg of chlorofluorocarbon-11 (CFC-11) equivalents]
- Photochemical smog formation [kg of ozone (O_3) equivalents]

3.3 ESG

To develop the ESG presented in P4, life cycle impact assessment (LCIA) data and biodegradation data for 38 excipients were compiled in an Excel™ spreadsheet. Raw (non-normalized) LCIA scores across seven environmental impact categories and biodegradation percentages were compiled. To enable comparison, internal normalization was performed using min-max feature scaling (0-1 range). Biodegradation values were inverted (based on the OECD threshold of 60 %) to align with the direction of the LCIA impact scale, where lower scores indicate better environmental performance.

Normalized values were then aggregated into a composite score (0-8), with each of the eight metrics being given equal weighting. A maximum score of 8 indicates the comparatively least environmentally preferred excipient, while a score of 0 indicates the most preferred. A heatmap was generated to visualize the data and allow intuitive comparison of excipients based on their environmental profiles, ranging from green (preferred) to red (least preferred).

4. BbD: Approaches to Greener and more Sustainable Excipients

4.1 Environmental Biodegradability

4.1.1 Cellulose-based Polymers

Cellulose-based excipients were assessed to establish a baseline for structure-biodegradability relationships. Fourteen pharmaceutical-grade cellulose derivatives were evaluated using standardized OECD 301D and 301F test conditions (OECD, 1992) (P1). The results showed that none of the cellulose derivatives tested, except for native MCC, met the OECD threshold for classification as "readily biodegradable" (≥ 60 % mineralization). Most compounds exhibited low biodegradation rates, many below 20 % and in some cases below 5 %, indicating considerable persistence in the environment (Table 1). These findings demonstrate that, although cellulose-based excipients are bio-based, the assumption that "bio-based" implies biodegradable can be misleading. This is consistent with previous reports showing that extensive chemical modifications strongly influence environmental biodegradability (Kwon et al., 2023).

The use of a consistent inoculum source and harmonized methodology ensured that data from all 14 cellulose derivatives were directly comparable. This provided the basis for a broader biodegradability classification framework applicable to future excipient development and regulatory evaluation (RQ1, RQ5). Based on the biodegradation curve profiles and extent of mineralization, the modified cellulose-based excipients were classified into two categories:

- Group I: compounds that showed signs of ongoing but incomplete biodegradation
- Group II: compounds that reached a plateau early, indicating negligible biodegradation.

This grouping facilitated the identification of compounds likely to be persistent in the environment (Table 1) (RQ1). The study further elucidated the relationships between structure and biodegradability, showing that higher degree of substitution (DS), molecular substitution (MS) and polymer chain length were associated with reduced biodegradability (Fig. 2) (RQ2). It is well documented that the biodegradation of modified cellulose derivatives decreases as their derivatization, i.e., DS/MS increases (Erdal and Hakkarainen, 2022).

To aid practical application, a "traffic-light" system was introduced to visually categorize excipients according to degradability into the following subgroups: "readily" according to OECD test guidelines (≥60 %), "moderately" (20-59 %), "slightly/poorly" (5-19 %), and "non-biodegradable" (<5 %) (Table 1). This system provided predictive value: compounds in Group

I are likely to benefit from extended testing (e.g. CMC, as demonstrated by Menzies et al, 2023), while those in Group II are unlikely to degrade further, even with prolonged exposure (RQ1). However, the study cautioned against whether extended testing will always result in higher mineralization. Given the potential challenges associated with extended testing, which significantly increases the time and resources required for the evaluation process, the scoring system provides valuable insights, particularly for industry and regulators. This addressed a growing concern that the standard 28-day test may underestimate the biodegradability of certain excipients, particularly polymers (McDonough et al., 2023). By identifying structural features associated with potential persistence, the study provided guidance for molecular redesign of excipients with reduced environmental impact in line with the BbD concept (RQ5).

P1 generated novel and harmonized biodegradation data for a key class of pharmaceutical excipients (RQ1), established robust structure-biodegradability relationships (RQ2), introduced a practical tool for compound prioritization (RQ2, RQ5), and provided a predictive basis for excipient selection and molecular redesign based on environmentally sustainable principles (RQ5). The approach was also in line with ongoing efforts to integrate environmental data into future regulatory frameworks for SSbD chemicals (RQ5).

Table 1. Biodegradability classification of cellulose derivatives according to OECD 301 results. This classification uses Methocel, a trade name for HPMC derivatives. (Source: P1)

Readily biodegradable ≥ 60 %	**Moderately biodegradable 20 - 59 %**	**Slightly biodegradable 5 - 19 %**	**Non biodegradable < 5 %**
MCC	Methocel K3 LV CMC cross-linked	Methocel K100 LV Methocel K4M Methocel K100M Methocel E5 LV HPMCAS CMC linear	HPC SL HPC L HPC M HEC MC EC
	Group Ia	**Group Ib**	**Group II**

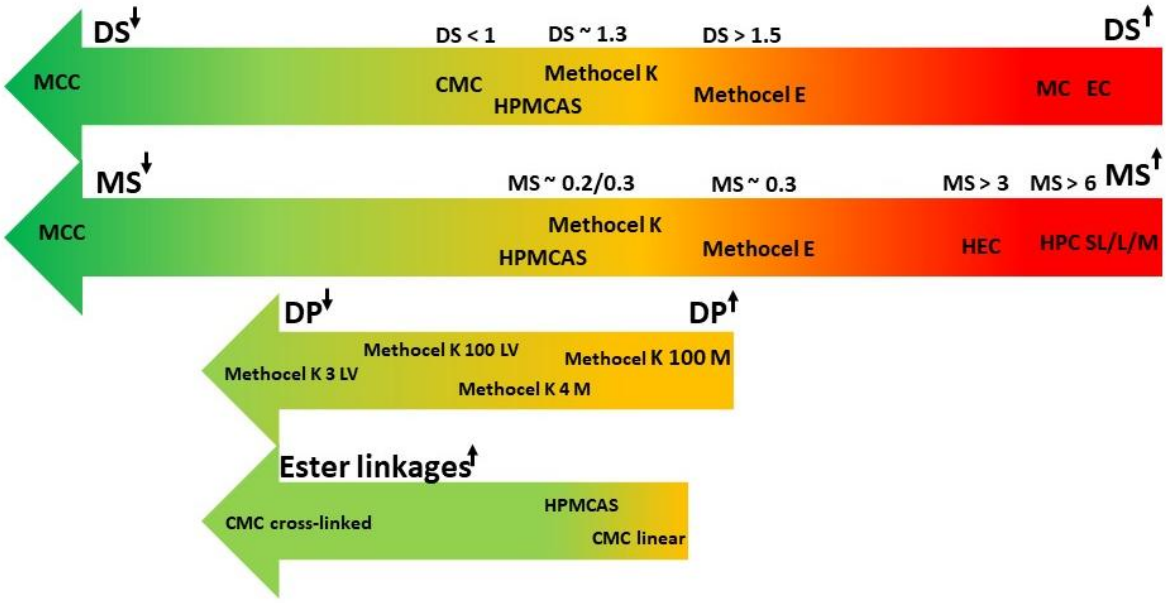

Fig. 3. Color-coded relationship between biodegradability and DS, MS, DP, Ester linkages of cellulose derivatives. (Source: P1)

4.1.2 Synthetic and Bio-based Non-cellulosic Polymers

P2 extended biodegradability assessment beyond cellulose-based excipients by focusing on a wider range of synthetic and natural polymers commonly used in pharmaceutical formulations. The research aimed to provide a comprehensive assessment of the environmental fate of common PEx, including synthetic polymers such as PVP, copovidone, PVA, Macrogol 6000 (PEG) and polymethyl methacrylate derivatives (Eudragit EPO and Eudragit L100-55), as well as natural polymers such as corn starch, its derivative sodium starch glycolate (SSG) and chitosan (RQ1). Using the classification framework previously developed (P1), excipients were classified based on extent of degradation. Maize starch, chitosan, Macrogol 6000, PVA and SSG were classified as “readily biodegradable” (Table 2A).

In the case of PVA and PEG, considerable differences in biodegradation rates were observed depending on the OECD 301 screening tests used (Fig. 3) (P2, Table 1). The lack of biodegradation observed in the OECD 301D test for PEG and PVA contrasts with the readily biodegradable results obtained in the OECD 301F using secondary effluent and activated sludge, respectively. This suggests that the OECD 301D test conditions may not be sufficient to fully assess their potential for "readily" biodegradation (Fig. 3) (RQ1, RQ2). The use of OECD 301 F with secondary effluent as inoculum may also be limited in assessing the full extent of biodegradation for readily biodegradable compounds within the standard 28-day

timeframe, as shown for PVA. Thus, the outcome of biodegradation assessments is context dependent, highlighting the critical role of both inoculum source diversity and inoculum concentration. This is further supported by CFU measurements. According to our results, the initial CFU concentrations varied significantly between the test setups, spanning four orders of magnitude. The concentrations were approximately 10^0 cells mL^{-1} in OECD 301D, about 10^3 cells mL^{-1} in OECD 301F with secondary effluent, and about 10^4 cells mL^{-1} in OECD 301F with activated sludge. These differences help to explain the variation in biodegradation results between test conditions. Importantly, microbial cell counting experiments were carried out after this research was published; the results are included in P3.

The variability observed, often attributed to the so-called "biodegradation lottery," was a result of the random presence or absence of competent microbial degraders within the test systems (Davenport et al., 2022; Goodhead et al., 2013; Martin et al., 2017b). The low cell densities in standard OECD 301D tests limited the inclusion of such degraders, leading to false negative assignments of biodegradability. Conversely, increasing inoculum size and diversity in the OECD 301F tests resulted in positive degradation results for some polymers within the 28-day period. These observations confirm that inoculum sources play a key role in determining the biodegradation potential of the investigated polymers (RQ2). This is in line with assumption that increasing the number of microbial cells in biodegradation screening tests to environmentally representative levels can reduce this variability and enhance test reliability (Martin et al., 2017a; Martin et al.2017b).

SSG showed substantially higher biodegradability than CMC, likely due to its lower degree of substitution (Table 2) (DS = 0.25 vs. 0.8 for CMC). As noted above (P1), the degree of chemical derivatizations plays a critical role in determining biodegradability. The lower DS in SSG likely facilitated microbial degradation within the 28-day test period.

For some groups of excipients, specifically PVP, copovidone and the two Eudragit polymers, no evidence of biodegradation was detected under any test conditions. None of these compounds reached the lag phase, defined as the time required to achieve 10 % biodegradation (OECD, 1992). The lag phase is characterized by the initial adaptation or selection and growth to reach critical mass of microorganisms and the commencement of the biodegradation process (adaptation phase). The absence of a lag phase in this group confirms their low susceptibility to microbial degradation (RQ1, RQ2). Even though the test period was extended to 42 days, no further degradation was observed. This lack of progress strongly suggests that these substances are persistent in the environment, although confirmation by higher tier simulation tests would

be required for regulatory classification (RQ2, RQ5). The absence of degradation in these materials may be attributed to the lack of oxidizable or hydrolysable groups in their molecular structures, particularly in the carbon backbone. This contrasts with PVA and PEG, for which biodegradation is supported by well-established oxidative pathways in literature (Eubeler et al., 2010; Wilkes and Aristilde, 2017) (RQ2). Based on these results, the study refined the classification system originally proposed in P1 by setting 10 % degradation as the threshold to be categorized as "slightly/weakly" biodegradable. This addition accounted for the absence of a lag phase for these compounds highlights their lack of susceptibility to microbial degradation.

In summary, P2 has addressed several key research questions by demonstrating that the environmental biodegradability of PEx is highly dependent on both chemical structure and test conditions (RQ1, RQ2). It highlighted critical limitations of standard OECD screening methods, especially when applied to complex or synthetic materials, and advocated for improved inoculum strategies to better reflect environmental conditions (RQ2, RQ5). It also provided a refined biodegradability classification system and established a scientific basis for prioritising excipients for further study or substitution, ultimately contributing to the sustainable design of pharmaceutical products (RQ4, RQ5).

Table 2. Biodegradability classification according to OECD 301 results. A) old classification system B) new classification system. (Source: P2)

A			
Readily biodegradable ≥ 60 %	**Moderately biodegradable 20 - 59 %**	**Slightly biodegradable 5 - 19 %**	**Non biodegradable < 5 %**
Maize starch Chitosan SSG PVA Macrogol 6000 (PEG)		Eudragit L100-55 Eudragit EPO	PVP Copovidone Kollidon CL
B			
Readily biodegradable ≥ 60 %	**Moderately biodegradable 20 - 59 %**	**Slightly biodegradable 10 - 19 %**	**Non biodegradable < 10 %**
Maize starch Chitosan SSG PVA Macrogol 6000 (PEG)			PVP Copovidone Kollidon CL Eudragit L100-55 Eudragit EPO

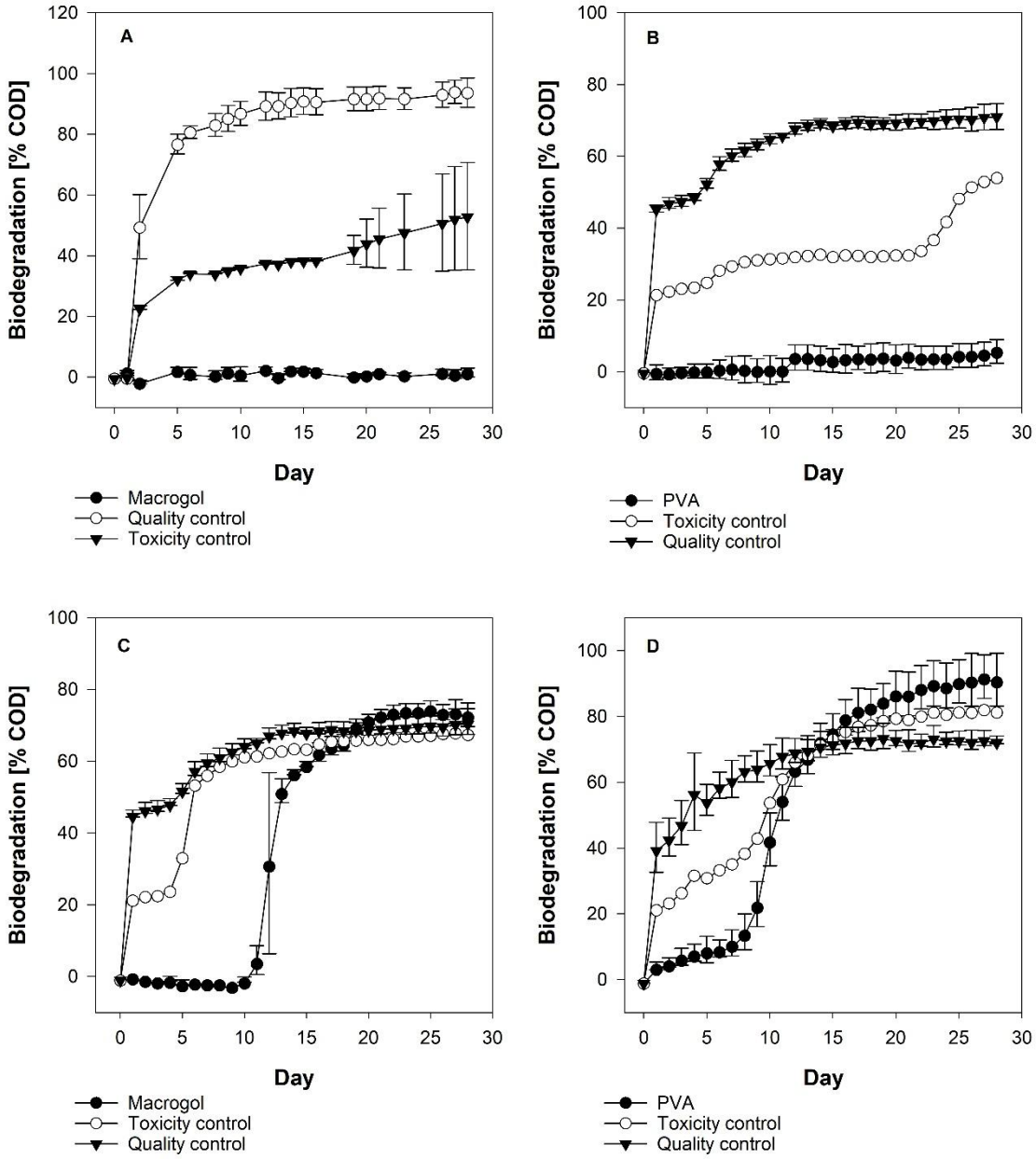

Fig. 4. Biodegradation of A) Macrogol (PEG) in an OECD 301D test B) PVA in an OECD 301F test C) Macrogol (PEG) in an OECD 301F test and D) PVA in an OECD 301F AS test. Presented as an average of replicates(n=2) with error bars showing standard deviation. (Source: P2)

4.1.3 Non-ionic Surfactants and their Derivatives

P3 focused on non-ionic surfactants, specifically PS and poloxamers and their derivatives (see Fig. 5), chosen because of their widespread pharmaceutical use and the limited systematic data on their environmental fate. Existing studies on soil degradation suggest that, for PS, microbial activity can hydrolyze the fatty acid ester groups, whereas the more hydrophilic ethoxylated components remain largely persistent (Lee et al., 2013). A similar metabolic pattern occurs in the human body: following drug administration, PS undergo partial hydrolysis, releasing POE

sorbitan moieties that are poorly absorbed in the gastrointestinal tract and ultimately excreted (Maher et al., 2023). The environmental behavior and fate of these POE sorbitan structures in aquatic systems remain largely unexplored. It is unclear whether biodegradation extends beyond the initial fatty acid hydrolysis or whether the POE sorbitan moiety is resistant to further transformation.

PS were found to be moderately biodegradable, with partial degradation observed in both OECD 301D and 301F tests. In the 28-day OECD 301F test, PS 80 and PS 20 degraded by 45.5 % ± 5.5 % and 45.3 % ± 4.3 %, respectively, with only a slight increase after 42 days (50.3 % ± 5.3 % for PS 80, 49.1 % ± 6.2 % for PS 20) (Fig. 7) (RQ1). This partial degradation reflects a stepwise process: initial rapid hydrolysis of the fatty acid ester groups, followed by slower oxidative transformation of the ethoxylated moieties. PS degradation leads to the formation of ethoxylated sorbitan residues, which may not be readily mineralized and could form “dead-end” intermediates. While the environmental relevance of these residues requires further study, their persistence suggests that complete PS degradation cannot be assumed (RQ2). Two potential degradation pathways for the sorbitan POE moiety were identified (see Fig. 7) (RQ2).

P3 investigated the biodegradability of high-molecular-weight poloxamer 188 (P188) and poloxamer 184 (P184). In the 28-day OECD 301D and 301F tests, as well as the extended 42-day OECD 301F test, P188 showed no measurable degradation. However, P184 was readily biodegradable in the 28-day OECD 301F test (RQ1). These results suggest that biodegradability is not solely determined by water solubility or the inherent degradability of the PEG and polypropylene glycol (PPG) blocks, but rather by the specific ratio of the PEG-PPG-PEG blocks. The larger PEG flanks in P188 appear to induce polymer conformations that limit microbial uptake and enzymatic accessibility, thereby preventing degradation despite the solubility of the building blocks and their degradable nature (RQ2).

In line with the SSbD framework, P3 provides a harmonized dataset based on OECD 301 for structurally related surfactant derivatives. This enables a structured comparison of the molecular features influencing environmental degradation (see Table 2). Differences in lag phase duration, which are used as an indicator of microbial adaptability and the ability of microbial communities to adjust to specific molecular structures over time, formed the basis for deriving structure-specific dependencies that govern biodegradability. Based on this analysis, the influence of each molecular feature on biodegradability was categorized as low, medium or high. This approach serves two essential functions: it enables direct comparisons between surfactant derivatives and pinpoints structural features that enhance biodegradability. This

supports the development of environmentally degradable pharmaceutical surfactants and polymeric materials.

Results of P3 also demonstrated the importance of test conditions in assessing biodegradability (RQ2). Significant discrepancies were observed between results from the OECD 301D and 301F protocols. OECD 301D, which uses low microbial cell densities, often underestimated the biodegradation of certain compounds, resulting in many potential false negatives. In contrast, OECD 301F, with its higher microbial load, provided a more realistic picture of degradation potential, particularly for polymeric compounds that require longer adaptation periods. Reliable biodegradability testing requires not only standardized methods, but also sufficient microbial activity to accurately reflect environmental conditions (RQ2).

Together, these results support the broader goal of developing environmentally friendly surfactants through data-driven design. The combination of experimental screening, mechanistic investigation and structure-based analysis provides a foundation for safer, more sustainable innovation in pharmaceutical formulation science.

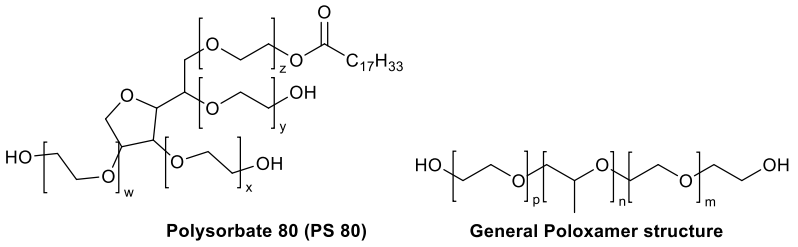

Fig. 5. Structures of two major pharmaceutical surfactants showing PS 80 (w+x+y+z=20), and poloxamer (P188: p, m=75, n=30, MW ~8200; P184: p, m=15, n=30, MW ~2400).

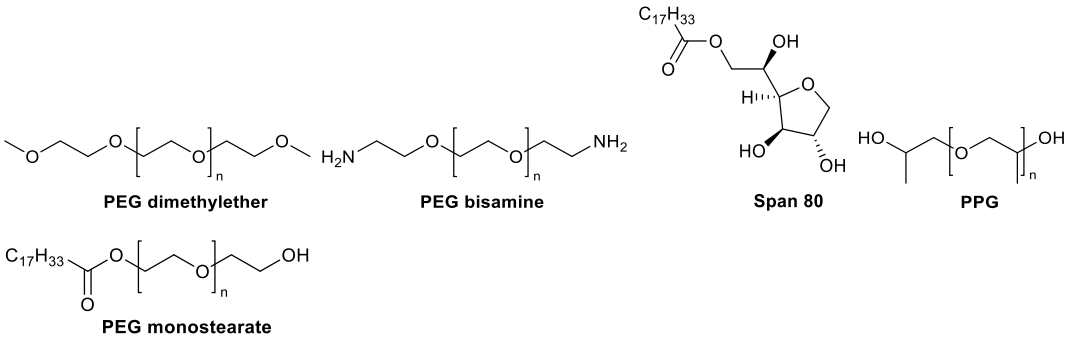

Fig. 6. Structures of study compounds showing PEG dimethylether (MW ~240), PEG bisamine (MW ~2000, Span 80, PPG (PPG 400: MW ~400; PPG 4000: MW ~4000), and PEG monostearate (MW ~2044).

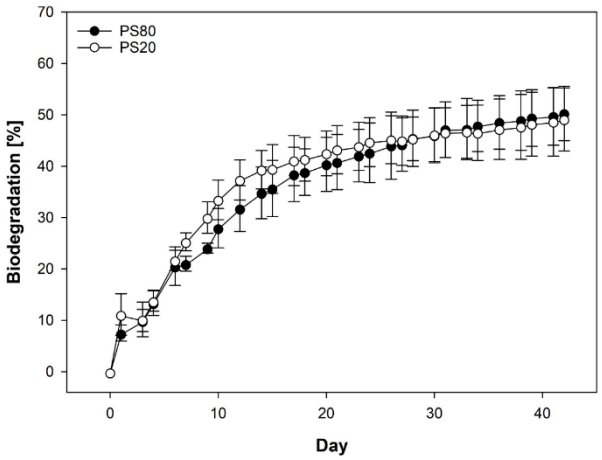

Fig. 7. Biodegradation of PS 80 and PS 20 in a 42-days OECD 301F test. Presented as an average of replicates (n≥2) with error bars showing standard deviation.

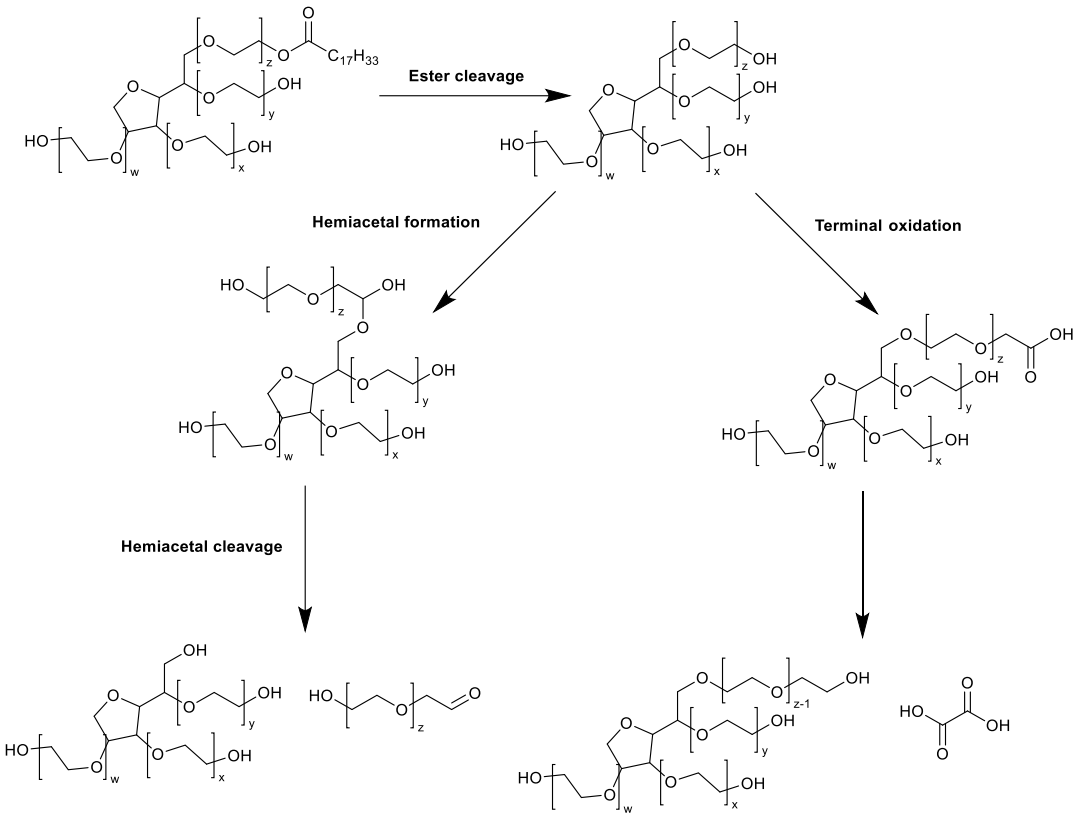

Fig. 8. Proposed pathways for the degradation of the sorbitan POE moiety, with ester hydrolysis occurring first (rapid step), followed by slower oxidative conversion of the POE moiety. (Source: P3)

Table 3. Comparative overview of the main molecular features influencing the biodegradability of non-ionic surfactants, including their dependencies, the observed biodegradation behavior (including lag phases) and representative examples. The degree of dependency is ranked from low (✓) to medium (✓✓) to high (✓✓✓), reflecting the relative influence of each parameter on microbial adaptation and degradation outcomes. (Source: P3)

Parameter	Degree of Dependency	Impact on Biodegradability	Representative Example
Molecular Weight	PEG/POE: ✓	Readily biodegradable at low and high MWs	PEG (up to 30,000 Da)
	PPG: ✓✓✓	High MW impact	PPG 400 (4-day lag phase), PPG 4000 (non-biodegradable)
	POE-PPG POE: ✓✓	MW-dependent transport limits; longer adaptation phase	P184 (9-day lag phase) P188 (non-biodegradable)
Spatial Configuration	✓✓	Steric hindrance limits enzymatic access; slower adaptation; slower or incomplete biodegradation	Ethoxylated PS
Functional End Groups	✓✓✓	Ester groups: rapid enzymatic cleavage; short lag phase	PEG monostearate, Span 80 (< 2-day lag phase)
		Amine (-NH_2): requires oxidation/transamination; moderate to long lag phase	PEG-bisamine (13-day lag phase)
		Ether (-O-): resistant to biodegradation; no adaptation	PEG dimethylether (non-biodegradable)
Solubility	PEG/POE: ✓	High solubility facilitates biodegradation	
	PPG: ✓✓✓	Low solubility hinders biodegradation	PPG 4000 (not degraded)
	POE-PPG-POE: ✓	Steric hindrance limits degradation	P188 (high solubility, no degradation)
Conformation/ Microbial Uptake	PEG/POE: ✓	Generally favorable uptake	
	PPG: ✓	Favorable at low MW	PPG 400
	POE-PPG-POE: ✓✓✓	Unfavorable conformation; poor uptake; no degradation	P188

4.2 LCA and Development of ESG

P4 extended previous work by integrating experimental biodegradability data with CTG LCA results to develop a comprehensive environmental profile of pharmaceutical excipients (RQ3, RQ4). Fig. 7 illustrates this comparison visually in a bar chart, showing the excipient impact relative to the overall average across categories. This approach highlighted critical environmental trade-offs between bio-based/agricultural and petroleum-based excipients across four categories: very high, high, moderate, and low (RQ3). In particular, the analysis showed that most of the contributions to the assessed environmental impacts were dominated by NREc, reflecting energy-related emissions. These emissions were primarily from the combustion of fossil fuels required to meet the electrical and thermal energy demands of excipient manufacturing. These results are in line with previous studies showing that energy consumption accounts for 50 % to 80 % of the total environmental impact of the chemical manufacturing sector (Parvatker and Eckelman, 2020).

Excipients that did not fall into the "low impact" category typically had either high process-related emissions (e.g., release of hazardous chemicals during synthesis) or, in the case of agricultural-based excipients, high blue water consumption associated with irrigation requirements. The results showed that the high (total score 10-15) environmental impact categories consisted of both bio-based and petroleum-based substances, each with different environmental impacts. Within the agriculturally based group, lactose and sucralose were classified as having very high impact. Lactose had an exceptionally high blue water footprint, 13 times higher than average, mainly due to irrigation requirements for corn used in dairy feed and emissions from dairy production. Similarly, the environmental profile of sucralose was dominated by high NREc and smog formation linked to chlorine emissions from phosgene production and irrigation-intensive sugar cane cultivation. In contrast, petroleum-derived excipients such as PVP, copovidone, and crospovidone also fell into the high impact group due to extremely high NREm values - three to four times higher than average. These values reflect the embedded fossil energy content of the materials, highlighting the heavy reliance on crude oil and natural gas feedstocks in the production of synthetic polymers. A notable trade-off emerged for methylated cellulose derivatives (e.g., methylcellulose, MC; hydroxypropyl cellulose, HPC; hydroxypropyl methylcellulose, HPMC), which are bio-based but chemically modified. These compounds exhibited significant ozone depletion potential due to the use of methyl chloride during synthesis, which is a known source of stratospheric chlorine. The degree of methylation correlated with increased ozone impact, with more methylated derivatives being

in the high or even very high impact group. For the agricultural excipients in the low impact group, Starch 1500, sorbitol, dextrose, mannitol and sucrose, the largest contribution to the total environmental impact came from blue water consumption due to irrigation. For magnesium stearate (MgSt) and sodium starch fumarate (SSF), their high eutrophication potential was particularly striking. This was mainly due to nitrate and phosphate emissions to water sources from the use of fertilizers during the plantation phase of the production of fresh fruit bunches for crude palm oil. The excipient PS80 (agricultural) and the petroleum-based excipients PEG 6000 and sodium lauryl sulfate (SLS) had the highest NREm values within this group, reflecting the total energy value of the fossil fuels extracted from the ground for the materials themselves (RQ3).

A key innovation of this work was the development of ESG, a novel decision support tool to assist in the selection and design of more environmentally sustainable excipients (RQ4). The ESG combines normalized LCA impact scores and biodegradability data, a key parameter in ERA. This is in alignment with recommended approaches to shift from decoupled assessments of risk and sustainability for decision making is needed to identify and minimize risk-sustainability trade-offs through a more overarching and holistic approach (systems thinking) (Hauschild et al., 2022; Moermond et al., 2025) (RQ4, RQ5).

The ESG developed in this study serves two main purposes: (1) to provide a comprehensive framework for the selection of more environmentally sustainable excipients and (2) to support the development of greener and more sustainable alternatives for the future (RQ4, RQ5). Visually, the ESG is presented as a heat map, providing an intuitive, color-coded overview of the environmental performance of each excipient (Fig. 8). This visualization facilitates quick comparisons and ranks excipients from most to least environmentally friendly, making complex sustainability assessments more accessible and actionable for decision-makers (RQ3). In addition to its role as a selection tool, the ESG also serves as a valuable resource for excipient development and process optimisation. By analyzing process-related emissions, excipient suppliers can target specific GTG stages for impact reduction and improved environmental performance. For example, ammonia emissions from PVP and copovidone, chlorine emissions from HPC and methyl chloride emissions from HPMC have been identified as major contributors to environmental impact. Targeting such emissions could significantly improve environmental performance and reduce the environmental burdens associated with these excipients. These findings highlight the need for process optimization and environmental considerations in excipient production (RQ5).

The integrated approach revealed critical trade-offs between environmental performance during manufacture and biodegradability after use. For example, Eudragit derivatives had comparatively lower environmental impacts during manufacture, but raised concerns due to their poor biodegradability and persistence in the environment after use. In contrast, the production of lactose, which is environmentally benign at the end of its life cycle, was associated with significant environmental impacts, in particular high-water consumption and global warming potential. These findings highlight the need for full LCAs and the application of SSbD principles to effectively manage and minimize such trade-offs (RQ5).

Ultimately, while this ESG was presented with a focus on the pharmaceutical sector, its application extends far beyond, offering a practical tool to support decision-making in industries reliant on excipients, including food, cosmetics, and personal care sectors. It can also serve as an example of how to combine multiple aspects of environmental sustainability in a systems-thinking way to avoid point solutions that lead to rebound effects, and to broaden the BbD approach from its current focus on the environmental biodegradability of chemical compounds, including pharmaceuticals and many others. This combined approach addressed existing gaps in sustainability assessments where biodegradability and LCA have typically been considered separately. It also supported a more holistic, systems thinking perspective in line with SSbD principles (RQ5).

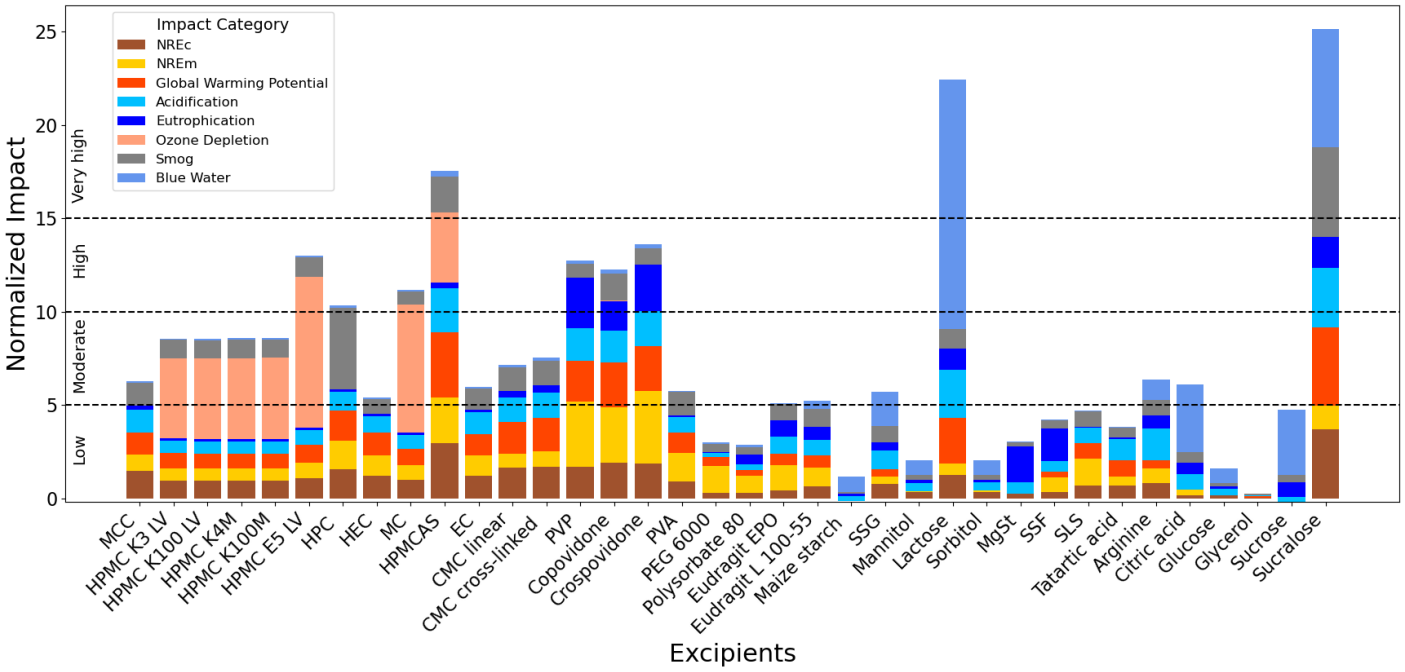

Fig. 9. Environmental impact characterization (normalized impact 0-30) of the 36 analyzed excipients for the eight impact categories, Global Warming Potential, acidification, eutrophication, ozone depletion smog, blue water, NREm and NREc (NREm+NREc =NREt). (Source: P4)

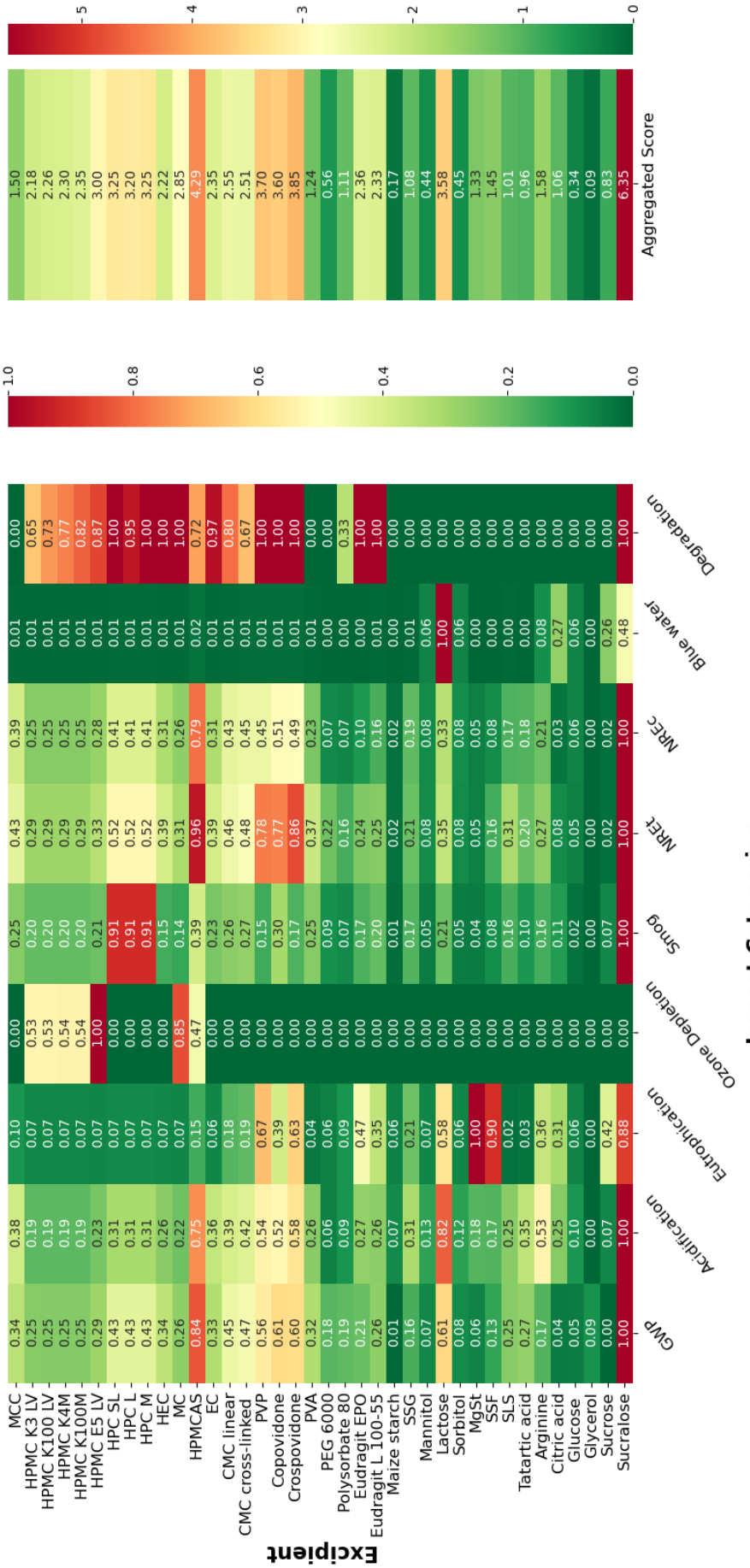
Excipient
MCC
HPMC K3 LV
HPMC K100 LV
HPMC K4M
HPMC K100M
HPMC E5 LV
HPC SL
HPC L
HPC M
HEC
MC
HPMCAS
EC
CMC linear
CMC cross-linked
PVP
Copovidone
Crospovidone
PVA
PEG 6000
Polysorbate 80
Eudragit EPO
Eudragit L 100-55
Maize starch
SSG
Mannitol
Lactose
Sorbitol
MgSt
SSF
SLS
Tatartic acid
Arginine
Citric acid
Glucose
Glycerol
Sucrose
Sucralose
GWP
Acidification
Eutrophication
Ozone Depletion
Smog
NRET
NREC
Blue water
Degradation
Impact Categories
Aggregated Score

Fig. 10. ESG represented as a heatmap, showing normalized values for the assessed impact categories: GWP, Acidification, Eutrophication, Smog, Total NRE, Blue Water NREc, and Environmental Biodegradation. The far-right side displays the aggregated score (0 = most preferred, 8 = least preferred). NREc is excluded from the score to avoid double counting, as it is included in NREt. (Source: P4)

5. Implications of Findings for Practice and Future Research

WSPs have recently emerged as a pressing concern within the broader context of polymer pollution (Arp and Knutsen, 2020; Huppertsberg et al., 2020; Albright and Chai, 2021; Zumstein et al 2022; Robison-Smith et al., 2024). Against the background of the ongoing REACH revision, policymakers are addressing this oversight by establishing criteria to identify and register polymers that require regulatory control. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Task Force "Assessing the human health and environmental safety of polymers" has proposed a three-tiered approach that define standard information requirements for polymers subject to registration under REACH. Tier 1 focuses on environmental fate assessments, including biodegradability as a core parameter (Otte et al., 2023). This study was one of the first to carry out comprehensive biodegradation screening of 25 polymeric pharmaceutical excipients under OECD 301 conditions (P1-P3). This addressed the lack of validated assessment methodologies and reliable data for polymeric materials. P1-P3 also examined the applicability of OECD 301 screening tests for polymers, revealing necessary adjustments to test conditions for future assessments. The results showed considerable variability in biodegradation performance: Twelve excipients were classified as non-biodegradable, five as slightly biodegradable, three as moderately biodegradable and five as readily biodegradable.

These findings provided valuable empirical data that advanced scientific understanding and regulatory discourse in two key areas. Firstly, P1-P3 demonstrated technical applicability of the OECD 301 screening tests to polymeric substances, while also highlighting their limitations. Specifically, the tests significantly underestimated biodegradation when standard secondary effluent was used as the source of the inoculum. In contrast, using activated sludge, which is characterized by a higher microbial density and diversity, substantially improved the detection of biodegradation. This highlights the importance of adapting test protocols to ensure meaningful and representative results, particularly by optimizing the microbial composition of the inoculum. Secondly, the P1-P3 contested the binary pass/fail criteria currently used in OECD 301 studies for classifying substances as either readily biodegradable or non-biodegradable. Based on a screening of diverse sets of PEx, the studies demonstrated that this binary classification system fails to reflect the range of biodegradation behaviors observed in standardized tests. To address this issue, a new classification system has been developed that categorizes substances as non-biodegradable, slightly biodegradable, moderately biodegradable, or readily biodegradable (P1, P2). This categorization is based on

biodegradation curve patterns, mineralization percentages and mechanistic insights. It enables a more accurate interpretation of results from screening-level tests. Furthermore, structure-biodegradability relationships were elucidated across the tested excipients, providing additional validation of the classification system. The resulting scoring system offers several enhanced practical benefits. It prioritizes polymers for further assessment or substitution and acts as a decision-support tool for identifying structural features linked to improved biodegradability. This enables the development of molecular redesign strategies to enhance the environmental biodegradability of polymeric materials supporting the rational design of "greener" substances.

To integrate biodegradation profiles with life cycle metrics, the novel decision-support tool ESG was created (P4). Recent global and regional policy developments, including the European Green Deal, the CSS and the Zero Pollution Action Plan, emphasized the urgent need to incorporate life cycle thinking into the innovation of chemicals and materials (EC 2019; EC 2020; EC 2021). These strategic frameworks advocate shifting away from fragmented risk assessments towards systemic approaches that consider the full environmental footprint of substances throughout their lifespan. Against this policy backdrop, the SSbD framework, developed by the European Commission's Joint Research Centre, offers a conceptual structure to promote sustainability-driven innovation (Caldeira et al., 2022). It promotes the proactive integration of safety and environmental sustainability criteria from the earliest stages of chemical and material development (Caldeira et al., 2022; Puhlmann et al., 2024; Apel et al., 2024). Step 4 of the SSbD framework explicitly calls for an environmental sustainability assessment based on LCA methodology. However, the limited availability of toxicity and LCA data in the early stages of innovation poses a significant challenge to the implementation of SSbD (Caldeira et al., 2022; Apel et al., 2024). Consequently, industry and regulators currently lack practical tools to inform decision-making based on environmental performance, and prospective sustainability assessments are rarely conducted at the pre-market or R&D stage. This thesis addresses the critical shortage of life cycle data for pharmaceutical excipients by presenting the first comprehensive study that systematically evaluated the environmental impact of 38 substances through CTG LCAs (P4). The ESG tool is based directly on environmental data and demonstrates systems thinking in practice. It puts into operation the key principles of SSbD, translating the conceptual framework into a scalable, data-driven tool that supports environmentally sustainable innovation.

The following sections expand upon the key contributions/implications of this doctoral thesis, outlining short-, medium- and long-term strategies for translating its findings into practical

measures that can advance environmental sustainability in the pharmaceutical sector. These pathways demonstrate how the study's data, tools and methodological insights can influence industry practices, guide regulatory development and support future research.

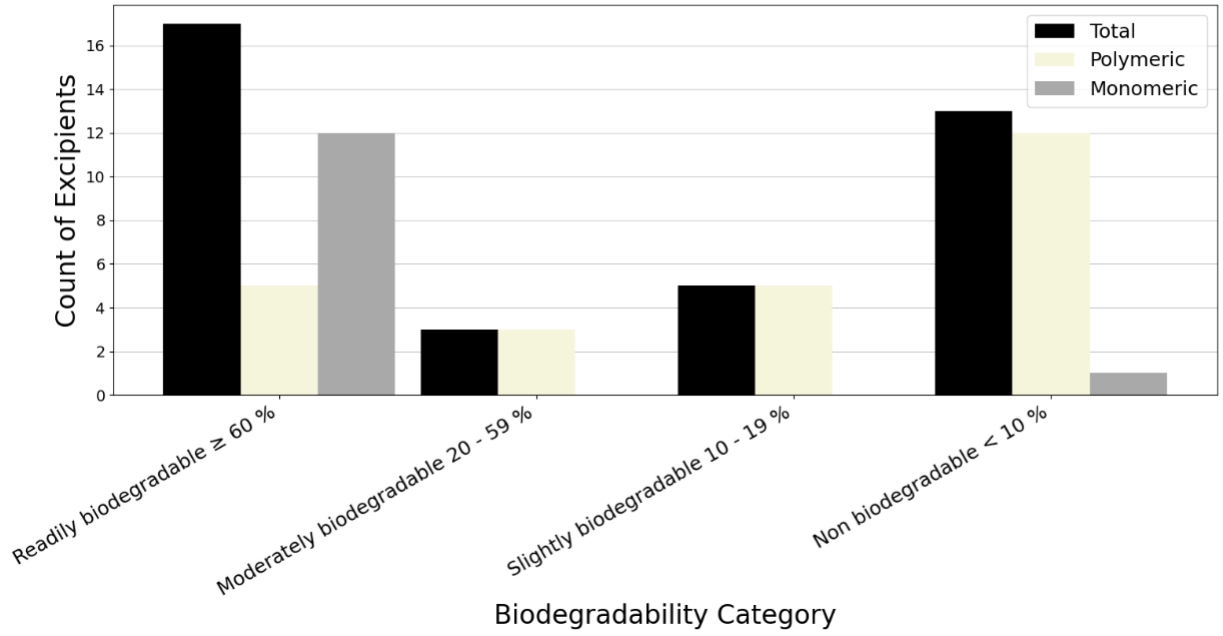

Fig. 11. Biodegradability distribution of excipients into readily, moderately, slightly, and non-biodegradable.

5.1 Short-Term: Strengthening the Basis for Sustainable Excipient Selection

In the short term, the results of this study provide a strong scientific and strategic basis for changing the way excipients are evaluated and prioritized within the pharmaceutical industry. For the first time, 38 commonly used pharmaceutical excipients were systematically evaluated and ranked according to key environmental parameters, which were derived from biodegradation screening and CTG life cycle impact data. This dual evaluation revealed substantial differences in the environmental impact of excipients, providing previously unavailable insights and directly leading to the development of a practical selection guide (ESG) (P4). This tool enables the classification of excipients according to their environmental footprint, distinguishing between greener, more sustainable options and less sustainable ones. This is in line with the EU Taxonomy Regulation and its technical screening criteria, which define the conditions under which economic activity qualifies as substantially contributing to environmental sustainability, such as pollution prevention and control (EC, 2023a).

Consequently, the ESG facilitates the incorporation of environmental considerations into the initial stages of R&D and formulation development, aiding companies in meeting regulatory expectations and internal sustainability objectives. In practice, the ESG also catalyses internal dialogue on portfolio management and reformulation by identifying excipients with unfavourable environmental profiles for potential substitution. For example, within the disintegrants group, sodium starch glycolate (SSG) ranked 1.08, cross-linked CMC 2.51 and crospovidone 3.85, highlighting clear opportunities for improvement and informed substitution decisions. By enabling science-based prioritization and promoting early trade-off discussions, the ESG helps lay the foundations for broader reformulation strategies, greener product portfolios and more environmentally focused innovation pathways (P4).

The cross-industry applicability of the ESG, which is used to assess many excipients that are also used in cosmetics, food and personal care products, promotes greater consistency in sustainability assessment and helps to avoid unfortunate substitutions that shift environmental burdens between sectors. Overall, this work highlights the importance of assessing environmental impacts holistically, considering multiple categories together as part of an integrated sustainability strategy, rather than in isolation.

Key short-term research and development priorities include finalizing a regulatory classification scheme based on biodegradability, in collaboration with industry partners, and refining standardized biodegradability test protocols. It is particularly important to harmonize OECD methods and enhance the environmental relevance of test conditions, for example by improving microbial diversity, if consistent and meaningful biodegradation data sets are to be generated. A deeper understanding of how intrinsic polymer properties influence microbial degradation will also be crucial. This knowledge will support more accurate biodegradation assessments and contribute to the validation of the proposed classification system. The system is designed to go beyond the current binary pass/fail approach, identifying polymers that continue to biodegrade when testing is extended beyond 28 days. This allows for a more accurate interpretation of degradation behavior and helps prioritize substances that may require modified testing or further assessment (P1-P3).

5.2 Mid-Term: Transitioning to Reformulation and Novel Excipient Development

In the medium term, the pharmaceutical sector should move from the mere evaluation of existing excipients to active reformulation efforts and the development of the next generation of environmentally optimized ingredients. A key step is the application of identified more sustainable excipients in real formulations. Testing under practical formulation conditions will

allow a comprehensive assessment of functionality, stability and compatibility, while identifying technical or regulatory barriers to adoption. This translational phase is essential to build confidence in sustainable alternatives and develop scalable reformulation pathways.

In parallel, research efforts must focus on the rational design and synthesis of novel excipients, guided by principles of biodegradability and low life cycle impact. Insights from structure-biodegradability relationships identified in this work provide a scientific basis for designing materials with environmental performance in mind from the outset, without compromising technological performance (P1-P4).

To facilitate wider industry adoption, sustainability policies and regulatory frameworks need to evolve alongside technological innovation. This includes embedding standardized tools for multi-criteria decision making, building on the ESG developed in this thesis. A harmonized framework for evaluating excipients based on both their functional and environmental profiles will ensure transparent, science-based decisions. Importantly, galenic formulation processes should also be included in environmental assessments, moving the industry towards full life-cycle thinking in excipient development and formulation design.

Taken together, this medium-term transformation rests on two interdependent pillars:

- Targeted substitution of existing excipients with greener alternatives, providing immediate, incremental improvements to current formulations and supply chains.
- Rational design of next-generation biodegradable excipients, driving disruptive, transformative innovation by rethinking excipient chemistry, functionality and end-of-life behavior from the ground up.

By enabling both evolutionary and disruptive advances, the tools and insights of this work bridge environmental assessment with practical reformulation strategies, paving the way for a more sustainable pharmaceutical industry.

5.3 Long-Term: Embedding Sustainability into Pharmaceutical Regulation

In the long-term, achieving truly sustainable pharmaceuticals will require a fundamental redesign of the current regulatory framework in order to systematically integrate environmental considerations at every stage of the pharmaceutical life cycle (Van Wilder et al., 2024; Puhlmann et al., 2024). The European Commission has recognized environmental sustainability as one of the five key objectives of its proposed revision of pharmaceutical legislation (EC 2023b.). This thesis addresses this emerging agenda by focusing on pharmaceutical excipients,

which are an essential, yet frequently overlooked component of life cycle analyses. However, several key stages remain insufficiently assessed, including:

- API synthesis
- Galenic formulation
- Packaging
- Use and end-of-life

Currently, environmental assessments in EU pharmaceutical regulation are narrow in scope. The mandatory ERA for marketing authorization only considers the ecotoxicological risks of APIs once they have been excreted by patients. However, it does not address broader sustainability issues, such as greenhouse gas emissions, water consumption and resource use. Pharmaceutical excipients are not considered at all (EMA 2024). The ongoing reform of EU pharmaceutical legislation provides a rare and time-sensitive opportunity to recalibrate the system (EC, 2023b). This thesis contributes directly to the ongoing debate on regulatory issues by focusing on the environmental impact of pharmaceutical excipients. It addresses a critical blind spot in current frameworks. Crucially, the work recognizes the cross-border nature of pharmaceutical pollution by quantifying emissions throughout the entire supply chain. This approach aligns with the Commission's acknowledgement that environmental risks do not stop at national borders (EC, 2023b). These results may spark discussions about the environmental trade-offs involved in pharmaceutical development and raise awareness among regulatory stakeholders. By compiling vital environmental data, such as biodegradability, emissions and resource use, into a structured, multi-criteria dataset, the ESG developed in this thesis offers a practical tool for integrating sustainability into evolving regulatory frameworks.

6. Conclusion

Pharmaceutical excipients are essential for drug formulations, yet their environmental impacts demand greater attention. This thesis revealed that PEx exhibited a broad spectrum of biodegradability outcomes influenced by structural features, microbial adaptation potential and testing conditions. This highlights the necessity for optimized protocols and more precise evaluation criteria. Standard OECD 301 tests alone are insufficient to capture the entire spectrum of biodegradation behaviors, thus demanding the adoption of the proposed graded "traffic-light" system. Beyond biodegradability, life-cycle assessments revealed that energy use, process emissions and agricultural inputs make a significant contribution to environmental

impact, highlighting the importance of adopting a holistic approach to excipients. Integrating these perspectives advances the concept of “Benign-by-Design”, offering the possibility of linking molecular design with environmental sustainability outcomes in the future. A notable contribution is the development of the Excipient Selection Guide, which serves as a practical decision-making tool, helping researchers and industry professionals to balance biodegradability with life cycle impacts during the initial stages of formulation development.

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List of Publications

Publication 1	Bading, M., Olsson, O., Kümmerer, K., 2024. Analysis of environmental biodegradability of cellulose-based pharmaceutical excipients in aqueous media. Chemosphere 352, 141298.
Publication 2	Bading, M., Olsson, O., Kümmerer, K., 2024. Assessing the aquatic biodegradation potential of polymeric excipients for pharmaceutical formulation. Chemosphere 368, 143739.
Publication 3	Bading, M., Suk, M., Olsson, O., Kümmerer, K., 2025. Biodegradation of pharmaceutical surfactants: polysorbates, poloxamers, and derivatives in OECD 301 screening tests and insights into “Benign-by-Design” rules. Ready for Submission.
Publication 4	Bading, M., Griffing, E., Olsson, O., Harris, J., Scher, J., Sakurai, A., Overcash, M. R., Kümmerer, K., 2025. Assessments of life cycle and biodegradation properties uncovered distinct profiles of pharmaceutical excipients guiding selection for drug formulations. Under Review at Green Chemistry.

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Analysis of environmental biodegradability of cellulose-based pharmaceutical excipients in aqueous media

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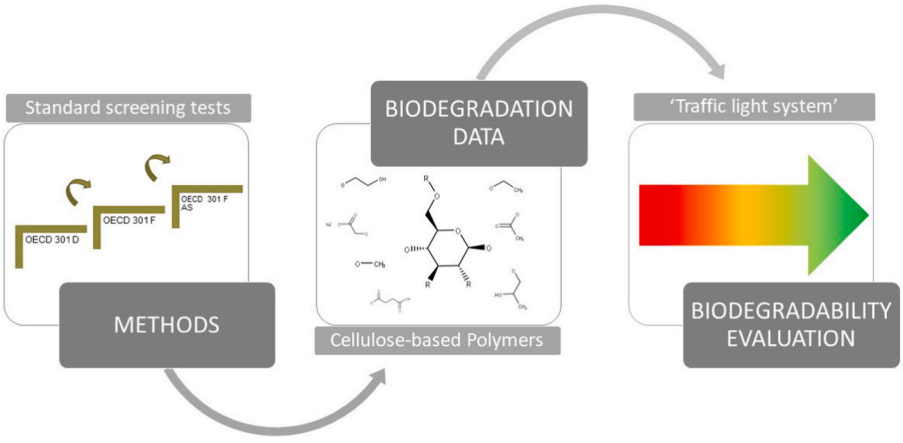
HIGHLIGHTS

- First systematic study on biodegradation of 14 cellulose-based pharmaceutical excipients using OECD 301 standard methods.
- None of the tested cellulose derivatives met the criteria for 'readily biodegradable' classification.
- Identification of potential inhibitory effects on inoculum respiration for 10 compounds.
- Development of a 'traffic light system' for grouping the substances, highlighting structure-biodegradability relationships.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical cellulosic polymers will inevitably reach natural water systems if they are not removed after entering wastewater. Biodegradation of organic chemicals in sewage or in the aquatic environment is an important removal mechanism. In this study, we investigated the environmental biodegradation of 14 cellulose derivatives commonly utilized as pharmaceutical excipients using three different test systems that are based on the closed bottle test (OECD 301D) and the manometric respirometry test (OECD 301F). For the different cellulose derivatives tested, we observed varying degrees of biodegradation ranging from 0 to 20.4 % chemical oxygen demand (COD). However, none met the criteria for classification as 'readily biodegradable'. In addition, 10 out of 14 cellulose derivatives and/or their possible transformation products formed during the experiments, may exhibit possible toxic inhibitory effects on the inoculum. This includes one or several derivatives of hydroxy propyl methyl cellulose, hydroxy propyl cellulose, methyl cellulose, ethyl cellulose, and hydroxy ethyl cellulose. Based on the results obtained, we have developed a graded classification score ('traffic light system') for excipient biodegradation. This could help streamline the assessment and classification of cellulose derivatives concerning risk of persistence and potential adverse environmental effects, thereby assisting in the prioritization

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of more favorable compounds. In the long term, however, excipients should be designed from the very beginning to be biodegradable and mineralizable in the environment ('benign by design').

1. Introduction

Polymers, both water-soluble and water-insoluble, represent a major group of formulation excipients in solid oral dosage forms, referred to as 'polymeric excipients (PEs)'. Their functionality ranges from their use as binders and disintegrants to film coatings, to modifiers of release kinetics of the active pharmaceutical ingredient (API) (Debotton and Dahan, 2017). Absorption of PEs by the gastrointestinal track is generally considered negligible. PEs are regarded as pharmacologically inert, non-metabolizable, and excreted primarily in feces as part of the unabsorbed material (Bauer and Lehman, 1951). Consequently, their potential high release into the aquatic environment after excretion could result in considerable environmental concentrations of PEs if they are not removed within sewage treatment or the natural environment. Concerning the overall environmental exposure to PEs, the recent restriction proposal on microplastics by the European Chemicals Agency (ECHA) has estimated the annual usage volume of a single, not further specified PE to range between 500 and 2700 tons, specifically within diffusion-controlled systems (i.e., controlled release dosage form) in Europe (ECHA Annex XV Restriction Report, 2019). However, this estimation does not include water-soluble polymers and polymers used in immediate-release forms, suggesting that the total environmental pollution from PEs could be substantially higher than this estimated range.

Despite extensive industrial applications of polymers, which extends far beyond their use in pharmaceutical industry, quantitative analytical methods to detect polymers in the environment, especially for water-soluble ones, are currently lacking (Huppertsberg et al., 2020). Therefore, quantitative data on their occurrence in the aquatic environment are scarce. Recently, for the first time, environmental data on the concentration of polyethylene oxide (PEO), a commonly used water-soluble polymer within pharmaceutical formulations, in wastewater and surface water samples have been reported (Pauelsen et al., 2023). The authors found that even the readily biodegradable polymer, PEO reached concentrations in the range of μg L^{-1} and raised concerns about the potential environmental impact of non-biodegradable polymers (Pauelsen et al., 2023). Biodegradation represents a key factor determining environmental persistence of chemicals. However, currently only a few studies have evaluated environmental biodegradation of PEs. Their focus was mainly on petroleum-based polymers such as PEO, polyvinyl alcohol and polyvinyl pyrrolidone (Menzies et al., 2023; McDonough et al., 2023; Julinová et al., 2012; Julinová et al., 2018; Vaňharová et al., 2017; Trimpin et al., 2001). However, polymers based on natural occurring substances ('bio-based') constitute the majority of pharmaceutical excipients. This includes in particular cellulose derivatives, such as Hydroxypropyl Methyl Cellulose (HPMC) and Carboxy Methyl Cellulose (CMC). They represent one of the broadest classes of 'bio-based' PEs within pharmaceutical dosage forms (Klein, 2009). Importantly, however, 'bio-based' does not necessarily imply environmental biodegradability (Kwon et al., 2023). Despite their large production volumes in the hundred thousand tons range (Thielking and Schmidt, 2006) and widespread use in various industrial sectors, including the pharmaceutical industry, environmental (bio)degradation remains largely unexplored. In addition, available data on cellulose derivatives with pharmaceutical application are mainly limited to Microcrystalline Cellulose (MCC) Cellulose Acetate (CA) and CMC, which have demonstrated environmental biodegradability (Menzies et al., 2023; Komarek et al., 1993; Van Ginkel and Gayton, 1996). It is well-documented that the biodegradation of the latter two cellulose derivatives declines as their derivatization, i.e., degree of substitution (DS), increases (Erdal and Hakkarainen, 2022). Yet, a systematic study to assess biodegradation of cellulose derivatives is missing. In this study, we used standardized conditions in line with the OECD 301 guidelines to determine environmental biodegradation of 14 pharmaceutical grade cellulose derivatives (Table 1). We employed a modified version of the closed bottle test (CBT, OECD 301D) (OECD, 1992; Friedrich et al., 2012) and the manometric respiratory test (MRT, OECD 301F) (OECD, 1992). This provided novel insight into biodegradation potential and behavior of modified celluloses under different 301 test conditions and revealed structure-biodegradability relationships.

2. Materials and methods

2.1. Chemicals

Table 1 lists the cellulose derivatives used along with their chemical structure, chemical name, trade names, substituent content(s) in percent, degree of substitution (DS), molar substitution (MS) and chemical formulas. Viscosity data and molecular weights of the analyzed cellulose derivatives can be found in the supplementary material (Table S1). Five different viscosity grades of Hydroxypropyl Methyl Cellulose (HPMC) and one viscosity grade of Methyl Cellulose (MC) were obtained from Colorcon Ltd. (Dartford, UK). Hydroxypropyl Cellulose (HPC) in the grades L, SL and M were provided by Nisso Nippon Soda Co., Ltd. (Tokyo, Japan). The Hydroxyethyl Cellulose (HEC) grade Natrosol 250 HX pharm was obtained from Ashland Industries Nederland B.V. (Zwijndrecht, Netherlands). Ethyl Cellulose (EC) Ethocel Standard 10 FP Premium was purchased from Nutrition&Biosciences Ltd. (Beaminster, UK). Hydroxypropyl Methylcellulose Acetate Succinate (HPMCAS) was obtained from Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). CarboxymethylCellulose (CMC) cross-linked and Microcrystalline Cellulose type 101 (MCC) were obtained from JRS pharma (Rosenberg, Germany). CMC linear was purchased from Ashland. Sodium azide, sodium acetate and N-allylthiourea were purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Evaluation of ready biodegradability

The environmental biodegradability was investigated employing the CBT (OECD, 1992) and the MRT (OECD, 1992), the latter in two different variants using different inoculum sources. The inoculum used for the CBT derived from secondary effluent of a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325,000 eq. inhabitants). The MRT, on the other hand, was performed with both activated sludge and secondary effluent. Substances that did not pass the CBT test were submitted to MRT using secondary effluent. Substances neither biodegrading in the CBT nor the first MRT were tested in the MRT using activated sludge (MRT-AS). Our experimental design pursues a gradual increase in the probability of biodegradation from the CBT to the MRT-AS, firstly by increasing the inoculum volume and secondly, the microbial diversity with AS (Table S2). The importance of microbial diversity and density for biodegradability was demonstrated in a previous study, where a reduced total cell number accompanied by reduced detectable diversity led to a decrease in 4-nitrophenol biodegradation (Goodhead et al., 2013). Due to the polydisperse nature and heterogenous substitution pattern of the cellulose derivatives assessed, calculation of the theoretical oxygen demand (ThOD) was not possible. Therefore, their biodegradation rate was calculated as the ratio of biological oxygen demand (BOD) to the chemical oxygen demand (COD). The COD values were measured by a reaction kit, i.e., Merck Spectroquant® photometric COD cell tests in the range of 5–80 mg L^{-1} according to DIN ISO 15705. For water-insoluble

cellulose derivatives, we directly transferred the test substance in the COD cells with a concentration range of 300–3500 mg L^{-1}. Table S3 presents a comparison between experimentally determined COD values and ThOD calculations for the cellulose derivatives. The ThOD values were calculated approximately by assuming an endless chain and using the single building block (monomer) as the basis, as previously described (Kümmerer et al., 2011).

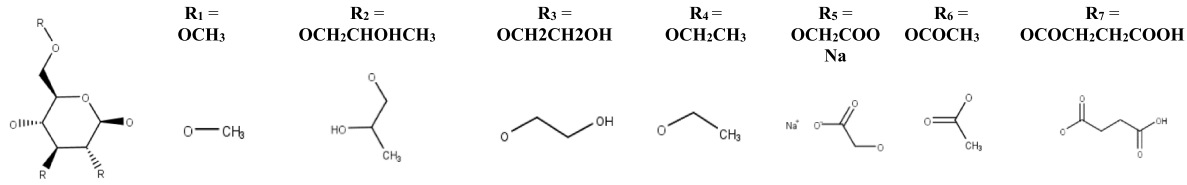
The pass levels to be classified as readily biodegradable are at least 60 % of the measured chemical or calculated theoretical oxygen demand meeting the required 10-day window (OECD, 1992). In addition, the biodegradation of the toxicity test containing both the reference compound and the test substance must exceed 25 % within 14 days (OECD, 1992).

According to the OECD guidelines, the ready biodegradability of the test compound was investigated in closed flasks at a constant temperature (20 ± 1 °C) in the dark within 28 days (OECD, 1992). In Table S2 the different test conditions are summarized (Table S2). Biodegradation of less than 5 % within 28 days was defined as 'no biodegradation'.

2.2.1. Closed bottle test (OECD 301D)

A solution of the PE corresponding to a chemical oxygen demand (COD) of 5 mg L^{-1} prepared in a mineral medium according to OECD guidelines was inoculated with secondary effluent (OECD, 1992). The inoculum concentration of 200 μL L^{-1} was selected to ensure that the oxygen consumption in the blank flasks after 28 days remains below 1.5 mg dissolved oxygen L^{-1}according to the OECD guidelines (OECD, 1992). The test system consisted of "blank", "quality control" using sodium acetate as a readily biodegradable substance, the "test series" and the "toxicity controls". An optode-based technique (Fibox 3, PreSens, Regensburg, Germany) was used to monitor the degree of aerobic biodegradation by measuring oxygen concentration at each day (modified CBT) (Friedrich et al., 2012).

Table 1
Structures of cellulose derivatives including the amount of derivatization in [%] expressed in terms of DS (degree of substitution) and MS (molar substitution).



Chemical name	Trade name	Content [%] *	DS [a]	MS [b]	Chemical formula
Hydoxypropyl methyl cellulose	Methocel K3 LV	R_1 22.9 R_2 7.7	1.3	0.2	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel K100 LV	R_1 22.9 R_2 8.7	1.3	0.3	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel K4M	R_1 22.5 R_2 7.6	1.3	0.2	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel K100M	R_1 21.8 R_2 9.9	1.3	0.3	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel E5 LV	R_1 29.5 R_2 9	1.7	0.3	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydroxypropyl cellulose	Nisso HPC SL	$R_2$70.2		6.6	$[C_6H_7O_2(OH)_x(R_2)_y]_n$
Hydroxypropyl cellulose	Nisso HPC L	R_2 72.5		7.4	$[C_6H_7O_2(OH)_x(R_2)_y]_n$
Hydroxypropyl cellulose	Nisso HPC M	R_2 73.5		7.8	$[C_6H_7O_2(OH)_x(R_2)_y]_n$
Hydroxyethyl cellulose	Natrosol 250 HX pharm	R_3 46.8		3.2	$[C_6H_7O_2(OH)_x(R_3)_y]_n$
Ethyl cellulose	Ethocel 10 FP	R_4 48.4	2.5		$[C_6H_7O_2(OH)_x(R_4)_y]_n$
Hydroxypropyl methyl cellulose acetate succinate	Hypromellose acetate succinate	R_1 22.2 R_2 6.2 R_6 7.9 R_7 14.7	1.3 0.2	0.2 0.2	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z(R_6)_m(R_7)_i]_n$
Carboxymethyl cellulose (linear)	Carmellose	not specified	0.8		$[C_6H_7O_2(OH)_x(R_5)_y]_n$
Carboxymethyl cellulose (cross-linked)	Croscarmellose	not specified	0.7		$[C_6H_7O_2(OH)_x(R_5)_y]_n$
Methyl cellulose	Methocel A4C	R_1 29.8	1.7		$[C_6H_7O_2(OH)_x(R_1)_y]_n$

* Certificates of analysis

[a] DS = Degree of substitution

[b] MS = Molar substitution

2.2.2. Manometric respiratory test (OECD 301F)

The test is performed with higher inoculum density and test concentration compared to the CBT (Table S2). For each test compound two test bottles, one toxicity and one sterile control and three blank and three quality controls were run. The sterile control contained sodium azide (320 mg L^{-1} NaN_3) to monitor abiotic degradation. The concentration of each test substance corresponded to a COD of 30 mg L^{-1}. All test bottles were inoculated with an aliquot of effluent derived from a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325,000 eq. inhabitants). To 1 L of medium 80 mL of inoculum were added resulting in a higher bacterial density in each test bottle compared to the CBT (Table S2). The OxiTop® (OC110-system, WTW GmbH, Weilheim, Germany) was used as measuring system. Biodegradation was determined by measuring the negative pressure in the closed bottle system that occurs when oxygen is taken up by the microbial population (biological oxygen demand, BOD) to transform organic carbon into CO_2 as described elsewhere in more detail (Trautwein and Kümmerer, 2011).

2.2.3. MRT with activated sludge (MRT-AS)

An additional MRT was performed utilizing 30 mg suspended solids L^{-1} of activated sludge from the same sewage treatment plant as mentioned above. The sludge was washed three times to lower the content or organic matter.

3. Results and discussion

3.1. Biodegradability

In this study, we monitored the ready biodegradability of 14 pharmaceutical grade cellulose derivatives and MCC. All biodegradation curves are available in the supplementary material (Fig. S1, Fig. S2, Fig. S3). For all tests, the validity criteria were met according to the OECD guidelines (OECD, 1992). In all studies, the positive control, sodium acetate, reached > 60 % mineralization by day 14 indicating inoculum activity and meeting test validity criteria (OECD, 1992). The biodegradation extents of all toxicity controls exceeded 25 % indicating no general toxicity to the inoculum of the test compounds (OECD, 1992). In all OECD 301D studies the oxygen uptake values of the blank flasks were below 1.5 mg L^{-1} following OECD requirements (OECD, 1992). The inoculum blanks of all 301F tests reached < 60 mg L^{-1} oxygen uptake at day 28 and over the duration of the study aligning with the OECD criteria (OECD, 1992). For all OECD 301 studies, the replicate variability of biodegradation extent at day 28 was < 20 % meeting the OECD 301 validity criteria (OECD, 1992). The obtained biodegradation results are summarized in Table 2. We found that MCC, tested in the MRT only due to its insolubility in water, biodegraded rapidly reaching 92.3 ± 3.3 % with secondary effluent and 88.1 ± 4.0 % with AS at day 28 (Fig. 1, Table 2). All cellulose derivatives investigated were classified as 'not readily biodegradable' according to the guidelines (Table 2) (OECD, 1992). Virtually no biodegradation (< 5 %) was observed for all cellulose derivatives tested in the CBT with secondary effluent. Similarly, in the MRT with secondary effluent, i.e., increased microbial density and thus diversity (Table S2), most modified celluloses showed no biodegradation (Table 2). Exceptions to this were HPMCAS (17.2 ± 2.6 %), CMC cross-linked (18.4 ± 3.3 %) and CMC linear (14.3 ± 2.6 %) (Fig. 2A, Table 2). For the Methocel K group (HPMC) only, the MRT-AS yielded a readily detectable increase in biodegradation (Fig. S3A, Table 2). The low viscosity grades, K3 and K100 LV (Table S1) yielded the highest biodegradation values of 21.4 ± 6.6 % and 15.8 ± 8.0 %, respectively. Methocel K100 M (highest viscosity grade) (Table S1) showed the lowest biodegradation rate of 11.2 ± 2.7 % within this group (Table 2). The molecular weight corresponds to the viscosity of each derivative within the Methocel K group (Table S1). The results obtained for the Methocel K group showed decreasing biodegradation with increasing molecular weight (reflected in increasing viscosity) suggesting that higher molecular weight compounds in this group hinder biological degradation.

The lack of biodegradation (< 5 %) observed for all cellulose derivatives in the CBT can most likely be attributed to the low microbial density and reduced diversity. The significant impact of inoculum source on biodegradation is supported by a previous study in which 25 %

Table 2
Biodegradability of cellulose derivatives in OECD 301D (secondary effluent), OECD 301F (secondary effluent) and OECD 301F AS (activated sludge). Presented as an average of replicate systems (n ≥ 2) with ± showing standard deviation.

	OECD 301 D	OECD 301 F	OECD 301 F AS	Results according to OECD
Biodegradation level [% COD]				
MCC	n.a.	92.3 ± 3.3 [a]	88.1 ± 4.0 [a]	readily biodegradable
Methocel K3 LV	−2.7 ± 0.9 [b]	−11.9 ± 8.0 [a]	21.4 ± 6.6 [b]	not readily biodegradable
Methocel K100 LV	2.1 ± 1.3 [a]	−18.6 ± 0 [a]	15.8 ± 8.0 [a]	not readily biodegradable
Methocel K4M	−2.7 ± 1 [b]	−5.5 ± 2.1 [a]	13.8 ± 2.6 [b]	not readily biodegradable
Methocel K100 M	−2.1 ± 0.7 [b]	3.8 ± 7.3 [a]	11.2 ± 2.7 [b]	not readily biodegradable
Methocel E5 LV	−2.7 ± 2.6 [b]	−9.2 ± 0.7 [a]	7.6 ± 11.9 [b]	not readily biodegradable
HPC SL	0.5 ± 2.3 [a]	−13.7 ± 10.6 [a]	−7.4 ± 6.0 [b]	not readily biodegradable
HPC L	−5.6 ± 1.7 [b]	−10.7 ± 1.4 [a]	2.5 ± 4.0 [b]	not readily biodegradable
HPC M	1.3 ± 1.4 [a]	−17.9 ± 4.7 [a]	−1.8 ± 0.7 [b]	not readily biodegradable
Hydroxyethyl Cellulose	3.9 ± 3.9 [a]	−12.9 ± 1.4 [a]	−5.0 ± 2.6 [a]	not readily biodegradable
Methyl Cellulose	0.4 ± 0.9 [a]	−17.1 ± 2.1 [a]	−8.7 ± 5.4 [a]	not readily biodegradable
Hydroxypropyl Methyl Cellulose Acetate Succinate	n.a.	17.2 ± 2.6 [a]	6.4 ± 2.8 [a]	not readily biodegradable
Ethyl Cellulose	n.a.	−13.7 ± 2.6 [a]	2.1 ± 6.6 [a]	not readily biodegradable
CMC linear	−3.3 ± 2.9 [a]	14.3 ± 2.6 [a]	12.3 ± 9.4 [b]	not readily biodegradable
CMC cross-linked	n.a.	18.4 ± 3.3 [a]	20.4 ± 9.4 [a]	not readily biodegradable

n.a. = not applicable (water-insoluble test compounds).
[a] n = 2.
[b] n = 4.

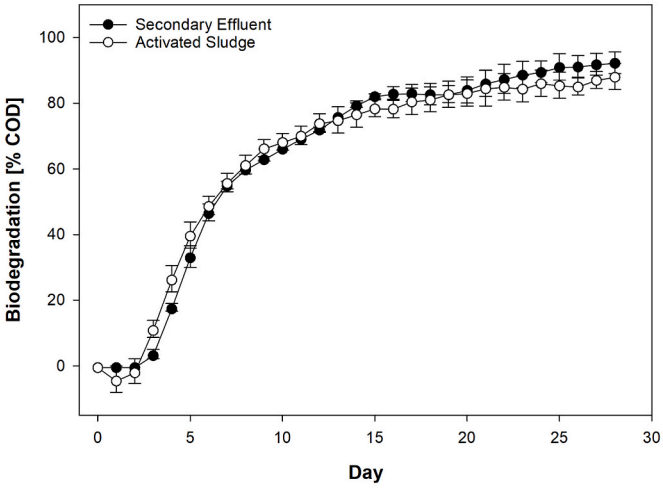


Fig. 1. Biodegradation of MCC in an OECD 301F. Presented as an average of replicate systems (n = 2) with error bars showing standard deviation.

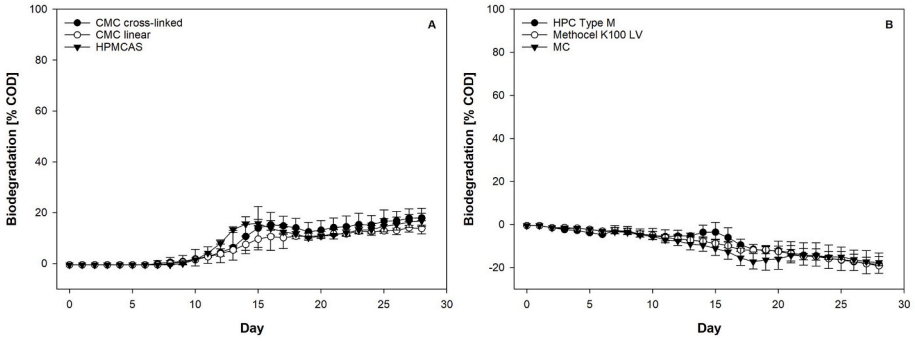


Fig. 2. Biodegradation of cellulose-based polymers in an OECD 301F using secondary effluent inoculum A) CMC cross-linked, CMC linear, HPMCAS B) HPC type M, Methocel K100 LV, MC. Presented as an average of replicate systems (n = 2) with error bars showing standard deviation.

biodegradation of a CMC polymer with DS of 0.7 (in our study: 0.8) was observed in a CBT with AS after 28 days (Van Ginkel and Gayton, 1996). In contrast, our findings revealed no biodegradation in the CBT with secondary effluent (Table 2). This suggests that under the CBT test conditions used in our experiments, a sufficiently high biomass of competent degraders may not have been reached to enable CMC biodegradation within the 28-day period. In addition, given that cellulose-based polymers cannot easily be taken up into bacterial cells due to their size, a sufficiently high production of exoenzymes catalyzing depolymerization is needed prior to microbial uptake and intracellular degradation. This probably involves microbial adaptation processes to CMC, which additionally may contribute to the extended lag phase in comparison to the prior CBT study (Van Ginkel and Gayton, 1996). Consistent with this hypothesis are our MRT and MRT-AS results obtained for a subset of cellulose derivatives comprising CMC polymers (linear and cross-linked), HPMCAS, and Methocel K derivatives, which showed a considerable increase in biodegradation compared to the CBT (Table 2). In contrast to CBT, both tests have a substantially higher bacterial diversity and density in their initial phase, increasing the likelihood that suitable degraders will be present at the critical mass needed for measurable degradation within the 28-day period.

3.2. *Negative deflection in MRT*

A striking feature of the MRT biodegradation curves with secondary effluent observed for most of the cellulose derivatives tested was their negative deflection after day 12. HPC type M, MC and Methocel K100LV (HPMC) reached the highest negative biodegradation values of -17.9 ± 4.7 %, -17.1 ± 2.1 % and -18.6 ± 0 % respectively, at day 28 (Fig. 2-B–Table 2). The negative biodegradation curve is a result of subtracting the blank respiration of the inoculum ('baseline respiration') from the oxygen uptake values of the test substance. This negative deflection indicates that endogenous oxygen-consuming processes in the inoculum may be inhibited, at least in part as respiration values were below baseline respiration. Under our experimental conditions, endogenous respiration of the microorganisms (MO) increases significantly from day 12 (blank flask) suggesting that nitrification processes take place while ammonium is oxidized from the added growth medium (Fig. 3). To support this hypothesis, we conducted a MRT with secondary effluent with the nitrification inhibitor, ATU as the only test substance to measure the inhibition of oxygen uptake. We found that the oxygen uptake rates of ATU decreased significantly from day 12 onwards after subtracting the baseline respiration values (Fig. 3A). This decrease in oxygen consumption from day 12 onwards, after subtracting baseline respiration was observed for 10 out of 14 cellulose derivatives, as shown, for example, for Methocel K100LV in Fig. 3B. Thus, either nitrification processes and/or growth processes of nitrifying bacteria may be disturbed by the presence of these compounds. Nitrifying bacteria are known to be highly sensitive to nitrification inhibition by the presence of toxic compounds (Chhetri et al., 2022).

A possible explanation for the decline in metabolic activity may be the accumulation of non-metabolizable monomeric units after enzymatic cleavage in bacteria. Alpha-methyl glucoside (αMG) is an example of a non-metabolizable glucose analog (Pikis et al., 2006). Non-metabolizable sugar compounds can accumulate intracellularly after uptake by the bacterial phosphotransferase system (Pikis et al., 2006). Accumulation of sugar-phosphates, which can be taken up but not further metabolized like αMG, has been shown to be toxic to bacteria and inhibit their growth (Richards et al., 2013). However, our data do not establish whether extracellular fragmentation occurs and thus the issue remains unsolved. Extracellular cleavage is primarily influenced by the type of the substituent and the degree of substitution (DS) or molar substitution (MS), which affect the accessibility to enzymatic fragmentation (Richardson and Gorton, 2003). In general, lower DS/MS values are associated with a higher enzymatic degradation rate (Erdal and Hakkarainen, 2022). MC is the derivative with the smallest substituent group for which enzyme accessibility and thus fragmentation was expected to be high. MC can be effectively enzymatically cleaved at

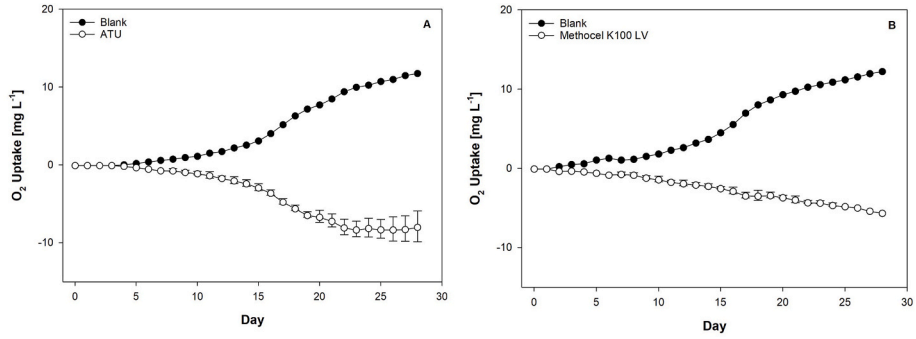


Fig. 3. O_2 Uptake [mg L^{-1}] in an OECD 301F using secondary effluent inoculum A) ATU B) Methocel K 100LV. Presented as an average of replicate systems (n = 2) with error bars showing standard deviation.

DS values of up to 2.1 (Saake et al., 1998). In our study, MC with an average DS of 1.7 was investigated. Accordingly, enzyme-induced chain scission is highly likely to occur. A study conducted by Dow Chemical Co. reported 73 % $^{14}CO_2$ conversion of MC (DS ~ 1.9) with AS obtained from the Dow chemical Co. wastewater treatment plant (Blanchard et al., 1976). This finding contrasts with the 'negative degradation percentages' obtained in our study with MC (DS ~ 1.7). A possible explanation, for this apparent discrepancy is that the activated sludge used in the Dow Chemical study, was pre-adapted to the compound due to high exposure to MC and related compounds in the wastewater treatment plant. The impact of microbial adaptation on biodegradation has been described previously/is well known (Poursat et al., 2019). It is important to note that pre-adaptation of the inoculum prior to biodegradability testing will invalidate results according to OECD test guidelines (OECD, 1992). Enzymatic depolymerization has been demonstrated for highly substituted HEC (MS > 3) and HPC derivatives (Cheroni et al., 2012). Thus, taking into consideration these previous findings, partial fragmentation of the modified cellulose backbone cannot be ruled out in our experimental conditions. Consequently, this may have led to the formation of nonmetabolizable degradation products with potential adverse, i.e., toxic effects on MO (see also above).

3.3. Potential biodegradable cellulose derivatives

In contrast to the 'negative degradation percentages' of the Methocel K group obtained from the MRT with secondary effluent, they showed a significant increase in mineralization rate with activated sludge (Table 2). This indicates their potential for biodegradation in presence of activated sludge containing competent microbial degraders. Like MC (DS ~ 1.7), the analyzed Methocel K derivatives have predominantly methyl content (DS ~ 1.3), albeit to a lesser extent (Table 1). Their MS values (hydroxypropyl) of ~ 0.2–0.3 can be considered low in comparison to HPC (MS ~ 7) and HEC (MS ~ 2.5) (Table 1). The reduced DS and lower MS values compared to the mentioned cellulose derivatives explain the observed increase in biodegradation for the Methocel K group. In addition, we found that the degradation value of Methocel E5 LV, despite lower viscosity and hence molecular weight compared to Methocel K100LV, K4M and K100 M (Table S1) but with a higher degree of methyl group functionalization (DS ~ 1.7), exhibited the lowest degradation of 7.6 ± 11.9 among the HPMC derivatives in MRT-AS (Table 2). This suggests that the degree of polymerization (DP), represented by viscosity, may have a lesser influence on biodegradability compared to the extent of substitution. However, as discussed in the 'biodegradability' section for the Methocel K group, molecular weight might still have an influence on biodegradability. The fact that no plateau was reached after 28 days for the Methocel K group indicates that the degradation process was still ongoing, and the MO had not achieved complete biodegradation of HPMC derivatives within this period. This suggests that HPMC-degrading MO may need a longer adaptation time and have slower growth rate under the test conditions evaluated. Other cellulose derivatives for which partial degradation was observed and was not complete after 28 days (no plateau reached) are the CMC polymers and HPMCAS (Fig. 2A). The results of this study for linear and crosslinked CMC (DS 0.8 and 0.7) are in good agreement with the findings of a recently published study (Menzies et al., 2023). In this study, a CMC polymer with a DS of 0.6 was subjected to an 'extended' OECD 301 B test (for 148 days) and reached 20 ± 2.4 % $ThCO_2$ after 28 days and ultimately 70 ± 2.3 % (Menzies et al., 2023). This partial biodegradation is well reflected in our results from the MRT where we achieved 14.3 ± 2.6 % for linear CMC and 20.4 ± 9.4 % for cross-linked CMC (Fig. S2C, Fig. S3C, Table 2). This strongly indicates the ability of MO to utilize carboxymethylated oligomers as a carbon source after an extended adaptation phase. However, it remains unclear whether test extension would result in a plateau of incomplete degradation or of full mineralization. HPMCAS contains both ester (acetate and succinate) and ether substituents (methyl and hydroxypropyl) (Table 1). Ester linkages are commonly known to be more easily hydrolyzed than ether linkages and generally increase biodegradability (Boethling et al., 2007). The increase in biodegradation due to the interplay of esterase activity and cellulose degrading enzymes has been found in previous studies for cellulose acetate (Yadav and Hakkarainen, 2021). Hence, the increased biodegradation of HPMCAS could be due to the existence of esterase-enzymes, facilitating the initial steps of degradation, and exposing the cellulose backbone to further enzymatic degradation.

3.4. Classification of biodegradability

Based on the collective data presented here, we suggest that the extent of degradation and the features of the biodegradation curves can serve as a framework to categorize the biodegradability of the studied modified celluloses into two distinct groups (Table 3). The first group (Group I) had not reached a plateau phase indicating possible ongoing biodegradation. The components of Group I can be further classified into the subgroups 'moderately' (Ia) and 'slightly/weakly' biodegradable (Ib) based on the percentage of biodegradation as previously proposed (Beiras and López-Ibáñez, 2023). In contrast, the second group (Group II) displayed a negative deflection or negligible degradation percentages (< 5 %) with an evidenced plateau phase. Thus, given lack of biodegradation in the OECD 301 screening assays, our results suggest that degradation of Group II compounds may also not occur in surface water with low bacterial density and diversity. Therefore, we recommend classifying these compounds as 'non-biodegradable' (Table 3).

Furthermore, the relationships described here between the biodegradability and factors such as DS, MS, DP, and the increase in ester linkages (see section 'potential biodegradable celluloses') allow for an extended scoring system of cellulosic excipients beyond the initial Group I/Group II categorization (Fig. 4; biodegradability is color-coded ranging from green 'readily biodegradable' to red 'non-biodegradable'). An important benefit of the traffic light scoring system is highlighted by recent data demonstrating that test extension yielded higher mineralization of polymers, indicating the inadequacy of the 28-day test duration in fully assessing biodegradation extent (McDonough et al., 2023). The scoring system allows to make predictions on which compounds are likely to undergo further biodegradation with extended test period. For example, Group I categorized compounds are likely to undergo further degradation as, indeed, has been described for CMC (Menzies et al., 2023), whereas an extension of Group II molecules may not result in further degradation. However, it is important to emphasize that one should not generally assume that additional biodegradation will occur within a longer test period. Considering the potential challenges associated with extending the testing duration, which significantly increases the time and resources required for the assessment process, the scoring system offers valuable insights in particular for both industry and regulators. Our research has demonstrated the suitability of using the '28-day OECD 301 based classification approach' (Fig. 4, Table 3) as an aid in determining whether an extended period will lead to further degradation. Moreover, it provides an initial method to prioritize PEs in pharmaceutical formulations, considering their potential environmental persistence.

4. Conclusions

To the best of our knowledge, this study is the first comparison of biodegradability data for the cellulose derivatives most used in pharmaceuticals. The data were acquired under standardized conditions and using the same source of inoculum. The outcome has laid the groundwork for a biodegradability classification system. It provides several benefits including a decision-making tool in the selection of both further investigations of biodegradability and for incorporation into pharmaceutical formulations. Moreover, the traffic light system can aid the selection of structures that may serve as potential candidates for the rational design of variants with increased biodegradability, following

Table 3
Biodegradability classification of cellulose derivatives according to OECD 301 results.

Readily biodegradable ≥ 60 %	Moderately biodegradable 20 - 59 %	Slightly biodegradable 5 - 19 %	Non biodegradable < 5 %
MCC	Methocel K3 LV CMC cross-linked	Methocel K100 LV Methocel K4M Methocel K100M Methocel E5 LV HPMCAS CMC linear	HPC SL HPC L HPC M HEC MC EC
	Group Ia	**Group Ib**	**Group II**

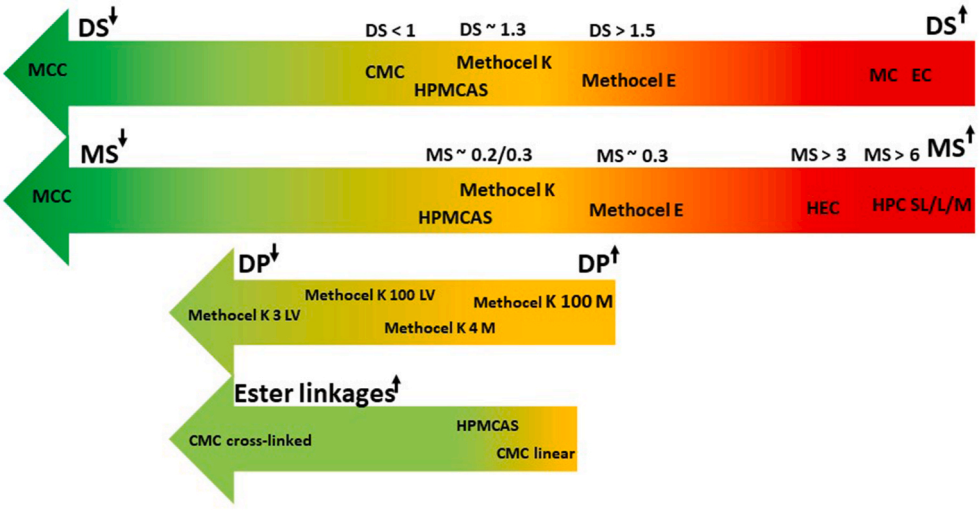


Fig. 4. Color-coded relationship between biodegradability and DS (degree of substitution), MS (molar substitution), DP (degree of polymerization), Ester linkages of cellulose derivatives.

the principle of 'benign by design'.

CRediT authorship contribution statement

Mila Bading: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Oliver Olsson:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Klaus Kümmerer:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141298.

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Publication 2

Mila Bading, Oliver Olsson, Klaus Kümmerer (2024)

Assessing the aquatic biodegradation potential of polymeric excipients for pharmaceutical formulation

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Assessing the aquatic biodegradation potential of polymeric excipients for pharmaceutical formulation

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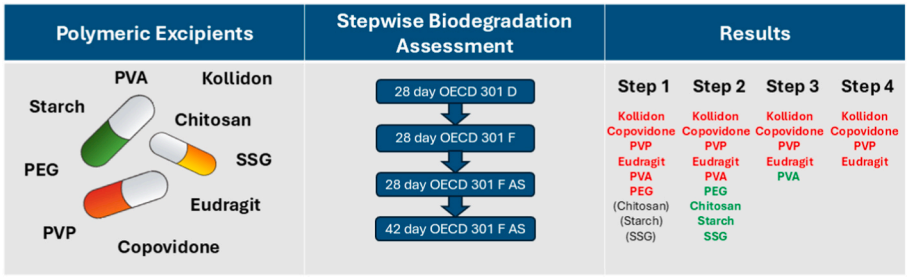
HIGHLIGHTS

- Biodegradation assessment of ten polymeric excipients using OECD 301 standard tests.
- Variations in biodegradation extents of PEG and PVA under different test conditions.
- Environmental persistence identified in five out of the ten polymeric excipients.
- Prolonged tests for 'non-biodegradable' compounds did not increase biodegradation.

GRAPHICAL ABSTRACT



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Nitrification

ABSTRACT

Polymeric excipients (PEx) are essential in drug formulation but raise environmental concerns upon wastewater release post-administration due to their potential detrimental effects to life-histories of freshwater vertebrates and invertebrates. Ten pharmaceutical polymeric compounds were assessed in a stepwise environmental biodegradation assessment according to standard OECD 301 guidelines to thoroughly evaluate biodegradability of these compounds. Polyvinyl alcohol (PVA), polyethylene glycol (PEG), chitosan, maize starch, and sodium starch glycolate (SSG) were found to be 'readily biodegradable,' although PVA and PEG showed variation across employed test systems. PEG and PVA did not degrade in OECD 301D tests having low microbial density and diversity. In contrast, in the OECD 301F tests i.e., higher microbial density and diversity, PEG exhibited 73.0 ± 3.3 % biodegradation, while PVA showed 91.2 ± 8.0 % biodegradation with secondary effluent and activated sludge, respectively. Polyvinyl pyrrolidone (PVP), Copovidone, Kollidon CL, and Eudragit derivatives EPO and L100-55 were categorized as 'non-biodegradable' (< 10 % biodegradation). No increase in degradation was observed after 42 days. This indicates their environmental persistence. This study lays the groundwork for a comprehensive understanding of the biodegradation potential of pharmaceutical polymers. It considers the influence of test conditions, inoculum sources, and compound characteristics. The environmental persistence of certain PEx underlines the urgent need to use more environmentally biodegradable alternatives in drug formulation.

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1. Introduction

Polymers are key formulation excipients for pharmaceutical drug products. Pharmaceutical excipients are ingredients that are considered pharmacologically inert and play crucial roles in drug formulations. Their functionalities range from their use as binders, disintegrants, and diluents to coatings, among others (Debotton and Dahan, 2017). The environmental risks associated with water-soluble polymers (WSPs) have recently gained attention due to concerns of their persistence and toxicity in freshwater ecosystems, however their environmental impact is still largely unexplored. (Arp and Knutsen, 2020; Huppertsberg et al., 2020; Albright and Chai, 2021; Robison-Smith et al., 2024). The WSPs polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyacrylic acid (PAA) and polyethylene glycol (PEG) are extensively utilized as excipients by pharmaceutical industry (Debotton and Dahan, 2017). Apart from their use in the pharmaceutical industry, these polymers find applications in various sectors such as personal care products, cosmetics, agrochemicals, household cleaning products, and water treatment (Nigro et al., 2023). The production volume of WSPs in Europe can be estimated by examining the annual production of the respective monomers, as these monomer products, mainly used in polymer synthesis, are registered under REACH (Huppertsberg et al., 2020). In Europe, the estimated production volumes exceed 10^3 tons per year (PVP) (ECHA (I)), between 10^5-10^6 tons per year (PVA and PAA) (ECHA (II); ECHA (III)) and more than 10^6 tons per year (PEG) (ECHA (IV)). Despite extensive industrial applications of polymeric excipients (PEx), quantitative data on their environmental concentrations are currently scarce (Huppertsberg et al., 2020). Available studies revealed PVP concentrations ranging between 0.9 mg L^{-1} and 7 mg L^{-1} in effluents from wastewater treatment plants, as well as concentrations around 0.1 mg L^{-1} in river water (Antić et al., 2011). In addition, polyethylene oxide (PEO) concentrations in effluent and surface water samples have been recently reported to reach up to 20 μg L^{-1} in effluent and concentrations exceeding 1 μg L^{-1} in surface water samples (Pauelsen et al., 2023). The ubiquitous presence of water-soluble polymers in the environment raises concerns about their potential ecotoxicological effects (Nigro et al., 2023; Robison-Smith et al., 2024). A recent study reported reproductive impairment in the aquatic organism *Daphnia magna* when exposed to PVP, PVA, PEG or to PAA at concentrations ranging from 5 to 10 mg L^{-1} (Mondellini et al., 2022). In addition, Nigro et al. observed ecotoxicological effects of PVP, PVA, and PAA on zebrafish embryos at three concentrations (0.001, 0.5, and 1 mg L^{-1}), calculated as expected environmental concentrations, by monitoring alterations in swimming behavior. Therefore, PEx that reach the aquatic environment pose potential risks to aquatic systems (Nigro et al., 2023).

Under the umbrella of the 'European Green Deal', the EU has implemented the 'Zero Pollution Action Plan for Air, Water and Soil' (EC, 2019; EC, 2021). As part of this action plan, the 'Chemicals Strategy for Sustainability' has been adopted, which catalyzed the current REACH revision to include the registration of polymers (EC, 2020). Further, the EU Taxonomy, a classification system identifying environmentally sustainable economic activities, is guiding companies towards aligning with green standards and fostering environmental responsibility. The pharmaceutical industry has recently come under the scope of this regulation, and in this context, the EU Taxonomy emphasizes the preference for the use of more biodegradable ingredients in drug products (EC, 2023). Against this background of the EU's policy at transitioning towards a toxic-free environment, it is necessary to provide standardized data on the biodegradation of PEx.

We recently carried out a comprehensive analysis in accordance with OECD 301 guidelines of cellulose-based polymers used in the pharmaceutical industry and developed a graded classification score of their biodegradability to lay the groundwork for aligning with the 'benign-by-design' concept (Bading et al., 2024). Building upon this work, our current study, aimed for a thorough biodegradation assessment focusing on non-cellulose-based polymers, which represent the most frequently used polymers in drug product development. We analyzed 10 different PEx including the vinyl polymers PVP, Copovidone, Kollidon, and PVA, along with Macrogol 6000 (PEG), the natural polymers chitosan, maize starch, its derivative sodium starch glycolate (SSG), and the polymethyl methacrylate polymers (PMMA) Eudragit EPO and Eudragit L100-55.

A recent multi-laboratory study (McDonough et al., 2023) demonstrated high reproducibility of OECD 301B and 301F test protocols for assessing polymer biodegradation, endorsing their suitability for polymeric substances. Among others, PEG and PVA were found to be readily biodegradable. While available data suggest that PVP may not biodegrade due to the lack of hydrolyzable or oxidizable functional groups in its carbon backbone, these data do not align with OECD 301 guidelines (Trimpin et al., 2001; Swift, 1994). However, the extent of PVP degradation may be influenced by factors such as the origin of activated sludge and the presence of other substrates (Julinová et al., 2012), rendering its biodegradability unclear. We included these polymers (PVA, PEG and PVP) to generate directly comparable biodegradation results, thereby establishing a consistent biodegradation dataset.

For this research study, we employed a stepwise method with sequentially conducted screening biodegradation tests, each incorporating increasing levels of microbial density and diversity. This approach aims not for authorization but provides in-depth insight into biodegradation performance and allows for the comparison of results obtained under different test conditions. By varying the size and source of the inoculum, we will gain insight into possible mechanisms influencing probability and extent of biodegradation (Bading et al., 2024).

This comprehensive systematic analysis of biodegradability according to different OECD 301 protocols supports the selection of environmentally benign excipients and promotes the development of pharmaceutical products with enhanced biodegradability, aligning with the 'benign-by-design' concept.

2. Materials and methods

2.1. Chemicals

Polyvinyl pyrrolidone, Kollidon CL (crosslinked polyvinyl pyrrolidone) and Kollidon VA 64 (Copovidone, polyvinyl pyrrolidone–vinyl acetate copolymer) were provided by BASF (Ludwigshafen, Germany). Macrogol 6000 (PEG) was purchased from Ter Hell & Co. GmbH (Hamburg, Germany). Polyvinyl alcohol 28–99 (degree of hydrolysis 99 %, MW ~ 130,000) was obtained from Merck Chemicals GmbH. Starch 1500 was provided by Colorcon Ltd. (Dartford, UK). Sodium starch glycolatewas purchased from Roquette (Lestrem, France). Eudragit EPO and Eudragit L100-55 were obtained from Evonik Operations GmbH (Essen, Germany). Sodium azide, sodium acetate and chitosan were purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Biodegradability in OECD 301D and OECD 301F tests

The assessment of biodegradation involved two OECD 301 screening tests, i.e., the optode-based (Fibox 3, PreSens, Regensburg, Germany) closed bottle test (CBT, OECD 301D) (OECD, 1992; Friedrich et al., 2012) and the manometric respirometry test (MRT, OECD 301F) using the OxiTop® system (OC110 system, WTW GmbH, Weilheim, Germany) (OECD, 1992). The latter was performed in two different variants using different inoculum sources. The analytical endpoint was oxygen consumption for both CBT and MRT. In addition, for soluble test compounds, the dissolved organic carbon (DOC) elimination (ASI-V autosampler, TOC-VCPN analyzer, Shimadzu, Germany) was monitored within the MRT test set-up after 28 days to confirm oxygen uptake results. As illustrated in Fig. 1, the experimental approach followed a stepwise procedure to increase the probability of biodegradation as follows. First, the test compounds were subjected to a CBT with secondary effluent and hence low bacteria density (step 1). It is important to note that the CBT is applicable to water-soluble test substances under

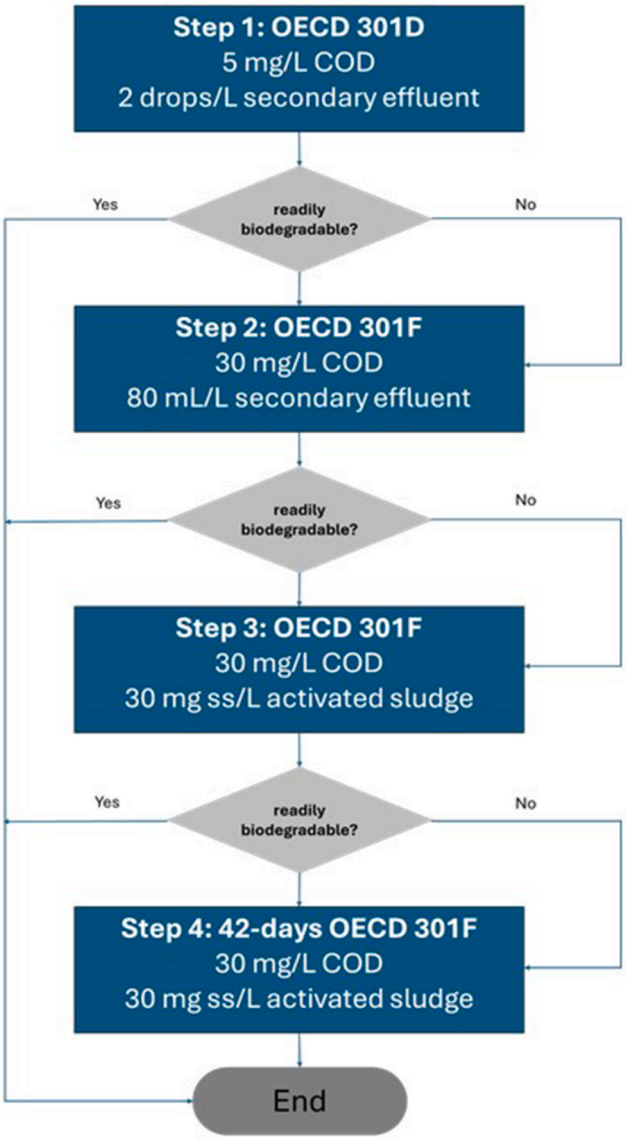

Fig. 1. Workflow of the biodegradability testing.

our test conditions. Substances that are not water-soluble were directly submitted to step 2. Substances that failed the CBT (step 1) were submitted to a MRT with secondary effluent and higher bacterial density (step 2). Substances neither biodegrading in the CBT nor in the MRT with lower bacterial mass were tested in a MRT with higher bacteria mass and diversity using activated sludge (MRT-AS, step 3). In addition, substances not meeting the ready biodegradability criteria in the MRT-AS over 28 days, were tested in an extended 42-day MRT (step 4). The CBT and MRT concentrations of the PEx were set at 5 mg L^{-1} chemical oxygen demand (COD) and 30 mg L^{-1} of COD, respectively (Fig. 1). These concentrations were determined through Merck Spectroquant® photometric COD cell tests in the range of 5–80 mg L^{-1} or 300–3500 mg L^{-1} according to DIN ISO 15705. Table S1 presents the determined COD values for the assessed PEx. The monomeric compounds used in this study included sodium acetate and N-methyl-2--pyrrolidone (NMP). Sodium acetate served as a reference compound, while NMP was included due to its structural similarity to the PVP monomer with respect to the presence of a lactam ring. For the monomeric compounds in this study, the initial concentrations were based on their theoretical oxygen demand (ThOD) and were set at 5 mg L^{-1} and 30 mg L^{-1} of ThOD for the CBT and MRT, respectively. The CBT and MRT were inoculated with 2 drops L^{-1} (~200 μL L^{-1}) and 80 mL L^{-1} of secondary effluent from a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325000 eq. inhabitants), respectively (Fig. 1). The secondary effluent was filtered through a filter paper before use. For the 28-day MRT-AS and 42-day MRT-AS 30 mg suspended solids L^{-1} of AS from the same treatment plant (see above) was utilized (Fig. 1). The sludge was washed three times with tap water before use to reduce the organic matter. The mineral media were prepared according to the corresponding OECD guidelines 301D and 301F (OECD, 1992). All tests consisted of 'blank', 'quality control' (sodium acetate), 'test series' and 'toxicity control'. The MRT also contained a 'sterile control' containing 320 mg L^{-1} NaN_3 to monitor abiotic degradation. CBT test runs were performed in parallel with two test bottles each for the blank value, quality control, test series and toxicity control. For MRT, each test compound was run in two test bottles, along with one toxicity control, one sterile control, and three blanks and three quality control bottles. Replicates of CBTs and MRTs ($n \geq 2$, as indicated in Table 1) were performed for each test compound. To obtain the 42-day MRT-AS, the duration of the test for two MRT-AS replicates was extended to 42 days to monitor prolonged biodegradation.

In all experiments, the pH values on days 0, 28, and 42 were measured to ensure they were within the range of 6.0–8.5, as required by the OECD guidelines (OECD, 1992). According to the OECD guidelines, the ready biodegradability of the test compound was investigated in closed flasks at a constant temperature (20 ± 1 °C) in the dark within the testing duration (OECD, 1992). Biodegradation extent was expressed as the ratio of biological oxygen demand (BOD) to COD. Biodegradation of less than 5 % at test end was defined as 'no biodegradation'. The lag phase was defined as time from start of a test until degree of biodegradation reached 10 %.

2.3. Classification of biodegradability

According to the comprehensive data gathered in this study, the PEx were categorized based on their degradation levels into the following subgroups as previously outlined: 'readily' in line with the OECD test guidelines (≥ 60 %), 'moderately' (20–59 %), 'slightly' (5–19 %), and 'non-biodegradable' (< 5 %) (Bading et al., 2024).

3. Results and discussion

3.1. Biodegradability according to OECD 301D and F

In our study, we investigated the environmental aquatic biodegradability of 10 pharmaceutical grade PEx. For all tests, the validity criteria were met according to the OECD test guidelines (OECD, 1992). None of the tested compounds was toxic to the inoculum according to the guidelines, biodegradation of the toxicity test vessels containing both the reference compound, and the test substance exceeded 25 % within 14 days (OECD, 1992). The obtained biodegradation results are summarized in Table 1. In the OECD 301D (closed bottle test, CBT) with secondary effluent as the inoculum source, we found no biodegradation (< 5 %) for any of the PEx tested (Table 1). Maize starch, chitosan, and SSG were not tested in the CBT due to their insolubility in water. The OECD 301F results revealed that Macrogol 6000 (PEG), PVA, maize starch, SSG, and chitosan classified as 'readily biodegradable' according to the OECD 301 test guidelines (OECD, 1992). By day 28 of testing, these compounds displayed oxygen consumption values > 60 % of COD. Importantly, the 10-day window criterion does not apply to polymers, as they are known to degrade sequentially (OECD, 2006). All other investigated PEx were classified as 'not readily biodegradable' (Table 1).

We observed a notable difference in the classification for 'readily biodegradability' of PVA and PEG between the OECD 301D and OECD 301F tests (Table 1). This suggests that under stringent CBT test conditions (10^4 - 10^6 approx. cells L^{-1}), a sufficiently high biomass of competent degraders may not have been reached. This could have

Table 1
Biodegradability of polymeric excipients in OECD 301D (secondary effluent), OECD 301F (secondary effluent) and OECD 301F AS (activated sludge). Presented as an average of replicates($n \geq 2$) with ± showing standard deviation.

	OECD 301D	OECD 301F	OECD 301F AS	42 d OECD 301F AS	Results according to OECD
	Biodegradation level [% COD]				
PVP	−0.2 ± 2.1 [b]	−4.0 ± 1.4 [a]	0.0 ± 1.4 [b]	1.8 ± 5.6 [a]	not readily biodegradable
Kollidon CL	n.a.	−4.4 ± 2.8 [a]	−6.7 ± 4.6 [c]	−0.8 ± 4.0 [a]	not readily biodegradable
Copovidone	−3.0 ± 2.6 [b]	−7.6 ± 4.7 [a]	−0.1 ± 3.9 [b]	0.8 ± 4.0 [a]	not readily biodegradable
Macrogol 6000 (PEG)	1.6 ± 1.4 [a]	73.0 ± 3.3 [a]			readily biodegradable
PVA	−2.9 ± 2.2 [b]	5.7 ± 3.3 [a]	91.2 ± 8.0 [a]		readily biodegradable
Maize starch	n.a.	69.2 ± 7.3 [a]			readily biodegradable
Sodium starch glykolate	n.a.	60.3 ± 10.6 [a]			readily biodegradable
Chitosan	n.a.	125.8 ± 0.0 [a]			readily biodegradable
Eudragit EPO	−2.1 ± 1.9 [a]	6.9 ± 5.2 [a]	−5.0 ± 6.3 [c]	−1.0 ± 9.5 [a]	not readily biodegradable
Eudragit L100–55	−1.5 ± 3.0 [a]	−3.7 ± 3.5 [c]	6.0 ± 4.7 [b]	6.5 ± 4.0 [a]	not readily biodegradable

n.a.=not applicable (water-insoluble test compounds).
[a] n=2.
[b] n=4.
[c] n=6.

prevented PVA and PEG biodegradation within the 28-day period. On the other hand, the OECD 301F tests contain higher levels of biomass (10^7 - 10^8 approx. cells L^{-1}) in their initial phase increasing the likelihood that suitable degraders will be present at the critical mass needed for measurable degradation within the 28-day period (OECD, 1992).

In the following sections, we will provide more details on the biodegradation potential of the different excipients using the different test set-ups (Fig. 1).

3.2. PVA and PEG mineralization: potential co-metabolic processes

No degradation was observed for either compound in the OECD

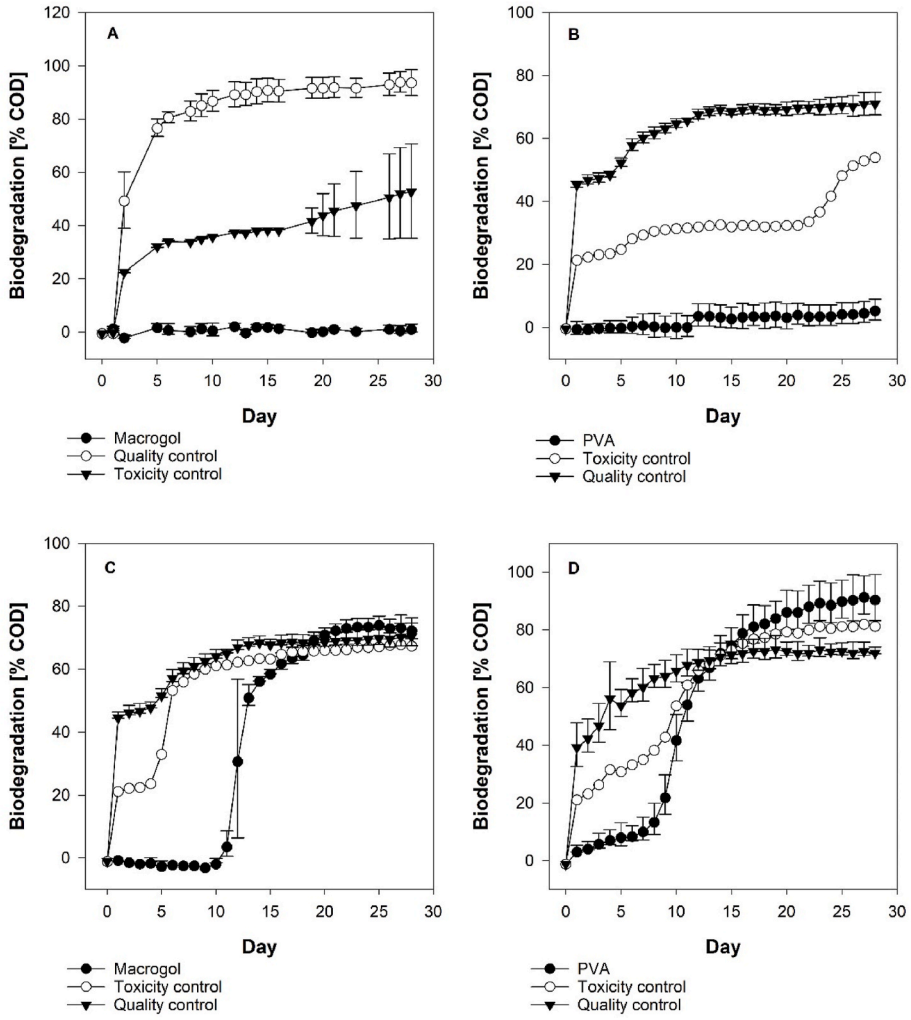

Fig. 2. Biodegradation of **A)** Macrogol (PEG) in an OECD 301D test **B)** PVA in an OECD 301F test **C)** Macrogol (PEG) in an OECD 301F test and **D)** PVA in an OECD 301F AS test. Presented as an average of replicates($n = 2$) with error bars showing standard deviation.

301D (CBT) (Table 1). Within the CBT toxicity vessels, however, PEG showed an increase in biodegradation from day 16 onwards (Fig. 2A). This observed increase in biodegradation in the toxicity flasks could be explained by two potential mechanisms, or a combination of both. Firstly, the observed increase could be linked to co-metabolic biodegradation by ammonia-oxidizing microorganisms (AOM). Several studies have observed co-metabolism of organic micropollutants along with ammonium oxidation (Kennes-Veiga et al., 2022). In our test set-up, nitrification processes (nitrification phase) typically occur between days 12 and 22 and are initiated by the oxidation of ammonium in the mineral salt medium (Bading et al., 2024). The presence of sodium acetate (reference compound) might have enhanced growth of nitrifying bacteria, thereby favoring co-metabolic biodegradation of PEG from day 16 onward with the onset of nitrification processes. Secondly, the availability of sodium acetate as an easily metabolizable substrate increased overall microbial growth, leading to the critical mass of competent strains required for biodegradation measurement in the CBT flasks. It is possible that both co-metabolic activities (ammonium oxidation) and enhanced microbial growth due to the presence of sodium acetate contributed to the observed increase in PEG biodegradation in the toxicity flasks. In this experiment, we noticed a high variability in the toxicity controls towards the end of the assessment period, as indicated by the high error bars showing standard deviation (Fig. 2A). One possible explanation for the apparent variability in the degree of biodegradation may arise from different onsets of the delayed nitrification processes. Nitrifying bacteria, which are known for their slow growth, typically begin nitrification towards the end of the testing period under our conditions (Bading et al., 2024; Chhetri et al., 2022). The precise starting point of this process within the 28-day test duration may vary and thus the length of this test period may not have been sufficient to fully capture these processes. This could account for the differing levels of PEG metabolism and co-metabolism observed.

Similar observations were noted for PVA in MRT with secondary effluent, where biodegradation increased from day 22 onwards in the toxicity flasks (Fig. 2B). The DOC removal of the toxicity control of 91.3 % (Fig. S1) confirmed full mineralization of PVA.

The subsequent OECD 301F tests (MRT and MRT-AS), i.e., increased microbial density and diversity, showed substantial biodegradation for PEG and PVA reaching 73.0 ± 3.3 % (96.2 % DOC removal, Fig. S2) and 91.2 ± 8.0 % (87.8 % DOC removal, Fig. S3) with secondary effluent and AS, respectively (Table 1). In the case of PEG within the MRT using secondary effluent, biodegradation increased rapidly after a 12-day lag phase. In addition, in the MRT toxicity flasks, the lag phase for PEG was reduced from 10 to 5 days (Fig. 2C). This reduction in lag phase could be attributed to the readily available sodium acetate facilitating the growth and establishment of a critical mass of PEG degraders. In summary, the lag phase duration decreased in the following order: CBT-toxicity vessel (19 days) > MRT-test vessel (12 days) > MRT-toxicity vessel (5 days).

PVA, on the other hand, achieved ultimate biodegradation (mineralization) in MRT with AS by day 28 after a lag phase of 7 days (Fig. 2D). In contrast, PVA biodegradation was neither observed in the CBT toxicity bottles nor in the MRT test bottles with secondary effluent as the inoculum due to lower microbial biodiversity and density compared to activated sludge. Consequently, the use of the CBT and MRT with secondary effluent as inoculum may be limited in assessing the full extent of biodegradation for PVA within the standard 28-day timeframe. The MRT-AS test system renders the test for ready biodegradability more potent and indicative of the biodegradation potential of PVA. The findings align well with a recent study evaluating different PEG and PVA materials biodegradation in an OECD 301B test setup with activated sludge. Low molecular weight PEG materials (MW 4000, 25,000, and 35,000) rapidly biodegraded, exceeding 70 % $ThCO_2$ in 28 days (Menzies et al., 2023). The lag time of the different PEG derivatives varied between 4 and 9 days and increased with higher MW (Menzies et al., 2023). However, the lag phase of 4 days determined in the study by Menzies et al. (2023) for the low MW of 4000 Da contrasts with our results from the MRT, which showed alag phase of 12 days for a 6000 Da PEG derivative. This observation could be due to the fact that secondary effluent was used as inoculum in our study, as opposed to AS, i.e. higher microbial density and diversity, which was used in the previous study.

The MRT-AS results on the biodegradation of PVA, with a degree of hydrolysis (DH) of 99 % and a molecular mass of ~130,000 Da, show that high DH and MW values do not significantly hinder biodegradation under these test conditions. Consistent with this result, other studies reported that PVA materials with a MW of 10,000 to 130,000 Da and a DH of 79 % and 88 % achieved ThCO2 degradation of more than 75 % by day 28, indicating no significant effect of MW and DH on the mineralization rate (Menzies et al., 2023).

The observed biodegradation of both PEG and PVA can be explained by established oxidative biodegradation pathways in literature. For PEG, the biodegradation process involves a series of sequential oxidation steps targeting the terminal hydroxyl group. This group is progressively oxidized to form carboxylic acid moieties. Subsequently, the terminal ether bond undergoes oxidative cleavage, resulting in the depolymerization of PEG by the removal of one glycol unit at a time (Eubeler et al., 2010). PVA, with its repeating 1,3 diol structure, follows a two-step biodegradation process. Initially, diketone structures are generated, which are subsequently broken down through hydrolase or aldolase reactions, leading to the production of acetic acid, which is further metabolized within the TCA cycle (Wilkes and Aristilde, 2017).

3.3. PVP, Copovidone and Kollidon CL

We observed no biodegradation for PVP, Copovidone (polyvinyl pyrrolidone–vinyl acetate copolymer), and Kollidon CL (crosslinked polyvinyl pyrrolidone) in both OECD 301D/F 28-day tests and in the extended 42-day 301F test with AS (Fig. 3A, Table 1). Our findings are consistent with current research indicating resistance of vinyl polymers, except for PVA, to biodegradation (Kawai, 2010). Generally, carbon backbone polymers lacking oxidizable/hydrolysable functional groups exhibit recalcitrance to biodegradation (Kawai, 2010). In line with this assumption are practical biodegradation studies indicating that PVP is largely resistant to biological degradation in aerobic aquatic environments (Trimpin et al., 2001; Julinová et al., 2012, 2013). However, certain positive results in PVP (bio)degradation have been reported. Julinová and colleagues proposed a theoretical three-step mechanism for PVP degradation, involving the opening of the lactam ring (1), breakdown of secondary amines via aminooxidases (2), and subsequent mineralization of breakdown products (3) (Julinová et al., 2012). Their study found that approximately 8 % and 14 % of PVP was degraded when aerobic activated sludge was pre-adapted with NMP, a compound containing a lactam ring also found in PVP (Julinová et al., 2012; Julinová et al., 2012). The proposed mechanism for PVP degradation (see above) implies the release of nitrogen-containing transformation products, potentially leading to the release of ammonium and an observable nitrification phase during PVP biodegradation. However, our biodegradation curves, including the 42-day curves for the three compounds, did not show any increase in oxygen consumption related to nitrification, indicating that no nitrification phase was observed (Fig. 3A). In contrast, the monomer NMP, analyzed in a CBT, exhibited over 90 % degradation, displaying an evident nitrification phase resulting from the release of ammonium after day 22 (Fig. 3B). The lack of a nitrification phases for the PVP-based polymers indicates their resistance to biodegradation under our test conditions. The potential environmental persistence is concerning from an ecotoxicological perspective. Chronic exposure to PVP has been associated with adverse effects on aquatic organisms, including impairments of the reproductive cycles in *Daphnia magna* (Mondellini et al., 2022). Additionally, behavioral changes in *Danio rerio* and disruptions to host-parasite interactions (i.e., *Gyrodactylus turnbulli* and *Poecilia reticulata*) within freshwater ecosystems have been reported (Nigro et al., 2023; Robison-Smith et al., 2024). Increasing evidence of PVP as an emerging

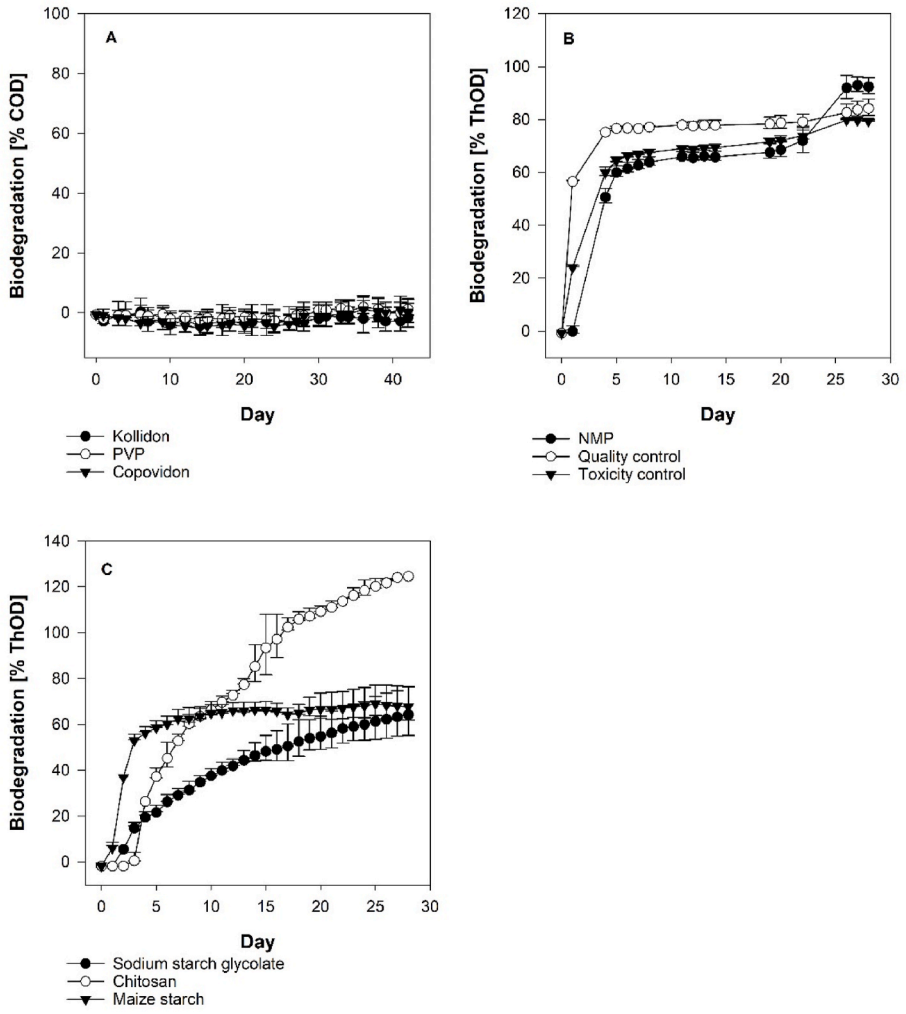

Fig. 3. Biodegradation of **A)** PVP, Copovidone and Kollidon CL in a 42-day OECD 301F AS test **B)** NMP degradation in an OECD 301D test and **C)** Natural and natural-based polymeric excipients in an OECD 301F test. Presented as an average of replicates(n = 2) with error bars showing standard deviation.

environmental contaminant underscores the importance of considering alternatives to vinyl pyrrolidone polymers in pharmaceutical formulations.

3.4. Eudragit derivatives

The assessed PMMAs, Eudragit EPO (MW 47 kDa) and Eudragit L100-55 (MW 320 kDa) showed no biodegradation in the OECD 301D (CBT). While Eudragit L100-55 did not better degrade in biodegradation within the OECD 301F (MRT) with secondary effluent, Eudragit EPO showed 6.9 ± 5.2 % biodegradation. In contrast to the results obtained with secondary effluent, Eudragit EPO showed no biodegradation in both the 28-day and prolonged 42-day MRT-AS, potentially due to its adsorption by AS solids, limiting its bioavailability to microorganisms. Hydrophobic interaction and hydrogen bond force have been found to play a major role in the adsorption of polyacrylate onto AS (Zhao et al., 2018). The 2:1:1 ratio of dimethylaminoethyl methacralyte, butyl methacrylate, and methyl methacrylate in Eudragit EPO, compared to the 1:1 ratio of free carboxyl groups to ester groups in Eudragit L100-55, might facilitate stronger hydrophobic interactions with activated sludge. For Eudragit L100-55, we observed hardly any increase in biodegradation during the MRT-AS after 42 days (Table 1). Concluding, the results show that the use of secondary effluent instead of activated sludge in the MRT may be relevant for substances prone to adsorption effects by activated sludge. Therefore, this study emphasizes the importance of considering the specific characteristics of polymers and their interactions with different test conditions when evaluating biodegradation potential through OECD 301 tests. As described for the assessed vinyl polymers in section 3.3, the limited biodegradation extents observed for the analyzed PMMAs might be attributed to the polymer's resistant carbon backbone, coupled with quaternary carbons hindering metabolism even more and their high MWs (Gaytán et al., 2021). Efficient mineralization of PAA has been reported with MWs below 1000 Da indicating the negative impact of the C–C backbone size in PAA biodegradation (Jackson et al., 2022). Several oxidative aerobic metabolic pathways have been proposed for PAA biodegradation leading to double bond formation between α- and β-carbons, analogous to the metabolic pathway of β-oxidation. However, the presence of methyl groups in the carbon backbone of the investigated Eudragit derivatives likely impedes the initial oxidation steps for double bond formation, thus inhibiting biodegradation of the carbon chain. Therefore, the action of enzymes e.g., mutases to shift the methyl group would be required, enabling PMMA biodegradation (Gaytán et al., 2021). The observed slight biodegradation (Table 1) may derive from initial sidechain removal and mineralization, aligning with the suggested two-phase degradation of PAA/PMMA involving enzymatic side group elimination (first phase) prior to backbone cleavage (second phase) (Gaytán et al., 2021). However, it is important to consider that the low biodegradation values may not definitively indicate biodegradation, as they could potentially fall within the variability and/or error range of the

analytical OxiTop® system.

3.5. Chitosan

Chitosan, manufactured by deacetylation of the natural polymer precursor chitin, was fully mineralized after 28 days in the OECD301F (MRT) with secondary effluent (Fig. 3C, Table 1). The degradation process of chitosan is well-established and involves enzymatic activity of chitinases, which hydrolyze glucosamine-glucosamine linkages (Qiu et al., 2022). The resulting amino sugar, glucosamine, undergoes further metabolism, generating substantial amounts of ammonia (Moye et al., 2014). Chitosan was fully mineralized after 28 days in the OECD 301F (MRT) with secondary effluent (Fig. 3C, Table 1). Following a 4-day lag phase, chitosan degradation manifested in two distinct phases, as indicated by a biphasic biodegradation curve (Fig. 3C). During the lag phase, it is likely that extracellular chitinases (hydrolases) were produced to fragment the polymer, which did not reflect in increased oxygen consumption monitoring as no oxygen is consumed during hydrolysis. The resulting amino sugar compounds are further mineralized within the microorganism's central metabolism, marking the onset of the first degradation phase. The observed increase in oxygen consumption from day 14 results from the nitrification of the ammonium produced during metabolism (nitrification phase), leading to over 100 % degradation by day 28 (Fig. 3C). Interestingly, in contrast to the findings reported here, previously published results indicated that chitosan exhibits limited mineralization under ASTM D5338 composting conditions (Gillece et al., 2024). Under the thermophilic conditions used in the study by Gillece et al. (2024), i.e., 58 ± 2 °C in a solid matrix, chitosan remained largely degradation-resistant and required acidic pretreatment to initiate biodegradation. This resulted in only approximately 40 % mineralization after 180 days (Gillece et al., 2024). These contrasting results illustrate the significant impact that environmental conditions such as temperature and medium have on the biodegradation potential of chitosan.

3.6. Maize starch and sodium starch glycolate

Both maize starch and SSG exhibited degradation exceeding 60 % in the OECD301F (MRT) with secondary effluent (Fig. 3C, Table 1). Maize starch displayed an immediate (day 1) increase in its biodegradation curve, reaching a distinct plateau phase after approximately 12 days (Fig. 3C). In contrast, SSG showed a 3-day lag phase and without evidenced plateau phase by day 28, indicating not yet finished but still slowly ongoing biodegradation (Fig. 3C). As it is well-established for cellulose derivatives, the extent of substitution of the glycosidic backbone significantly influences biological degradability. A previous study highlighted the critical role of the amount of derivatization expressed as degree of substitution or molar substitution in determining biological degradability (Bading et al., 2024). Further, studies confirmed inherent biodegradability of carboxymethyl cellulose (CMC) derivatives with DS values below 1 (Van Ginkel and Gayton, 1996; Menzies et al., 2023). SSG has same chemical modification as CMC, but with starch as polymer backbone. In our recent analysis, applying same test conditions to the present study, CMC linear with a DS of 0.8 exhibited 14.3 + 2.6 % degradation over 28 days (Bading et al., 2024). Extended biodegradation assessments revealed increased degradation for CMC derivative with DS < 1. For instance, CMC with a DS of 0.6 reached 20 ± 2.4 % $ThCO_2$ after 28 days and ultimately 70 % biodegradation after 148 days in an OECD 301B test (Menzies et al., 2023). In this study, we monitored biodegradability of SSG with a lower DS (0.25) compared to CMC DS (0.8). The considerably reduced level of derivatization in SSG could explain the notable increase in biodegradation observed within 28 days.

4. Biodegradability and applicability of OECD 301 screening tests

By applying the classification scheme established by Bading et al. (2024), the compounds, maize starch, chitosan, Macrogol 6000, PVA, and SSG used in the study can be classified as 'readily biodegradable' (Table 2). This classification scheme builds on our previous research, where we developed a framework to categorize biodegradability by considering not only the extent of degradation but also the specific characteristics of the biodegradation curves. Its aim is to predict a compound's biodegradation potential beyond the standard 28-day period. Additionally, it helps identify chemical structures that could be optimized for enhanced biodegradability (Bading et al., 2024).

In case of PVA and PEG, we observed differences in the biodegradation rates depending on the OECD 301 screening tests used (see section 3.2). The absence of biodegradation in the OECD 301D (CBT) obtained for PEG and PVA, which contrasts the readily biodegradation observed in the OECD 301F with secondary effluent and activated sludge, respectively, suggests that the OECD 301D (CBT) test conditions may not be sufficient to assess the full potential of their 'readily' biodegradation. PVA showed over 60 % biodegradation in the MRT-AS and hence classifies as 'readily biodegradable'. However, using the same test set up with secondary effluent instead of activated sludge, PVA does not undergo biodegradation (Fig. 2B) and, consequently, would not have been classified as 'readily biodegradable' according to the OECD 301 guidelines (OECD, 1992). Thus, the outcome of biodegradation assessments is context dependent, highlighting the pivotal role of both inoculum source diversity and inoculum concentration. This is in line with other studies showing that depending on the biodegradation screening

Table 2
Biodegradability classification according to OECD 301 results. **A)** old classification system **B)** new classification system.

A			
Readily biodegradable ≥ 60 %	**Moderately biodegradable 20–59 %**	**Slightly biodegradable 5–19 %**	**Non biodegradable <5 %**
Maize starch Chitosan Sodium starch glycolate PVA Macrogol 6000 (PEG)		Eudragit L100-55 Eudragit EPO	PVP Copovidone Kollidon CL
B			
Readily biodegradable ≥ 60 %	**Moderately biodegradable 20–59 %**	**Slightly biodegradable 10–19 %**	**Non biodegradable < 10 %**
Maize starch Chitosan Sodium starch glycolate PVA Macrogol 6000 (PEG)			PVP Copovidone Kollidon CL Eudragit L100-55 Eudragit EPO

test conditions, the outcome for a given substance can differ widely (Goodhead et al., 2013; Martin et al., 2017a). Also here, the variability is largely attributed to the inoculum and the random inclusion or exclusion of specific degraders, a phenomenon often referred to as the "biodegradation lottery" (Davenport et al., 2022). Increasing the number of microbial cells in biodegradation screening tests to environmentally representative levels can reduce this variability and enhance test reliability (Martin et al., 2017a; Martin et al., 2017b). The low levels of inoculum in the OECD 301D test, with approximately 10^1-10^3 cells mL^{-1}, may reduce the chances of including sufficient quantities of naturally occurring competent degraders (OECD, 1992). This reduction could explain the low biodegradation potential observed in these tests and may lead to 'false negative' assignments of biodegradability within the OECD regulatory framework. For example, PEG and PVA show biodegradability in the OECD 301F test but not in the OECD 301D test. Our experimental 'step-by-step' approach revealed such differences in the requirements for biomass and diversity, which we accomplished through increasing inoculum volume and by introducing more microbial diversity and density with activated sludge to the test system (Fig. 1). To expand on these findings, further research is required to determine if these results are applicable to other readily biodegradable polymers. Additionally, gaining a deeper understanding of how the intrinsic properties of polymers affect microbial adaptation and growth mechanisms is crucial for enhancing biodegradation assessments. Further, in the toxicity controls, both PEG and PVA underwent biodegradation in the CBT and the MRT with secondary effluent, respectively. The presence of easily degradable carbon sources played a significant role in enhancing the biodegradation of these two compounds.

Eudragit EPO and Eudragit L100-55 fall into the category of 'slightly biodegradable.' PVP, Copovidone, and Kollidon CL are identified as 'non biodegradable' (Table 2A). In contrast to PEG and PVA, compounds like PVP, Copovidone, Kollidon CL, Eudragit EPO, and L100-55 lack oxidizable/hydrolysable groups within their carbon backbone structure. As a result, breaking down the carbon backbone for these compounds becomes notably challenging. This aligns with our biodegradation findings within the OECD 301 tests. In contrast to the biodegradation outcomes observed for PEG and PVA, there was minimal variability in the biodegradation results obtained across different screening tests for the PEx categorized as 'non biodegradable' and 'slightly biodegradable.' This low variability indicates a high inherent resistance to biodegradation. Importantly, none of these substances surpassed the lag phase, defined as the time to achieve 10 % degradation (OECD, 1992). The lag phase is characterized by the initial adaptation or selection and growth to reach critical mass of microorganisms and the commencement of the biodegradation process ('adaptation phase'). The absence of a lag phase for these compounds highlights their lack of susceptibility to microbial degradation. Extending the test duration for these compounds, which either did not surpass 5 % degradation or only marginally surpassed this level within the initial 28 days, to 42 days for further assessment of their biodegradation potential did not result in a readily detectable increase in degradation. Therefore, we have to consider these PEx as potentially environmentally persistent. However, higher tier simulation tests may be needed to draw definitive conclusions for regulatory persistence assessment. For these reasons, we revised the previously published classification system (Bading et al., 2024) and set 10 % degradation as the threshold to be categorize as 'slightly' biodegradable (Table 2B). By setting the threshold at 10 % degradation, the classification system accounts for the lag phase, which is a crucial indicator of the initiation of the biodegradation process.

For readily biodegradable polymers according to the OECD guidelines, more stringent test conditions within OECD 301 guidelines could be applied to gain a more thorough picture of their biodegradation potential. More research needed due to the limited mechanistic understanding of the underlying microbial processes. However, for an initial screening for potential biodegradation, it may be more suitable to first analyze the test compounds in a screening test with a higher possibility of positive outcome in biodegradation, as in this study, the OECD 301F with activated sludge.

5. Conclusions

This study serves two important purposes: it enhances scientific understanding and contributes to discussions on regulatory and industrial practices. With increasing pressure to adopt sustainable practices, particularly under the EU Taxonomy Regulation, there is a growing need for readily biodegradable compounds in pharmaceutical formulations. Our systematic analysis identified potentially persistent compounds such as PVP, Copovidone, Kollidon CL and the Eudragit derivatives EPO and L100-55 as well as readily biodegradable compounds such as PVA, Macrogol 6000, SSG, chitosan and maize starch. These results thus provide an initial orientation for the selection of environmentally friendly excipients and offer a solid basis for the development of pharmaceuticals with improved biodegradability, in line with the concept of "benign-by-design". In addition, our stepwise approach has shown that susceptibility to biodegradation is highly dependent on the size and diversity of the inoculum. Future research should aim at a deeper mechanistic understanding of how the physicochemical properties of polymers affect microbial degradation and adaptation processes.

CRediT authorship contribution statement

Mila Bading: Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Oliver Olsson:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Klaus Kümmerer:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143739.

Data availability

Data will be made available on request.

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Publication 3

Mila Bading, Morten Suk, Oliver Olsson, Klaus Kümmerer (2025)

Biodegradation of Pharmaceutical Surfactants: Polysorbates, Poloxamers, and their Derivatives in OECD 301 Screening Tests - Insights into “Benign-by-Design”

Ready for Submission

Biodegradation of Pharmaceutical Surfactants: Polysorbates, Poloxamers, and their Derivatives in OECD 301 Screening Tests - Insights into "Benign-by-Design"

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Abstract

Non-ionic surfactants, such as polysorbates (PS) and poloxamers, are widely used in the pharmaceutical industry. However, data on their biodegradability in the environment remains limited. In line with the "Safe and Sustainable by Design" (SSbD) framework, this study presents a harmonized dataset based on OECD 301 screening tests to evaluate ready biodegradation behavior. Using lag phase duration as an indicator of microbial adaptation, we identified structure-specific degradation patterns. For poloxamers, the ratio of polyethylene glycol (PEG) to polypropylene glycol (PPG) blocks appears to be a key factor influencing biodegradability. Notably, high-molecular-weight poloxamers with larger PEG blocks showed no biodegradation. For the first time, we have elucidated the biodegradation pathway of PS and identified ethoxylated sorbitan residues as potential dead-end transformation products that may persist in the environment. These findings highlight the significance of polymer structure and transformation products in determining environmental fate. This approach combines

standardized testing, a systematic analysis of molecular features and indicators of microbial adaptation to provide insights that support the design of more environmentally friendly surfactants ("Benign-by-Design", BbD).

1. Introduction

Non-ionic surfactants are widely used as excipients in pharmaceutical formulations, including oral, topical and injectable drugs. These surfactants can be either polymeric or non-polymeric. Common classes of polymeric surfactants include ethoxylated sorbitan esters (e.g. polysorbates, or PS), and PEG-PPG-PEG block copolymers (e.g. poloxamers). Examples of non-polymeric surfactants include fatty acid esters of sorbitan, such as Span 80 (Maher et al., 2023; Fig. 1). In addition to their use in the pharmaceutical industry, non-ionic surfactants are widely used in various other industries, including food additives, cosmetic ingredients, oil spill remediation agents, and cleaning product components (Nazar et al., 2021; Yuan et al., 2024). In other words, they are used in many products that contribute significantly to the release of substances into the environment via wastewater. They have been detected and quantified ubiquitously in various environmental compartments up to 3370 mg L^{-1} in industrial wastewater, raising concerns about their environmental impact (Orlandi et al., 2019; Nunes et al., 2022). For example, the adverse effects of PS 80 on grazer life history traits and planktonic ecosystem stability have been reported, emphasizing the necessity of a better comprehension of how these substances biodegrade in the environment (Yuan et al., 2024). If these compounds were to degrade quickly and completely, they would not be present in the environment and therefore would not be able to expose organisms and pose a risk. Furthermore, a recent prioritization study of water-soluble polymers by Brunning et al. identified several high-emitting, down-the-drain polymer groups, some of which also function as non-ionic surfactants. Among these, polyethers and their copolymers, particularly PPG and PEG, were identified as significant contributors to emissions. Additionally, polyol ethoxylate esters were identified as another significant group of high-emitting polymers (Brunning et al., 2025). The two predominant pharmaceutical surfactants, poloxamers and PS, belong to the following groups: poloxamers are classified as polyether copolymers, while PS are classified as polyol ethoxylate esters (Bollenbach et al., 2022; Roy et al., 2024).

Despite their widespread use, data on the fate of these compounds in the aquatic environment remains scarce (Brunning et al., 2025). This creates a data gap that hinders the effective design of compounds for rapid and complete mineralization in the environment (Benign-by-Design,

BbD). This affects its application within the European Safe and Sustainable by Design (SSbD) framework (EC, 2022). The SSbD framework aims to promote the development of sustainable chemicals. Fast and full environmental degradation is an important building block of SSbD. However, the absence of robust, harmonized biodegradability data hinders the molecular redesign of environmentally degradable polymeric materials based on clear biodegradability descriptors (Kim et al., 2023). Challenges include the complexity and diversity of polymeric materials, the lack of standardized tests and inconsistent study designs. These factors hinder the reliable cross-comparison of biodegradability and the qualitative and quantitative modelling of biodegradability (Kim et al., 2023; Kintzi et al., 2024; Lin and Zhang, 2025).

Existing studies on soil degradation suggest that, for PS, microbial activity can break down the fatty acid ester groups while the more hydrophilic ethoxylated components remain largely persistent (Lee et al., 2013). A similar metabolic pattern is observed in the human body: following drug administration, PS undergoes partial hydrolysis, releasing POE sorbitan moieties that are poorly absorbed in the gastrointestinal tract and ultimately excreted (Maher et al., 2023). However, the environmental behavior and fate of these POE sorbitan structures in aquatic systems remain largely unexplored. It is unclear whether the biodegradation of PS in the environment extends beyond the initial hydrolysis of fatty acids, or whether the POE sorbitan moiety is largely resistant to further transformation. This study therefore places particular emphasis on elucidating the biodegradation pathways of PS 80, the most used PS excipient, with a specific focus on whether its POE sorbitan moiety undergoes further degradation under environmental conditions.

For poloxamers, existing research has focused on the environmental biodegradability of their individual building blocks, PEG and PPG, with the oxidative degradation pathways largely elucidated. The chemical structure of the terminal groups has been shown to play a key role in initiating oxidative biodegradation (Eubeler et al., 2010; Zgola-Grzeskowiak et al., 2007). In addition, molecular weight (MW) has a major influence on biodegradability: PEG is considered readily biodegradable up to a MW of 35,000, while PPG biodegrades up to 2,700 (West et al., 2007; Menzies et al., 2023). Studies suggest that transport across membranes and enzymatic activity of polyethers - particularly in the periplasm - are crucial factors influencing metabolism, as MW dictates uptake and subsequent degradation (Kawai, 2002; West et al., 2007). However, despite these findings, data for complete poloxamer block copolymers degradation is still not available. While these studies provide valuable insight into the degradation behavior of PEG and PPG individually, comprehensive biodegradation data for full poloxamer block copolymers is still lacking. This is a gap that must be filled, given the widespread use of these polymers in

the pharmaceutical industry and their structural complexity, which cannot be fully understood from an analysis of their monomeric components alone.

As highlighted in previous research, meaningful predictions of polymer biodegradability require accurate and comprehensive data on chemical structure and degradation behaviour. In addition to established surfactants, structurally related derivatives were included to improve our understanding of structure-biodegradability relationships. This approach aligns with the data-driven principles of the SSbD framework, generating robust, comparable data to inform future polymer design and regulatory assessment (EC, 2022; Apel et al., 2024).

These included:

- PEG derivatives with different terminal functional groups (e.g. polyethylene glycol bisamine, dimethylether and PEG fatty acid esters) were evaluated to determine their effect on oxidative biodegradation.
- PPG polymers of varying molecular weights to assess how chain length influences microbial degradation potential.
- Poloxamers (PEG-PPG-PEG block copolymers) with differing backbone lengths and architectures to examine combined effects of composition and molecular size.

Here, for the first time, we have systematically evaluated the ready biodegradation of PS, poloxamers, and their derivatives in the aquatic environment to fill data and knowledge gaps to allow for a better understanding of structure biodegradability relationships. All substances were tested under harmonized OECD 301 test conditions. By analyzing the biodegradation profiles, particularly the duration of the lag phase and the time taken to reach defined biodegradation thresholds, we aimed to gain mechanistic insight into how microorganisms adapt to different polymer structures.

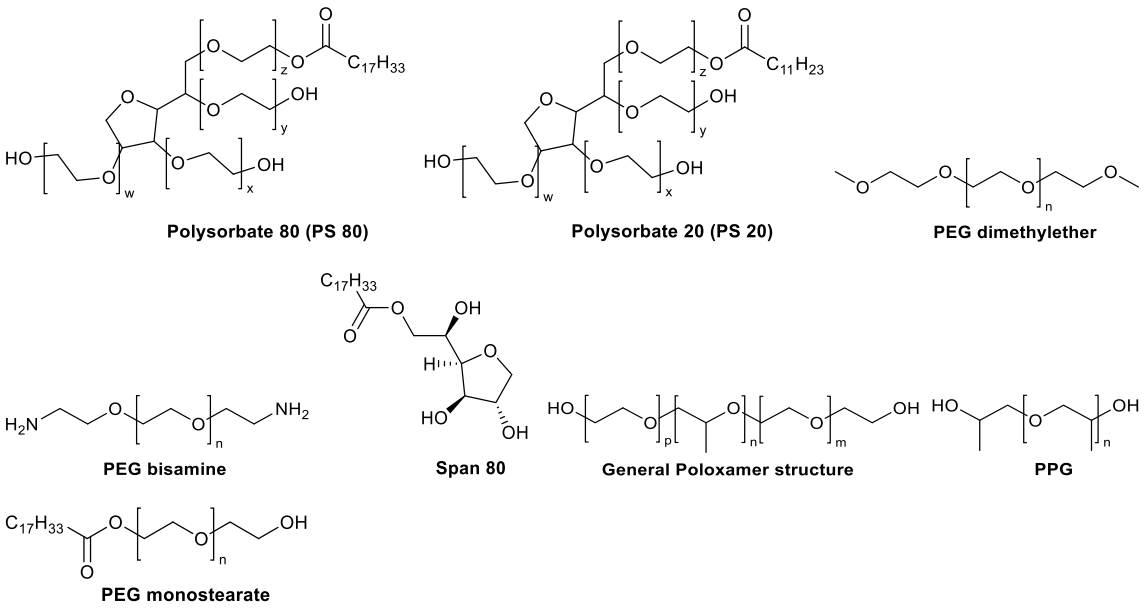


Fig. 1 structures of study compounds showing PS 80 (w+x+y+z=20), PS 20 (w+x+y+z=20), PEG dimethylether (MW ~240), PEG bisamine (MW ~2000), Span 80, Poloxamer (P188: p, m=75, n=30, MW ~8200; P184: p, m=15, n=30, MW ~2400) , PPG (PPG 400: MW ~400; PPG 4000: MW ~4000), and PEG monostearate (MW ~2044).

2. Materials and Methods

2.1. Chemicals

PS 80 and PS 20 were obtained from Merck Chemicals GmbH (Darmstadt, Germany). Sodium azide, sodium acetate, ammonium formate, PEG bisamine, and P184 were purchased from Sigma-Aldrich (Darmstadt, Germany).

PEG dimethylether, PEG monostearate, and Span 80 were obtained from Tokyo Chemical Industry (TCI) (Eschborn, Germany). P188 was supplied by BASF (Ludwigshafen, Germany). PPG 400 and PPG 4000, as well as Optima LC/MS grade isopropanol, were purchased from Fisher Scientific (Schwerte, Germany). Acetonitrile (ACN) was obtained from VWR (Darmstadt, Germany).

2.2. Biodegradability: OECD 301D and OECD 301 F Tests

The environmental biodegradation of the test compounds was assessed using an optode-based (Fibox 3, PreSens, Regensburg, Germany) closed bottle test (OECD 301D) (OECD, 1992; Friedrich et al., 2012) and a manometric respirometry test (OECD 301F) using the OxiTop® system (OC110 system, WTW GmbH, Weilheim, Germany) (OECD, 1992). The test concentrations in the OECD 301D and OECD 301F were set at 5 mg L^{-1} chemical oxygen

demand (COD) and 30 mg L^{-1} of COD, respectively. These concentrations were determined through Merck Spectroquant® photometric COD cell tests in the range of 5-80 mg L^{-1}. For the monomeric compounds in this study, the initial concentrations were based on their theoretical oxygen demand (ThOD) and were set at 5 mg L^{-1} and 30 mg L^{-1} of ThOD for the OECD 301D and OECD 301F, respectively. The OECD 301D was inoculated with 2 drops L^{-1} (~ 200 µL L^{-1}) of secondary effluent from a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325000 eq. inhabitants). The secondary effluent was filtered through a filter paper before use. In addition, we performed an OECD 301F utilizing 30 mg suspended solids L^{-1} of activated sludge from the same treatment plant (see above). Microbial enumeration of the secondary effluent and activated sludge was performed. Here fore, colony forming units (CFUs) were determined according to ISO EN 6222:1999 standard. Inoculum samples were serially diluted, plated in triplicate (with water controls) and incubated at 22 °C for 68 ± 4 hours. After incubation, CFUs were counted manually. The sludge was washed three times with tap water before use to reduce the organic matter. The mineral media were prepared according to the corresponding OECD guidelines 301D and 301F (OECD, 1992). All tests consisted of 'blank', quality control' (sodium acetate), test series' and 'toxicity control'. The OECD 301F also contained a 'sterile control' containing 320 mg L^{-1} NaN_3 to monitor abiotic degradation. OECD 301D test runs were performed in parallel with two test bottles each for the blank value, quality control, test series and toxicity control. For OECD 301F, each test compound was run in two test bottles, along with one toxicity control, one sterile control, and three blanks and three quality control bottles. Replicates of OECD 301Ds and OECD 301Fs ($n \geq 2$, as indicated in Table 1) were performed for each test compound. To obtain the 42-day OECD 301F, the duration of the test for two OECD 301F replicates was extended to 42 days to monitor prolonged biodegradation. In all experiments, the pH values on days 0, 28, and 42 were measured to ensure they were within the range of 6.0 to 8.5, as required by the OECD guidelines (OECD, 1992). According to the OECD guidelines, the ready biodegradability of the test compound was investigated in closed flasks at a constant temperature (20 ± 1°C) in the dark within the testing duration (OECD, 1992). Biodegradation extent was expressed as the ratio of biological oxygen demand to COD.

The test compounds were categorized into the following subgroups based on biodegradation levels:

- Readily biodegradable (≥60 %)

- Moderately biodegradable (20-59 %)
- Slightly/weakly biodegradable (10-19 %)
- Non-biodegradable (<10 %)

This classification system follows the OECD guidelines, with slight modifications introduced by Bading et al. (2024a). The lag phase is defined as the time from the start of the test until 10% biodegradation is reached. It is important to note that the 10-day window criterion (i.e. 60% biodegradation within 10 days of reaching 10% biodegradation) does not apply to polymers, given that they are known to degrade sequentially (OECD, 2006). OECD 301F tests were extended to 42 days to monitor potential increases in biodegradation beyond the standard 28-day period. Samples were taken on days 0, 28 and 42. Prior to analysis using an Orbitrap mass analyser, the samples were filtered through a 0.45 µm polyethersulfone membrane filter (Macherey-Nagel, Düren, Germany).

2.3. Assessment of transformation products of PS 80

The biodegradation process and transformation products of PS 80 were investigated using a HPLC method adapted from Thermo Fisher Scientific's fingerprinting protocol for PS (Thermo Fisher Scientific, 2021). The biodegradation process and transformation products of PS 80 were monitored by a UHPLC (Vanquish, Thermo Scientific, Dreieich, Germany) coupled to an Orbitrap Exploris 240 (Thermo Scientific Dreieich, Germany) equipped with a H-ESI source (Thermo Scientific, Dreieich, Germany) in full scan and positive ionization mode. The chromatographic separation was carried out on a Accucore C18 column (100 x 2.1 mm, 2.6 µm, Thermo Fisher Scientific, Germany) using gradient mode. The eluents consisted of 5mM ammonium formate adjusted to pH 4 with formic acid (A) and 50/50 isopropanol/acetonitrile (v/v) (B) with a flow rate of 0.4 mL min L^{-1}. The gradient program was taken from a Thermo Fisher Scientific method (Thermo Fisher Scientific, 2021). The column temperature was maintained at 50 °C and the injection volume was 10 µL. The H-ESI parameters were as follows: sheath gas flow rate, 50; auxiliary gas flow rate, 10; sweep gas flow rate, 1; spray voltage, 4400 V; ion transfer tube temp, 300 °C; vaporizer temp, 0 °C. Parameters of the full-scan analysis were: resolution, 30000 at m/z 200; AGC target, standard; max IT, auto; scan range, m/z 150-2000. The parameters of data-dependent MS2 were as follows: resolution, 60000 at m/z 200; isolation window, 1; HCD collision energy, 30 V; AGC target, standard; max IT, auto.

205

206 **3. Results and Discussion**

207 **3.1. Biodegradability according to OECD 301**

208 All tests met the validity criteria outlined in the OECD guidelines (OECD, 1992). None of the
209 compounds tested were toxic to the inoculum, as confirmed by the biodegradation of the
210 toxicity in the control vessels containing both the reference compound and the test substance,
211 which exceeded 25 % degradation within 14 days (OECD, 1992). In all studies, the positive
212 control, sodium acetate, reached > 60 % mineralization by day 14 indicating inoculum activity
213 and meeting test validity criteria (OECD, 1992). The biodegradation results are summarized in
214 Table 1. Among the compounds tested, P184, Span 80, PPG 400, PEG bisamine and PEG
215 monostearate (Fig. 1) were classified as readily biodegradable (i.e. > 60%) after 28 days
216 according to OECD 301F guidelines (Fig. 2A). In contrast, none of these compounds degraded
217 >10 % in the OECD 301D test, except PEG monostearate which showed 33.5 ± 0.7 %
218 biodegradation and was classified as moderately biodegradable according to the applied
219 classification scheme (Table 1). This variability in biodegradation results was most likely due
220 to differences in microbial cell densities and related bacterial diversity. CFU determinations
221 showed that the OECD 301D inoculum contained approximately 3 cells mL^{-1}, whereas the
222 OECD 301F test had a microbial density of 10^4 cells mL^{-1}. As observed in previous studies,
223 differences in biodegradation levels may happen under different test conditions including
224 bacterial density and diversity as well as test compounds' concentration which characterize
225 OECD 301D and OECD 301F (Bading et al., 2024a, b).

226 Notably, unlike PEG monostearate, Span 80 did not degrade in OECD 301D, even though both
227 compounds contain a fatty acid moiety linked via an ester bond. This contrast suggests that
228 biodegradability under these conditions is influenced by factors beyond the presence of ester
229 linkages alone. While PEG monostearate underwent degradation, the lack of degradation in
230 Span 80 suggests that its limited water solubility may limit bioavailability, thereby hindering
231 enzymatic access and biodegradation in the OECD 301D. This hypothesis is further supported
232 by the biodegradation behaviour of water-soluble PSs, which, like Span 80, contain a sorbitan
233 core structure, but were classified as moderately biodegradable in both the OECD 301D and
234 the 28-day and prolonged OECD 301F tests, based on the classification scheme. PS were found
235 to be moderately biodegradable in both the OECD 301D test and the 28-day and 42-day OECD
236 301F tests (Table 1, Fig. 2C). In contrast, compounds that were not biodegradable included

P188, PPG 4000 and PEG dimethylether. These results and their implications are discussed in detail in the following sections.

Table 1 Biodegradability of non-ionic surfactants and related polymeric materials in OECD 301D, OECD 301F, and 42-day OECD 301F. Presented as an average of replicates (n ≥ 2) with ± showing standard deviation.

	Biodegradation level [% COD]			Results according to OECD
Compound	**OECD 301 D**	**OECD 301 F**	**42-day OECD 301 F**	
PS 80	26.3 ± 2.1 [a]	45.5 ± 5.5 [c]	50.3 ± 5.3 [c]	not readily biodegradable
PS 20	17.6 ± 0.7 [a]	45.3 ± 4.3 [c]	49.1 ± 6.2 [c]	not readily biodegradable
P188	-0.18 ± 1.4. [b]	1.1 ± 10.0 [b]	4.0 ± 8.0 [b]	not readily biodegradable
P184	-6.5 ± 0.2 [a]	89.20 ± 8.0 [a]	93.2 ± 9.4 [a]	readily biodegradable
Span 80	7.3 ± 4.3 [a]	74.8± 2.6 [a]	75.7 ± 4.0 [a]	readily biodegradable
PPG 400	-10.5 ± 1.9 [a]	87.2 ± 2.6 [a]	94.2 ± 5.2 [a]	readily biodegradable
PPG 4000	-12.5 ± 1.9 [a]	6.2 ± 3.3 [b]	9.5 ± 6.7 [b]	not readily biodegradable
PEG bisamin	-5.9 ± 1.4 [a]	83.6 ± 8.0 [a]	93.9 ± 3.8 [a]	readily biodegradable
PEG dimethylether	-3.7 ± 0.8 [a]	2.8 ± 1.4 [a]	4.1 ± 1.2 [a]	not readily biodegradable
PEG monostearate	33.5 ± 0.7 [a]	98.2 ± 4.0 [a]	101.2 ± 5.2 [a]	readily biodegradable

a n = 2

b n = 4

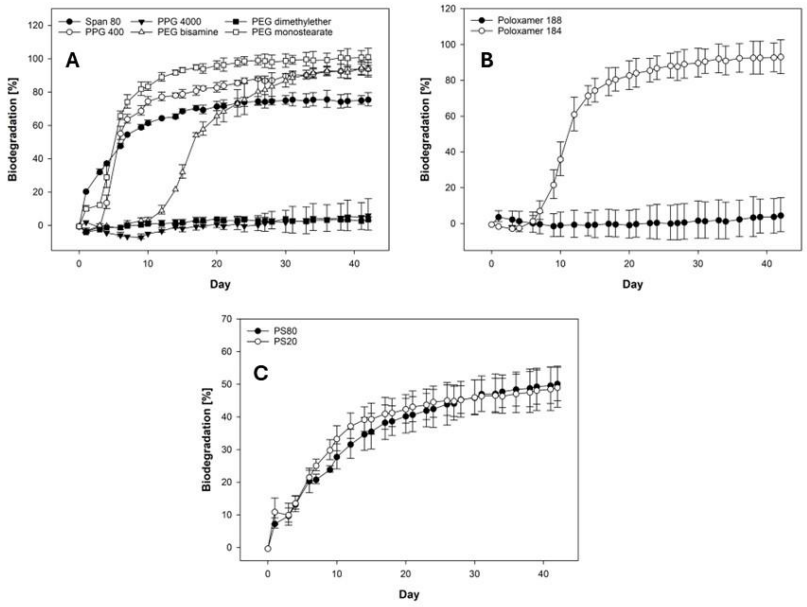


Fig. 2 Biodegradation of A) Span 80, PPG 400, PPG 4000, PEG bisamine, PEG dimethylether. PEG monostearate B) P188 and P184 and C) PS 80 and PS 20 in a 42-days OECD 301F test. Presented as an average of replicates (n≥2) with error bars showing standard deviation.

3.2 P184 and P188

No biodegradation was observed for either poloxamer in the OECD 301D test (Table 1). P184 (MW ~2400) was found to be readily biodegradable in the 28-day OECD 301F test, whereas P188 (MW ~8200) showed no degradation, even in the extended 42-day OECD 301F test (Fig. 2B). Poloxamers are triblock copolymers consisting of a central, hydrophobic PPG block, which is flanked by two PEG blocks. The block lengths of PEG differ specifically between P184 and P188: P184 has a PEG-PPG-PEG ratio of approximately 13-30-13, whereas P188 has a much higher PEG content, with a ratio of 75-30-75 (Fig.1).

The absence of biodegradation for P188 is unexpected given that both PEG and PPG are known to be biodegradable under standard OECD test conditions. PEGs degrade readily at MWs of up to ~30,000 Da under OECD 301B conditions (Menzies et al., 2023), and PPGs have been shown to degrade at molecular weights of up to ~2,700 Da in OECD 301F tests (West et al., 2007). PPG 2700 exhibited lower biodegradability, possibly due to its poor water solubility, which may limit its dissolution in the test medium (West et al., 2007). Our results support this, showing that the water-soluble PPG 400 (MW ~400 Da) was readily biodegradable in the OECD 301F test, whereas water-insoluble PPG 4000 was not (Fig. 2A). This confirms the molecular weight dependency of PPG biodegradation. The decreased degradability at higher

molecular weights is likely due to reduced bioavailability due to poor water solubility rather than a lack of enzymatic compatibility.

Both PEG and PPG are degraded by intracellular enzymes located in the periplasmic space or bound to bacterial membranes. For biodegradation to occur, these polymers must first be transported to the periplasmic space where membrane-bound oxidoreductases (often linked to respiratory pathways) facilitate oxidative degradation (Kawai et al., 1980). Microbial enzymes responsible for PPG degradation, such as membrane-bound oxidoreductases, are believed to have low structural specificity (Kawai et al., 2002). Hence, they rely on access to the substrate, which must first be transported to the periplasmic space (Kawai et al., 1980). The poor biodegradability of P188 cannot be attributed to its water solubility, since it is fully soluble under test conditions. Rather, it appears that the decisive factor is the PEG-PPG-PEG block ratio, as this determines polymer conformation, which in turn governs microbial uptake and enzymatic accessibility. Larger PEG flanks, as found in P188, may result in conformations that hinder interaction with microbial transport systems or enzymes despite solubility. This interpretation is consistent with the findings of West et al. (2007), suggesting that polymer conformation influences degradation by enabling higher affinity for microbial enzymes and/or facilitating more efficient transport across cell membranes. Conversely, the lower MW of P184 is likely to facilitate cellular uptake and subsequent degradation.

Differences in lag phases further highlight the importance of chemical structure in biodegradability of structurally related derivatives. According to the OECD 301 guidelines, the lag phase is the period required for microbial adaptation prior to measurable degradation (OECD, 1992). The lag phase reflects the period of microbial adaptation during which the community undergoes selection and growth until a critical biomass is reached, enabling the onset of biodegradation. The absence of a lag phase indicates that a compound is not susceptible to any microbial degradation in a measurable extent under the test conditions. Substances with readily accessible enzymatic cleavage sites, such as Span 80 and PEG monostearate (n=40; MW ~2044 Da), exhibited lag phases of just one day, indicating rapid microbial uptake (Fig. 2A). These compounds contain ester groups that are likely to be rapidly enzymatically hydrolysed and subsequent microbial oxidative degradation by microbial enzymes. By contrast, P184 exhibited a lag phase of nine days, indicating slower microbial adaptation despite its relatively low molecular weight. As discussed earlier, polymer conformation may influence how readily a compound is transported into cells and accessed by enzymes. With its smaller size, PPG 400 showed a shorter lag phase of four days, likely due to a more favourable conformation for membrane transport and enzymatic attack, and hence a faster onset of

biodegradation. PEG bisamine (MW ~2000 Da) exhibited a prolonged lag phase of 13 days, likely due to initial biochemical transformations, such as transamination reactions, converting it into more readily oxidisable carbonyl compounds, which are required for its terminal amine groups. PEG dimethylether showed no biodegradation (Fig. 2A), which is consistent with the absence of oxidisable functional groups such as hydroxyl, ester or amine groups, which are essential for microbial oxidation (Eubeler et al., 2010).

In summary, the biodegradability of poloxamers appears to be strongly governed by their PEG-PPG-PEG composition, which affects the polymer's conformation and, consequently, its bioavailability and interaction with microbial enzymes. While PEG and PPG units are biodegradable at specific molecular weights, their integration into block copolymers modifies their behaviour. The poor degradation of P188, despite its solubility and its building blocks being degradable, highlights that limitations in conformation and transport - rather than chemistry alone - can dominate biodegradation outcomes. These results emphasise the importance of block architecture and terminal functionality, as well as molecular weight, in predicting the environmental fate of amphiphilic polymers.

3.3 Biodegradation of PS:

PS were classified as moderately biodegradable, exhibiting partial degradation in both the OECD 301D and OECD 301F tests after 28 and 42 days. In the OECD 301D test, PS 80 degraded by 26.3 ± 2.1 %, while PS 20 showed 17.6 ± 0.7 % degradation. In the OECD 301F test, PS 80 reached 45.5 ± 5.5 % degradation, and PS 20 showed a similar extent of degradation at 45.3 ± 4.3 % (Table 1). Extending the OECD 301F test to 42 days did not lead to a significant increase in biodegradation, with PS 80 reaching 50.3 ± 5.3 % and PS 20 reaching 49.1 ± 6.2 % (Table 1, Fig. 2C). The difference in biodegradation between PS 20 and PS 80 in the OECD 301D was likely influenced by the different lengths of their fatty acid esters. PS 80 primarily contains oleic acid esters, while PS 20 consists mainly of lauric acid esters (see Fig. 1). This structural difference is also reflected in their COD, which is slightly higher for PS 80 (1.8 mg O_2 mg test substance $^{-1}$) than for PS 20 (1.7 mg O_2 mg test substance $^{-1}$). In this case, COD correlates with the amount of oxidizable carbon atoms present in the molecule. The longer oleic acid chains in PS 80 therefore require more oxygen per unit mass than the shorter lauric acid chains in PS 20. This trend aligns with the degradation rate of PEG monostearate, which exhibited 33.5 ± 0.7% degradation in the OECD 301D, further supporting the role of fatty acid metabolism in the observed biodegradation (Table 1). A plausible explanation for this observation is that in the OECD 301D, only the fatty acid moiety undergoes significant

metabolism, whereas the PEG moiety of both PEG monostearate and PS remains largely resistant to degradation.

Biodegradation pathway and transformation products:

As outlined in the Methodology section, our analytical approach, based on the Thermo Fisher Scientific method, enabled us to identify and characterise the various components of PS 80. Using this method, four distinct groups of peak clusters were classified and categorized as follows (Fig. 3A):

- Group I: Sorbitan-POE and isosorbide-POE derivatives
- Group II: Sorbitan and isosorbide monoesters
- Group III: Sorbitan and isosorbide diesters
- Group IV: Sorbitan and isosorbide triesters

The classification of these groups was validated by MS^2 spectra and confirmed by the identification of the characteristic fragment ion of oleic acid (the predominant fatty acid in PS 80) at m/z 309.279 (Fig. 3B). A blank spectrum is provided in the Supplementary Material (Fig. S1). This grouping will be used for further discussion and comparative analysis of the biodegradation process. Examples of detected ions for each group, along with their tentative identity assignments, are summarized in Table S1. The observed components appeared as singly, doubly, or triply ammoniated adducts, depending on the species. OECD 301F samples of PS 80 were analysed at days 0, 28 and 42. The mass spectra showed distinct degradation patterns of PS 80 over time (Fig. 4). At day 28, a decrease of the MS peaks corresponding to groups II-IV was observed, indicating biodegradation of the ester-linked fatty acid moieties. This finding is consistent with the biodegradation levels observed at day 28, suggesting that while ester hydrolysis occurs, the polyoxy ethylated sorbitan moiety (Group I) remained. By day 42, a further decrease in Group I moieties were observed, suggesting that the degradation of the POE chains is a slow and sequential process (Fig. 4). The rapid disappearance of groups II-IV (day 28) suggests that the first step in the degradation process is the enzymatic cleavage of the ester-linked fatty acids. In contrast, POE chains persisted longer and appeared to be more resistant to microbial degradation. This resistance may be due to the branched structure of the ethoxylated chains (“spatial configuration”), which may hinder enzymatic attack, whereas

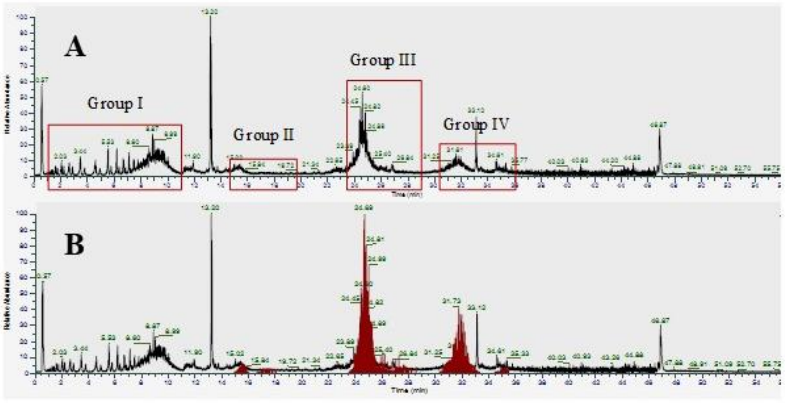
linear low MW PEGs are known to be biodegradable (Menzies et al., 2023; Bading et al., 2024a).

Interestingly, mass spectra from days 28 and 42 showed oxidized PEG derivatives that were not present at day 0. This suggests that a secondary transformation process occurred after the initial degradation of the fatty acids after hydrolysis. Notably, MS^2 spectra revealed the presence of carboxylated POE structures (e.g., m/z 710.413, see Fig, S2 A-C) that were not found at day 0, highlighting their formation as transformation products during PS biodegradation. These transformation products have not been previously reported for such POE-derived intermediates in the biodegradation of PS 80. This suggests that the enzymatic attack may occur at the ether linkage connecting the PEG to the sorbitan. Previous studies have shown that microbial degradation of ether oxygenates often involves hemiacetal intermediates (Hyman, 2013). A plausible reaction mechanism involves the formation of hemiacetals, supporting the hypothesis that PEG chains were cleaved directly from the sorbitan core. Based on these findings, we propose two potential pathways for the degradation of the sorbitan POE moiety (Fig. 5):

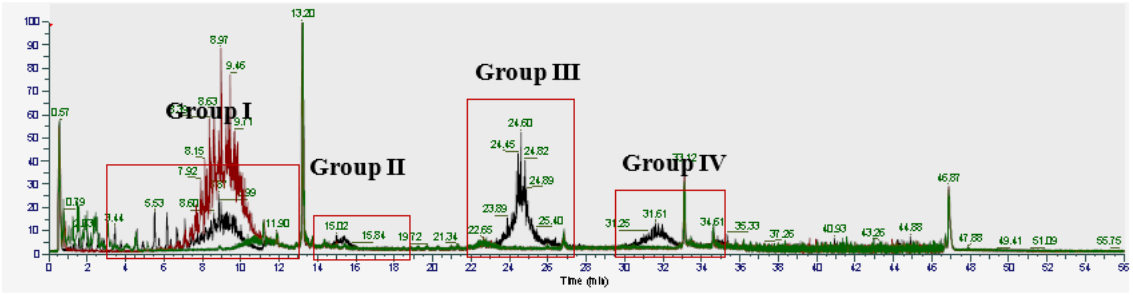
- Direct cleavage from the sorbitan core via gradual removal via hemiacetal formation

- Oxidative terminal removal of the POE moiety

These results indicate a stepwise degradation process, with ester hydrolysis occurring first and subsequent biodegradation of the resulting free fatty acids (fast step), followed by the slower conversion of the ethoxylated moieties by oxidation. The persistence of the POE moiety on day 28 suggests that while the PS are partially degraded, their resulting ethoxylated residues may accumulate in the environment.



391 *Fig. 3 Total Ion Chromatogram (TIC) of PS 80 showing identified groups: Group I - Sorbitan-POE and isosorbide-POE*
392 *derivatives, Group II - Sorbitan and isosorbide monoesters, Group III - Sorbitan and isosorbide diesters, Group IV - Sorbitan*
393 *and isosorbide triesters.*



394

395 *Fig. 4 TIC of PS 80 at day 0 (black), 28 (red), and 42 (green), showing the identified groups: Group I - Sorbitan-POE and*
396 *isosorbide-POE derivatives, Group II - Sorbitan and isosorbide monoesters, Group III - Sorbitan and isosorbide diesters,*
397 *Group IV - Sorbitan and isosorbide triesters.*

398

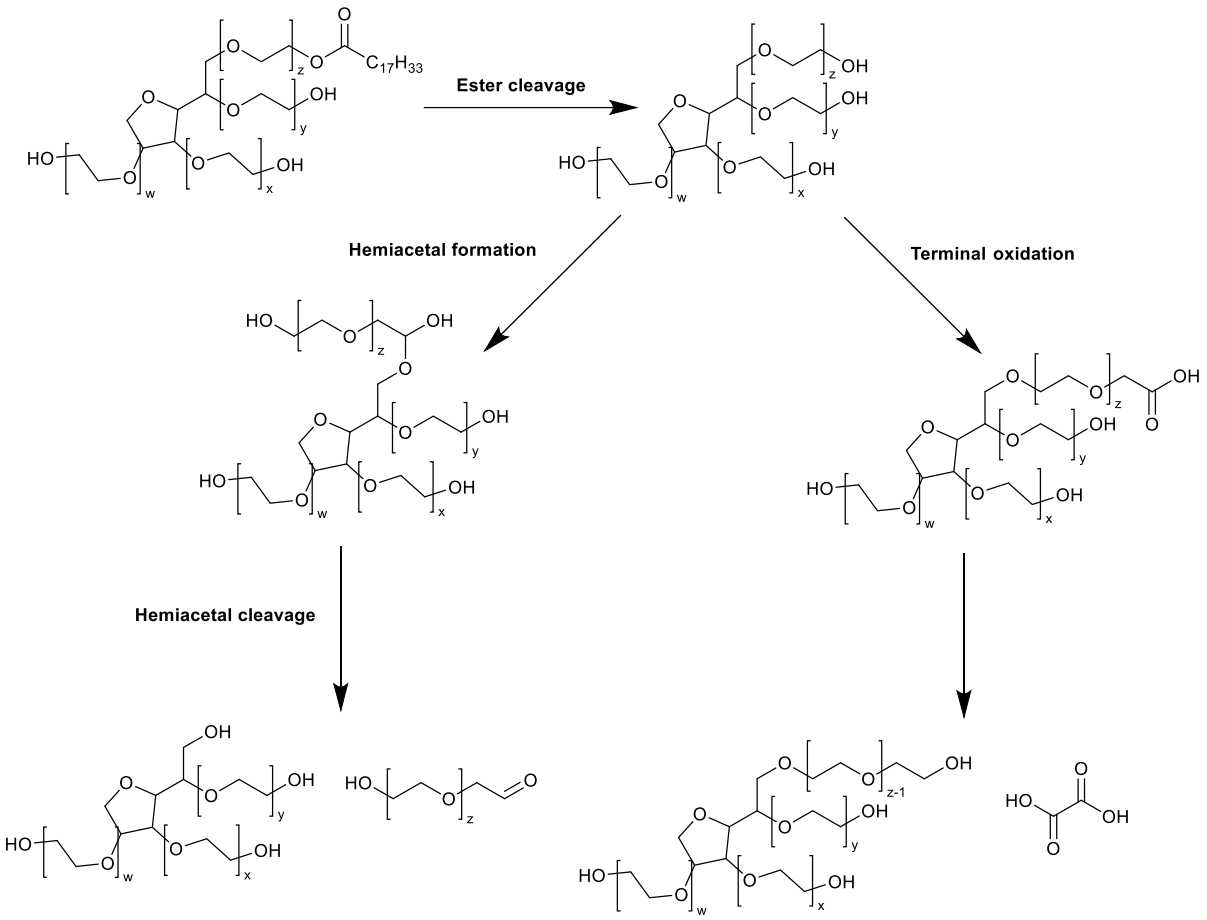


Fig. 5 Proposed pathways for the degradation of the sorbitan POE moiety, with ester hydrolysis occurring first (rapid step), followed by slower oxidative conversion of the POE moiety.

4. Structure-biodegradability relationships

In line with the SSbD framework, this study provides a harmonized dataset based on the OECD 301 guidelines for environmental biodegradability of non-ionic pharmaceutical surfactants and related compounds, allowing a structured comparison of molecular features that influence environmental degradation. By systematically comparing biodegradation data from this study, together with relevant findings from existing literature, we have identified structure-biodegradability relationships, which are summarized in Table 2. These include the influence of MW, functional end groups, spatial configuration, solubility, and molecular conformation. Further, we analyzed differences in lag phase duration as an indicator of microbial adaptation. This approach enabled the determination of the influence of these individual molecular parameters and their variation between different polymer types. Structure-specific dependencies could be derived that govern biodegradability. Based on this analysis, we rated the degree of influence of each molecular feature on biodegradability from low to medium to high. These results revealed clear differences in degradation behavior depending on polymer architecture. It fulfils two essential functions: it facilitates direct comparisons between different

surfactant derivatives, while identifying structural features that enhance biodegradability, thereby supporting the development of environmentally degrading pharmaceutical surfactants and polymeric materials.

In addition, our study showed that the selection of the specific OECD screening test has a significant impact on the assessment of biodegradability. CFU determinations showed that the OECD 301D inoculum contained approximately 3 cells mL^{-1}, whereas the OECD 301F test had a microbial density of 10^4 cells mL^{-1}, reflecting a clearly higher microbial load. This difference likely contributed to the variation in test results, with lower CFU counts in OECD 301D increasing the likelihood non biodegrading. The increased microbial density in OECD 301F provided information for substances requiring longer adaptation periods or sequential degradation mechanisms as in the case for PS. In view of these results, the choice of test plays a crucial role in the assessment of biodegradability and should be carefully considered when interpreting the results. From a modelling perspective, these findings on experimental conditions are particularly important for the successful development of QSAR models for polymer biodegradability. In order to ensure predictive accuracy, it is essential to avoid the inclusion of false negatives, as these can compromise model reliability. More realistic data inputs, especially from test conditions that better reflect real microbial environments, are necessary to improve model performance and predictive reliability.

Table 2 Comparative overview of the main molecular features influencing the biodegradability of non-ionic surfactants, including their dependencies, the observed biodegradation behavior (including lag phases) and representative examples. The degree of dependency is ranked from low (✓) to medium (✓✓) to high (✓✓✓), reflecting the relative influence of each parameter on microbial adaptation and degradation outcomes.

Parameter	Degree of dependency	Impact on biodegradability	Representative Example
Molecular Weight	PEG/POE: ✓	Readily biodegradable at low and high MWs	PEG (up to 30,000 Da)
	PPG: ✓✓✓	High MW impact	PPG 400 (4-day lag phase), PPG 4000 (non-biodegradable)
	POE-PPG POE: ✓✓	MW-dependent transport limits; longer adaptation phase	P184 (9-day lag phase) P188 (non-biodegradable)
Spatial configuration	✓✓	Steric hindrance limits enzymatic access; slower	Ethoxylated PS

		adaptation; slower or incomplete biodegradation	
Functional End Groups	✓✓✓	Ester groups: rapid enzymatic cleavage; short lag phase	PEG monostearate, Span 80 (<2-day lag phase)
		Amine (-NH_2): requires oxidation/transamination; moderate to long lag phase	PEG-bisamine (13-day lag phase)
		Ether (-O-): resistant to biodegradation; no adaptation	PEG dimethylether (non-biodegradable)
Solubility	PEG/POE: ✓	High solubility facilitates biodegradation	
	PPG: ✓✓✓	Low solubility hinders biodegradation	PPG 4000 (not degraded)
	POE-PPG-POE: ✓	Steric hindrance limits degradation	P188 (high solubility, no degradation)
Conformation/ Microbial Uptake	PEG/POE: ✓	Generally favorable uptake	
	PPG: ✓	Favorable at low MW	PPG 400
	POE-PPG-POE: ✓✓✓	Unfavorable conformation; poor uptake; no degradation	P188

5. Conclusions

This study provided new mechanistic insights into the environmental biodegradation behaviour of pharmaceutical non-ionic surfactants, in particular PS and poloxamers, under OECD 301 guideline conditions. By analysing key structural variables and their influence on biodegradation, we identified several critical factors that determine biodegradability, including MW, functional end groups, spatial conformation, water solubility and microbial uptake potential. We also showed that the duration of the lag phase is an effective indicator of microbial adaptive potential, reflecting the ability of microbial communities to adapt for degradation of specific molecular structures over time. Our work addresses a major data gap in the environmental biodegradability of these compounds, which are widely used not only in

pharmaceuticals but also in food and cosmetics. Specifically, we have elucidated the biodegradation pathway and transformation products of PS for the first time. Our findings revealed that high-molecular-weight P188 showed no biodegradation in either the 28-day OECD 301D/F test or the extended 42-day OECD 301F test. This lack of biodegradability is not due to its water solubility or the inherent degradability of its building blocks, but rather to its specific block architecture. The large PEG flanks in P188 likely induce conformations that hinder microbial transport and enzymatic access, thereby preventing degradation even when the polymer is soluble.

As observed in our results under OECD 301 test conditions, PS compounds undergo partial degradation, leading to the formation of ethoxylated sorbitan residues. This partial degradation may suggest that these transformation products are not readily mineralised and could form dead-end intermediates. While the environmental relevance of these residues remains to be clarified, their potential persistence indicates that complete degradation of PS cannot be assumed.

The systematic approach used in this study can be extended to other classes of compounds, as shown here for mainly polymeric compounds. By providing a structured comparison of molecular features and their impact on biodegradability, this study provides a basis for more targeted molecular re- and de novo design of surfactants with enhanced environmental biodegradation. It is crucial to identify and prioritize the influence of these parameters within specific polymer groups, as more data is needed to cluster, understand and confirm which molecular properties are most important for different polymer types.

In addition, the variability in biodegradation results due to different experimental conditions underscores the importance of careful test design. Our results highlight that although OECD 301 screening tests are valuable tools for assessing biodegradability, differences in microbial loading and test protocols, here demonstrated for OECD 301D and OECD 301F, can lead to inconsistencies. To improve the accuracy and reliability of biodegradability predictions, future research should refine the use of lag phase duration as an indicator of microbial adaptation potential and persistence potential. This will be an important additional information to further develop predictive models and support the development of more environmentally benign pharmaceutical surfactants.

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Supplementary Information (Publication 3)

Biodegradation of Pharmaceutical Surfactants: Polysorbates, Poloxamers, and their Derivatives in OECD 301 Screening Tests - Insights into "Benign-by-Design"

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Table S 1 Representative components of each identified group with corresponding chemical formula, detected ion, observed m/z, and retention time [min].

Component	Formula	Detected Ion	Observed m/z	Retention time [min]	Group
Sorbitan-POE-25	$C_{56}H_{112}O_{30}$	$[M+2NH_4]^{2+}$	650.395	3.58	I
Sorbitan-oleate-POE$_{15}$	$C_{84}H_{164}O_{36}$	$[M+2NH_4]^{2+}$	892.582	15.29	II
Sorbitan-dioleate-POE$_{21}$	$C_{84}H_{160}O_{28}$	$[M+2NH_4]^{2+}$	826.586	25.23	III
Sorbitan-trioleate-POE$_{26}$	$C_{112}H_{212}O_{34}$	$[M+2NH_4]^{2+}$	1068.772	31.61	IV

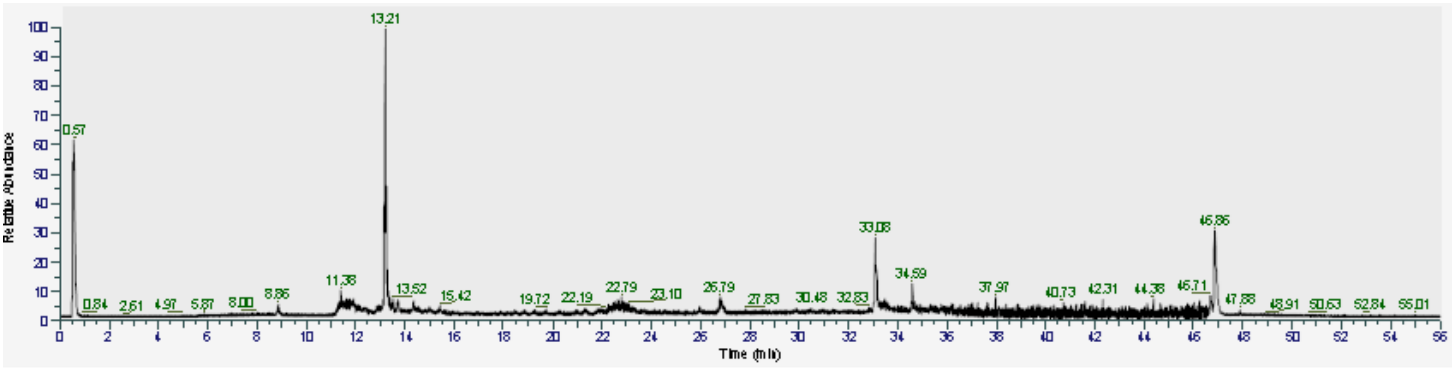

Fig. S 1 Blank TIC

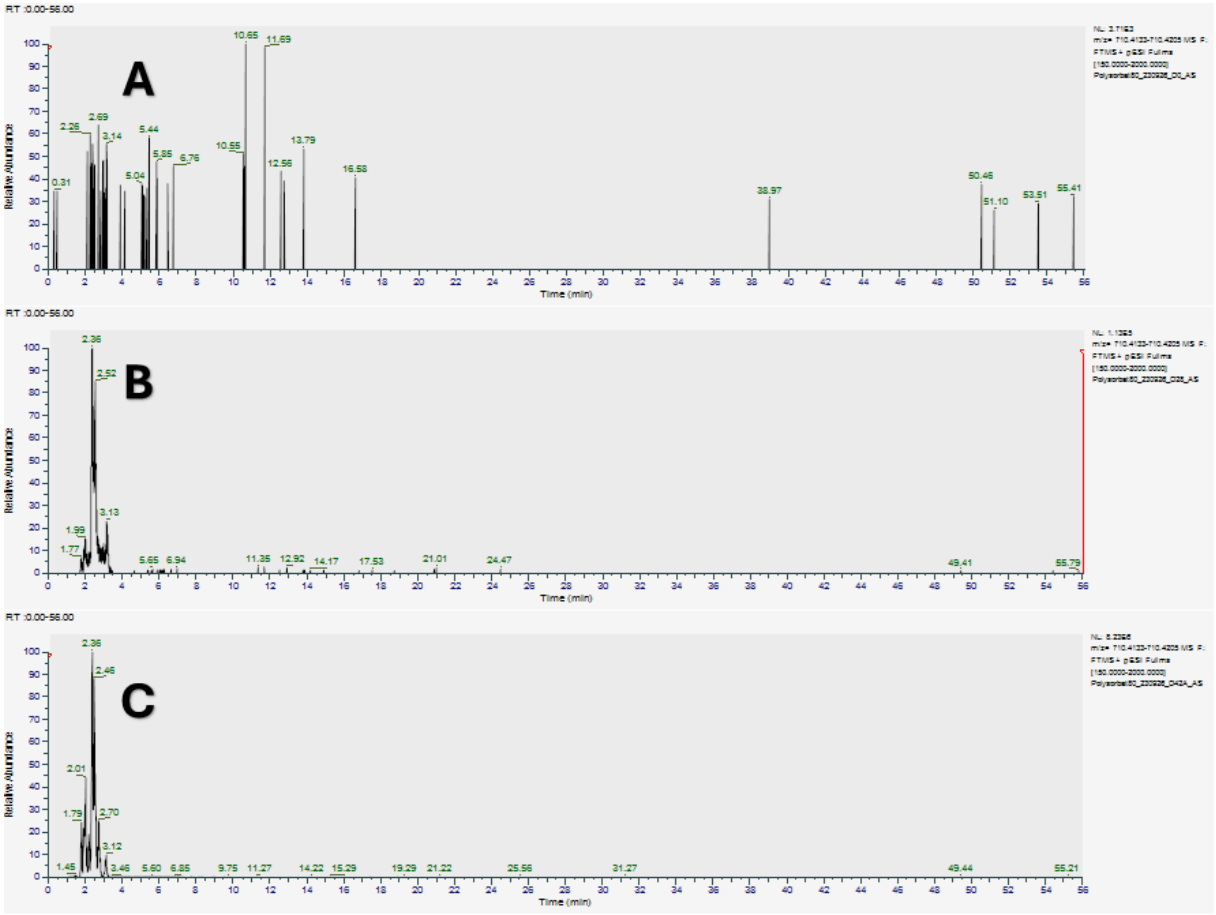

Fig. S 2 MS² Spectra at Day 0, 28, and 42: Detection of carboxylated POE structures (m/z 710.413)

Publication 4

Mila Bading, Evan Griffing, Oliver Olsson, Jake Harris, Jochen Scher, Atsushi Sakurai, Michael Overcash, Klaus Kümmerer (2025)

Assessments of life cycle and biodegradation properties uncovered distinct profiles of pharmaceutical excipients guiding selection for drug formulations

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Assessments of life cycle and biodegradation properties uncovered distinct profiles of pharmaceutical excipients guiding selection for drug formulations

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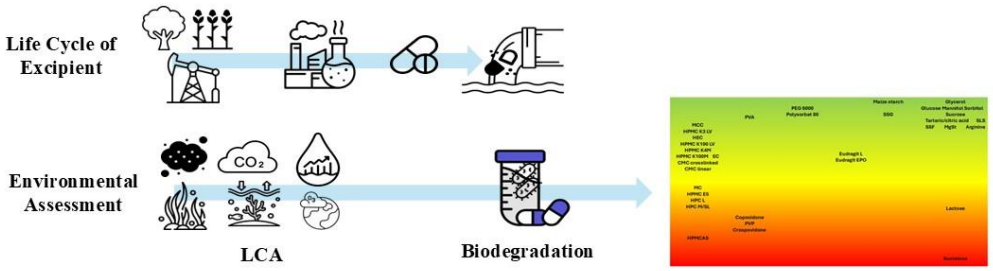
Graphical Abstract



Abstract

Urgent sustainability efforts are needed, particularly in resource-intensive industries such as the pharmaceutical sector. Pharmaceuticals (“drugs”) are made up of active pharmaceutical ingredients (APIs) and excipients. Excipients are essential components in drug formulations. They play a significant role for the applicability of drugs. In recent years the environmental impact of APIs received much attention. In contrast, the environmental impacts of excipients most often are not considered. Here, we systematically evaluate the environmental impacts of 38 pharmaceutical excipients through cradle-to-gate life cycle assessments (LCAs) and environmental biodegradability analysis. This integrated approach provides environmental scores for excipients. Our findings identify critical environmental hotspots, particularly in excipient application fields such as binders. This calls for greener, more sustainable alternative excipients. As a key outcome, the "Excipient Selection Guide" is introduced based on a database which provides data for relative ranking to environmental issues. It will enable the pharmaceutical industry to determine whether new or existing alternatives truly represent a more sustainable choice. The data and the method can be used to design novel, greener, and more sustainable excipients of the future (“Benign by Design”). While focused on the application for pharmaceuticals, the guide’s principles and data are applicable to other sectors, including food, chemistry, cosmetics, and personal care, supporting sustainability across industries where the same compounds are used.

1. Introduction

The contemporary Anthropocene is marked by increasingly global environmental changes that are exceeding the limits of planetary boundaries[1]. The planetary boundaries framework defines *safe* operating spaces for nine interlinked earth system processes encompassing climate change, biosphere integrity, biogeochemical flows, land-system change, ocean acidification, freshwater use, stratospheric ozone depletion, novel entities, and atmospheric aerosol loading[2]. The current transgression of six planetary boundaries including synthetic chemicals which are part of the novel entities urges a global shift towards a more sustainable development to stabilize the Earth system[1]. This urgent need for transformative change, including alignment with the overarching agenda of the European Green Deal, is increasingly evident[3]. As part of this effort, the EU has implemented the 'Zero Pollution Action Plan for Air, Water, and Soil,' intensifying calls for corporate sustainability actions across all industries[4]. Addressing environmental sustainability is becoming a core strategic imperative for the pharmaceutical industry, given its resource, waste- and energy-intensive nature and environmentally polluting profile, which must balance the benefits of individual and public health protection[5-7]. The recent inclusion of the pharmaceutical sector under the EU taxonomy, which sets technical screening criteria to determine environmental sustainability of economic activities, adds pressure to adopt environmental sustainability practices[8]. The EU Taxonomy outlines the preferential use of environmentally biodegradable ingredients, including both active and inactive ingredients (termed ‘excipients’), in drug products to mitigate environmental pollution. However, excipients, which are key components in galenical production have been little investigated with respect to their environmental biodegradation potential, let alone their broader environmental and sustainability profile. Polymers, both water soluble and water insoluble, represent a major group of formulation excipients. A recent inventory study on polymers in household use classified 339 substances into 26 groups. The ten most highly emitted down-the-drain groups include polyol ethoxylate esters, alcohol alkoxylates, polycarboxylates, polyethers and copolymers, starch and derivatives, silicones, polyquaterniums, polyvinyl alcohol, and cellulose derivatives[9]. Notably, among the 26 identified polymer groups, representatives of each group are also widely used in the pharmaceutical industry. Against this background, we recently carried out a comprehensive biodegradation assessment of polymeric excipients commonly used in the pharmaceutical industry[10,11]. However, selecting excipients with a lower environmental impact requires evaluating additional environmental dimensions beyond biodegradability to enable more sustainable choices and avoid regrettable substitutions[12, 13].

Sustainability is a property that refers to the functioning of an entire system and not just from individual aspects[14]. Approaches based on systems thinking in chemistry and pharmacy are essential to determine whether 'greener' excipients, which meet the biodegradability criterion, among others, really do represent a more 'sustainable' alternative[15]. Therefore, this work provides an initial framework for identifying and selecting environmentally friendly excipients, in line with the framework on *Safe and Sustainable by Design* developed by the European Commission[16]. In this regard, a valuable tool for evaluating a defined system in a broader approach is life cycle assessment (LCA). It quantifies environmental impacts associated with all inputs and outputs of a product system throughout the entire life cycle[7]. Still, the application of LCA within the pharmaceutical industry remains fragmented, primarily focusing on analyses of active pharmaceutical ingredients (APIs), encompassing their synthesis and manufacturing processes[17,18]. In contrast, pharmaceutical excipients, being essential components of drug formulations too, have thus far largely been overlooked. Recent studies emphasized the crucial environmental impact of excipient selection in drug formulations, using Ibuprofen tablet production as a model case study to compare two different excipient formulations[19, 20]. The environmental impact was linked not only to process-level energy consumption during manufacturing but also to the environmental footprints of different excipients, including their raw materials and manufacturing processes. The authors concluded that drug formulation scientists should consider environmental sustainability characteristics of excipients to mitigate the environmental impacts of pharmaceutical formulations.

The European Commission estimates that over 80 % of a product's environmental impact is determined at design stage[21]. By integrating systems thinking and related tools early in chemical and pharmaceutical product development along products' whole life cycle, the industry can effectively reduce this impact through sustainability design choices[22,23]. Despite this growing recognition, comprehensive decision-support tools to consider environmental impacts to aid in more sustainable excipient development ("design") and selection during early formulation stages are currently lacking. A significant barrier is the lack of complete life cycle inventory (LCI) data[24,25]. LCI data related to excipient manufacturing are badly documented in the literature, with only a few studies addressing this without publishing the actual inventories[20, 26]. Additionally, existing research often relies on heterogeneous and nontransparent aggregated LCI data of sometimes unknown quality and reliability from various databases like Ecoinvent, making it unclear whether gate-to-gate (GTG) or aggregated data were used to build cradle-to-gate (CTG) inventories, thus impeding quality checks[27].

To address this gap, the first part of this study developed comprehensive (CTG) LCAs to quantify the environmental impacts of manufacturing 33 chemically distinct excipients. A log file detailing the names of the excipients analyzed in this study is provided in the supplementary data for reference ("Excipient Naming List"). These excipients were selected to represent a diverse range of chemical classes and the most frequently used in pharmaceutical formulations. Their chemical diversity makes them particularly well-suited for comparing bio-based and petroleum-based excipients. An engineering design-based approach was used to develop all LCI data[28]. This methodology has the added benefit of consistency as all of the data have been generated with the same methods, boundaries, and calculations. This LCA study is the first to comprehensively assess and compare the multidimensional environmental impacts of pharmaceutical excipients, resulting in a valuable database of excipient LCI data. This database allows for full transparency throughout the entire supply chain, eliminating hidden mass and energy exchanges along the supply chain. Cellulose-based excipients with varying viscosities are among the most used excipient classes in pharmaceuticals[29]. For the first time, LCA analysis was utilized to evaluate the environmental impacts of viscosity reduction processes, addressing a gap in existing research.

In the second part, a novel decision-support tool, the 'Excipient Selection Guide' (ESG), was developed to address the need for holistic assessment methods. This tool combines selected LCA metrics with environmental biodegradability assessments for widely used pharmaceutical excipients. This approach acknowledges the growing importance of increasing the use of biodegradable ingredients in drug products, as outlined by the EU Taxonomy[8]. By integrating CTG LCIA data with biodegradability information following OECD standards, this tool provides a comprehensive framework to guide selection of existing and design of future more sustainable excipients in the early formulation stages. The findings of this research are expected to be applied during the early stages of pharmaceutical development to select or develop greener, and more sustainable excipients using a life cycle perspective. Given that excipients are universally used across pharmaceutical companies, mitigating their environmental impacts through this tool holds significant implications for the entire pharmaceutical industry. Moreover, their use extends far beyond pharmaceutical application, encompassing diverse sectors such as chemistry, food, cosmetics, personal care, and household products, broadening the guide's applicability and potential to drive environmental sustainability across industries.

2. Methodology

2.1 Life cycle assessment (LCA)

A unit process model-based methodology using manufacturing process flow diagrams and engineering design techniques was used to collect GTG inventory data (e.g., process inputs, process emissions, energy requirements, water use etc.). This methodology has been comprehensively detailed in previous work [28,30,31]. The GTGs are assembled to give a full CTG LCI. The boundaries for each CTG LCI included the extraction, production and transportation of raw materials, energy consumption for the full supply chain CTG, and the manufacture of the final excipient. The transportation distances and modes of transportation between GTGs were based on average US data[32]. The functional unit, or basis for all analyses, was 1000 kg of excipient. Chemical trees were created for all the excipients analyzed. A chemical tree is a graphical tool that shows the supply chain with all manufacturing processing steps, and the amount of each chemical product required for each processing step.

PEG 6000 is used as an example here to illustrate this methodology. The chemical tree for PEG 6000 shows all chemicals involved in its production (Table 1), with natural resources, represented in orange boxes, placed on the far right of the diagram. The LCI for PEG 6000 includes four chemicals used in the production process, with gate-to-gate (GTG) inventories performed for each (Table 1). The flow diagrams for PEG 6000, which illustrate all unit processes involved in the production of 1000 kg, are presented in Fig. S1 (Supplementary Data 1.docx). These diagrams formed the basis for calculating all necessary GTG inputs and outputs, such as energy consumption for each unit process, including reactors, pumps, and other equipment involved in the manufacturing process. The same approach was applied to all excipients, covering the chemicals involved in their production and the natural resources extracted from the Earth.

The LCI data were sourced from the Environmental Clarity (https://environmentalclarity.com/) and Environmental Genome Databases (https://environmentalgenome.org/). Detailed reports for all GTG LCIs used in this LCA are available from Environmental Clarity, Inc.

Mass allocation was applied throughout the study. The total energy for each GTG LCI was subdivided into six subcategories: 1. Electricity, 2. Steam, 3. Dowtherm heat transfer fluid, 4. High temperature indirect fuel use, 5. High temperature direct fuel use, 6. Transport fuel, and 7. Heat potential energy recovery. The last subcategory, heat potential energy recovery, is an estimate of energy recovery based on the cooling needs and temperature of each unit process. This lowered the net or total plant manufacturing energy when using heat integration design. Each LCI report included a summary of industrial process literature, process flow diagram, material and energy flows (so called 'elementary flows'), and chemical emissions.

Some of the excipients are derived in whole or in part from plant materials that absorb carbon dioxide from the atmosphere during growth. In LCA practice, these related carbon flows are referred to as biogenic (absorption or negative emissions). When using the cradle to end of life boundary, two accounting systems can be used, either including or excluding the biogenic flows from the GWP impact category. In this analysis, biogenic flows are counted as a negative emission, which is necessary to make a fair comparison between biological and synthetic products at the cradle to gate level. This biogenic carbon uptake partially offsets the carbon emissions associated with the production of these excipients, reducing their net carbon footprint. The excipient supply chains included cellulose, palm oil, corn, potato, sugarcane, soybean, and cotton. To accurately assess net CO_2 uptake from the atmosphere, calculations are based on the carbon content of each of these products, which was estimated based on the protein, carbohydrate, and oil contents of each product. Thus, at the excipient level, the credit was based on the amount used and composition of each of these crops.

This study quantifies the blue water footprint, defined as the net volume of surface and groundwater consumed through evaporation or chemical reaction during excipient production. For agricultural products in excipient supply chains, blue water consumption values were set to irrigation water, using global averages for primary crops as outlined by Mekonnen and Hoekstra[33].

Table 1 Chemical tree of PEG 6000 showing chemical supply chain inventory for producing 1000 kg of PEG 6000. Orange boxes denote natural resources; normal and bold text/numerical values respectively denote unallocated and allocated processes. Each block represents a manufacturing or extraction step.

Key	Natural resources	Unallocated	**Allocated**		
Level 1	**Level 2**	**Level 3**	**Level 4**	**Level 5**	**Level 6**
Polyethylene glycol 1.000	**Ethylene Glycol, di 17,6**	Ethylene oxide 12,8	**Ethylene 9,58**	Naphtha 9,77	blue water 27,4
					oil (in ground) 9,90
			Oxygen 9,37	Air (untreated) 9,38	
		Water for rxn 4,95	Water (untreated) 4,95		
	Ethylene oxide 1.049	**Ethylene 787**	Naphtha 803	blue water 2.251	
				oil (in ground) 813	
		Oxygen 770	Air (untreated) 771		

Life cycle impact assessment (LCIA) data

To analyze the results of the inventories, the environmental impacts of excipients were quantified using TRACI 2.1 (Tool for the Reduction and Assessment of Chemical and Environmental Impacts[34]. In selecting the most relevant impact categories, the approach was aligned with the Process Mass Intensity-LCA (PMI-LCA) streamlined tool, which was developed by the ACS GCI Pharmaceutical Roundtable and included the following seven LCIA metrics[35]:

- Total Natural resource energy (**NREt=NREc**+NREm; NREt: Total extraction of fossil resources.), [MJ HHV]; the total cumulative energy of all fossil fuels used to produce each of the seven process energies listed above. NREt is the sum of Natural Resource Energy Combusted (NREc) and Natural Resource Energy for Materials (NREm), reflecting the total energy value of fossil fuels extracted from the ground. It is akin to the non-renewable fossil component of the Cumulative Energy Demand (CED) metric that is widely used in life cycle assessment.
- Global warming potential, [kg of CO_2 equivalents]
- Blue water consumption, [kg of blue water]; blue water is measured as all water that is removed from the supply chain, including water lost to evaporation and water incorporated into the product.
- Acidification [kg of SO_2 equivalents]
- Eutrophication [kg of N equivalents]
- Stratospheric ozone depletion [kg of chlorofluorocarbon-11 (CFC-11) equivalents]
- Photochemical smog formation [kg of ozone (O_3) equivalents]

Post-assessment of LCIA data

For comparison, LCIA values were normalized relative to the average impact of all excipients across each impact category. Normalized values > 1 indicate impacts that exceed the average, reflecting a more negative environmental performance for the specific indicator. Conversely, values < 1 indicate impacts below the average, representing a comparatively more favorable environmental performance within the impact category. The overall score is the sum of the normalized values from each category. Thus, an excipient that had an average score in each of the seven categories would have an overall score of seven. The excipients were categorized by their overall environmental impact according to the following threshold values:

- Low environmental impact: Normalized values between 0 and 5.
- Moderate environmental impact: Normalized values between 5 and 10.

- High environmental impact: Normalized values between 10 and 15.
- Very high environmental impact: Normalized values exceeding 15.

In addition, emissions from elementary flows were classified as either energy-related or process-related. Process-related emissions refer to chemical releases into air, water, or soil that originate directly from chemical processes (e.g., in a manufacturing plant). These emissions result from chemical reactions, unreacted inputs due to yield limitations, byproducts, or fugitive emissions. To support this analysis, Chemical Contribution Sheets (CCSs) were developed to identify all relevant emissions and differentiate between process-specific and energy-related emissions for each impact category. Process-related emissions from specific GTGs that together contributed at least 10 % to the total impact for a specific impact category were represented in more detail. For each impact category where process-related emissions exceeded this 10 % threshold, we presented the total impact, energy-related impact, process-related impact, and the ratio of process-to-total impact for that specific category. The 10% threshold was chosen to ensure accuracy and focus on the most significant contributions. Additionally, the GTG stages of excipient production that were primarily responsible for these process-related emissions were identified and reported in a separate table. Addressing both energy-related and process-related emissions is crucial for gaining a comprehensive understanding of the environmental footprint in excipient manufacturing. Importantly, emissions are presented before waste management, as the latter includes air and aqueous treatment systems.

2.2.1 Viscosity Reduction Study

In this study, various viscosity grades of hydroxypropyl methyl cellulose (HPMC) were examined to evaluate whether viscosity differences significantly impact environmental considerations. Methocel K was selected from the HPMC type excipients to assess the approximate magnitude of LCIA differences across available market viscosities (viscosity measured as a 2wt% solution in water). The baseline is Methocel K300 M at 300,000 cp viscosity with a degree of substitution (DS) of 1.4 and a molar substitution (MS) of 0.2. All K-type HPMC products have the same 1.4 DS and a 0.2 MS. The progression to each of the four lower viscosities (100,000, 4,000, 100, and 3 cp) utilized a combination of grinding, hydrochloric acid reactant, hydrogen peroxide reactant, electricity, and/or steam. These utilities and chemical inputs (evaluated on a CTG basis) were used to provide the LCIs. A total of 12

patents were reviewed for this analysis that aided in the development of the GTGs here. On a basis of comparison, all GTGs begin with HPMC K 300 M, so that these can all be easily compared with one another. Table S1 shows the progression of the HPMC K grade GTGs, and a brief description of the methodology behind each viscosity reduction process. For all GTGs, sodium bicarbonate is used to neutralize acidic gas as this is the most common method reviewed. Sodium bicarbonate and hydrogen chloride react to sodium chloride, water, and carbon dioxide.

2.2 Biodegradation dataset

From the Institute of Sustainable Chemistry (INSC) at Leuphana University (Prof. Kümmerer's working group), a data set for all 38 excipients based on in-house OECD 301 D and OECD 301 F biodegradation experiments was provided. These OECD 301 screening tests are designed to assess ready biodegradability under aerobic conditions in aqueous environments. The in-house OECD 301D and OECD 301F tests and obtained results have previously been reported (Bading et al., 2024a, b). Briefly, a test compound is considered 'readily biodegradable' if it was degraded by ≥ 60 % within a 10-day window starting after 10% degradation was reached. The 10-day window criterion does not apply to polymeric compounds according to OECD guidelines, as polymers often undergo sequential biodegradation (OECD, 2006). The excipients were categorized based on the degradation levels into the following subgroups, as previously outlined: 'readily biodegradable' (≥ 60%), 'moderately biodegradable' (20–59%), 'slightly/weakly biodegradable' (10–19%), and 'non-biodegradable' (< 10%)[11].

The data were acquired under test conditions of standardized OECD 301 D and OECD 301 ensuring consistency and comparability. The use of OECD 301 methods aligns with regulatory requirements and EU taxonomy criteria for environmental sustainability[8].

2.3 Development of Excipient Selection Guide - ESG

For the development of the ESG for evaluating and comparing the environmental impacts of excipients, an Excel™ spreadsheet was utilized to compile the LCIA (section 2.1) and biodegradation data (section 2.2) of all 38 excipients (spreadsheet is provided in the Supplementary Data 1.xlsx, Sheet: Excipient Selection Guide). To enhance the overview of excipients, we grouped these into 'polymeric excipients' and 'non-polymeric excipients' along

with the CAS numbers and (main) pharmaceutical functions. The polymeric excipients were further categorized into 'cellulose derivatives', 'polyvinyl esters', 'polyethylene glycol derivatives', 'Eudragit derivatives', and 'Starch derivatives'.

The spreadsheet contains the raw (non-normalized) LCIA for each of the seven impact categories and biodegradation percentages for each excipient. To facilitate the comparison and aggregation of both the life cycle impact indicators and biodegradation results, we applied an internal normalization by a min-max feature scaling approach. This method transforms the values for each impact category to a standardized range between 0 and 1, using the equation for each impact category:

$$\boldsymbol{Normalized\ impact} = \frac{\textbf{Impact value} - \textbf{Minimum value}}{\textbf{Maximum value} - \textbf{Minimum value}} \quad \text{Eq. 1}$$

Here, the impact value refers to the specific excipient being analyzed, while the minimum value represents the lowest value across all excipients. The internally normalized LCIA and biodegradation values are listed in the column next to the respective raw values in the Excel™ spread sheet. A normalized score closer to 0 indicates a more favorable environmental profile, while a score closer to 1 suggests a less favorable profile in comparison to the other excipients analyzed for each environmental aspect i.e., specific LCA impact category or biodegradation. To ensure consistency with the 0-1 scale used for normalized LCA impacts, we applied the same min-max feature scaling to the biodegradation data. However, since higher biodegradation percentages indicate better environmental performance, we inverted the scale to align with the LCA impact scores, where lower values denote a more favorable outcome. Specifically, we subtracted the monitored biodegradation percentage from 60 %, as this threshold defines a chemical as ‘readily biodegradable’ according to the OECD 301 guidelines[37]. After normalization, the values were aggregated into a single score ranging from 0 to 8 as eight distinct metrics were assessed in this study, i.e., seven LCA metrics and one for environmental biodegradation. The maximum aggregated score of 8 would represent the comparatively least preferred excipient, while the minimum score of 0 would represent the most environmentally preferred. This procedure enables equal weighting across all categories, thus avoiding the introduction of subjective weighting factors. In addition, a heatmap was generated to visually represent the normalized impact data and aggregated final scores, serving as a graphical embodiment of the excipient selection guide. This heatmap enhances the interpretability of the selection guide by color-coding the environmental profiles of the excipients using a gradient

scale. The color scale allows for quick and intuitive comparison of excipients, with more favorable environmental profiles represented by greener shades and less favorable profiles represented by redder shades. This visual representation helps users easily identify excipients with the best overall environmental performance, providing a clear and accessible way to assess their relative sustainability.

3. Results and Discussion

3.1. Viscosity reduction study

For this study, the Methocel K grade line of products was evaluated to determine the environmental impact of producing less viscous excipients. The key mass inputs and energy requirements for each GTG developed can be found in Table S2. A LCIA was performed on each input component considered for the viscosity reduction process. The raw data are shown in Table S3 which shows the global warming potential (GWP, in kg CO_2), acidification (kg SO_2), eutrophication (kg N), ozone depletion (kg CFC-11), smog (kg O_3), and blue water consumption (kg H_2O) for each of the inputs described in Table S2. As shown in Table S3, going from HPMC K 100 M to HPMC K 3 LV slightly increases the GWP, acidification, and eutrophication. However, it also slightly decreases the ozone depletion, smog, and blue water metrics. The explanation of this is that the whole supply chain of the HPMC K 300 M has a greater influence over these metrics; thus, a slight decrease on the mass requirements decreases the total impact with respect to these three metrics. Even though these metrics slightly increase or decrease, the overall change in these metrics is insignificant. The percentage difference between HPMC K 100 M and HPMC K 3 LV was calculated for each metric with the range being from -0.9% for ozone depletion to 2.1% for Eutrophication. The average LCIA metric percentage difference between the K 100 M and K 3 LV was 0.5 %. Given this insignificance, the viscosity issue is excluded from further assessment of cellulose derivatives in this study. Since most depolymerization processes involve acid-catalyzed hydrolysis of the glycosidic bond, the findings of this study can be extended to other cellulose-based excipients as the cleavage mechanisms are the same. In summary, these results suggest that reducing the viscosity of cellulose-based excipients by chain shortening through hydrolysis has a minimal impact on the LCIA results and therefore viscosity changes can be considered negligible in the following LCA calculations.

3.2 LCA

The LCA covered the production from CTG for all excipients. Detailed chemical trees and CCSs are provided in the supplementary data (Supplementary Data 1.xlsx). Throughout this discussion, references to chemical emissions or related data should be understood as referring to the CCSs unless otherwise stated. For each excipient, Table S4 and Table S5 present both the raw LCIA values and the normalized LCIA values relative to the average of all excipients across the analyzed impact categories. Fig. 1 illustrates this comparison visually in a bar chart, showing the excipient impact relative to the overall average across categories (Fig. 1).

The process-related contributions were assessed based on data presented in Table S6, including total, energy-related, and process-related impacts, as well as the process-to-total impact ratio for each category. Table S7 summarizes the GTG stages that contributed at least 10 % to the total impact in each category. Discussions of process-related emissions refer to Table S6, while Table S7 provides an overview of the corresponding GTG stages.

The results in Fig. 1 show that most contributions to the assessed impact categories were dominated by NREc, representing energy-related emissions. These emissions originated from combustion of non-renewable fossil fuels to meet energy needs, including electricity and plant thermal processes, during excipient manufacturing. NREc emissions included key greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) responsible for the global warming impact category. In addition to greenhouse gases, NREc resulted in the emission of various air pollutants such as sulfur dioxide (SO_2), nitrogen oxides (NO_x). These pollutants were primarily contributors to the assessed environmental impact categories acidification and smog. These findings are consistent with previous research indicating that energy is a major contributor to environmental impacts in chemical manufacturing, with energy consumption accounting for between 50 % and 80 % of impacts in sector[25]. In addition, this analysis further revealed a strong correlation between NREc values and blue water consumption, indicating that higher NREc values are associated with increased blue water consumption. This finding aligns with prior research showing that fossil fuel-powered energy generation is highly water-intensive[36].

Given the dominance of energy-related emissions, NREc was identified as a key driver for the assessed environmental categories of GWP, acidification**,** smog, and blue water consumption. However, for certain bio-based excipients, irrigation water played a more significant role, which

will be further discussed in the following sections. Notably, sucralose showed the highest NREc normalized value at 3.7, almost four times higher than the average of all excipients. Additionally, HPMCAS, with a normalized NREc value of 3.0, was notably energy-intensive due to substantial solvent and sodium acetate salt flows in the GTG process, which require significant energy to recover and recycle acetic acid from large water streams.

For all excipients, we observed that excipients with NREc values ≥ 1 were categorized into either 'moderate' or 'high'/'very high' environmental impact groups, while all excipients classified as 'low' environmental impact had NREc values below 1. Excipients that were not categorized within the 'low' impact category exhibited either high process-related emissions and/or in the case of agriculturally based excipients high blue water due to irrigation.

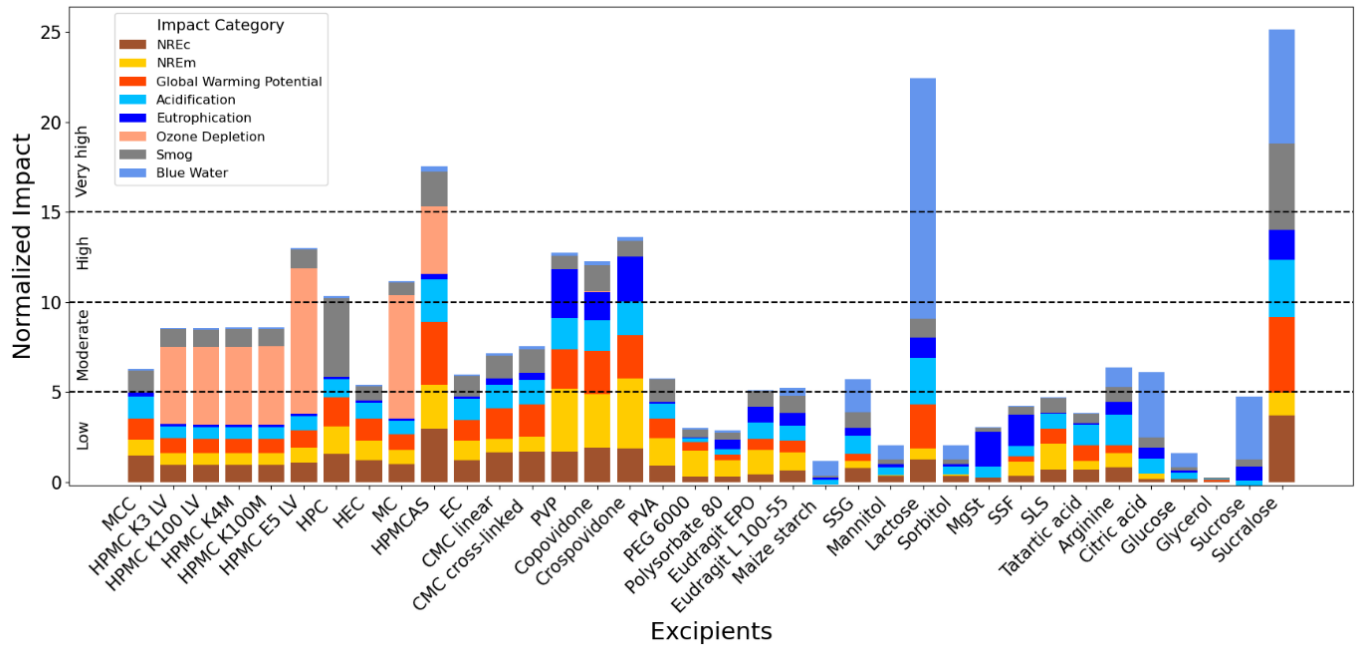


Fig. 1 Environmental impact characterization (normalized impact 0-30) of the 36 analyzed excipients for the eight impact categories, Global Warming Potential, acidification, eutrophication, ozone depletion smog, blue water, NREm and NREc (NREm+NREc =NREt).

3.2.1 High total score (10-15) and very high total score (> 15) impact excipients

Excipients in the 'high' and 'very high' categories represent the least environmentally sustainable options, reflecting substantial environmental impacts. The analysis of normalized LCIA results

across all impact categories revealed that sucralose, lactose, and HPMCAS, were categorized within the 'very high' environmental impact group, with aggregated normalized values exceeding 15. In contrast, the cellulose derivatives MC, HPC, and HPMC E, as well as the N-vinylpyrrolidone polymers PVP, Copovidone, and Crospovidone, were classified within the "high" environmental impact group, with aggregated normalized values ranging between 10 and 15.

Lactose and sucralose were found to be the excipients with the highest environmental impact based on the impact categories assessed. Lactose had the highest blue water footprint (146 6520 kg blue water), which was about 13 times greater than the average (109 873 kg blue water), due to the large amount of water needed to irrigate corn crops used as feed for dairy cows. Notably, lactose showed high process-related emissions in GWP and acidification. About half (51 %) of the GWP was attributed to process-related emissions, which were closely linked to milk production and mainly based on methane and N_2O emissions. Dairy production systems are a well-recognized source of greenhouse gas emissions[38]. In addition, 68 % of the acidification impacts associated with lactose production derived from raw milk production on dairy farms. In the case of acidification, ammonia emissions were the main contributor, with additional emissions from sulfur oxides (SO_x) and nitrogen oxides (NO_x). Dairy production systems are well-known hotspots for ammonia emissions, as livestock production generates substantial nitrogenous waste, primarily in the form of ammonia emissions from manure[39].

The 'very high' environmental impact of sucralose was largely driven by its elevated NREc, which was the highest among all excipients. This resulted in significant impacts across GWP, acidification, and smog categories. Sucralose also had the highest smog value, with process emissions contributing 64 % of the total smog impact. Most of these emissions stemmed from chlorine released into the air, primarily during phosgene production which in turn is needed as chlorinating reagent. Furthermore, as an excipient derived from agricultural sources, the production of sucralose required substantial irrigation water for sugarcane cultivation, which contributed notably to its high blue water footprint.

PVP, crospovidone, and copovidone demonstrated remarkably high NREm values (91637, 102528, and 77809 MJ HHV, respectively), approximately three to four times higher than the average (26268 MJ HHV extracted). NREm reflects the fossil energy resources extracted and embedded in these polymeric excipients, primarily derived from crude oil and natural gas used

as feedstocks in their synthesis. Unlike combustion-related energy use, NREm accounts for the material's embodied energy, contributing to resource depletion but not direct emissions from combustion. As this study focuses on CTG LCAs and not on cradle-to-grave assessment, the embodied energy potentially released at the end of life of excipients is not considered. However, since these substances are excreted by patients and discharged into wastewater, their end-of-life combustion is unlikely, making this issue less relevant.

For methylated cellulose derivatives, ozone depletion potential emerged as a critical factor in their environmental profiles, largely due to the use of methyl chloride as a methylation agent in their production processes. All methylated cellulose derivatives showed 100 % process-related emissions in this category, with higher methylation levels correlating with increased ozone depletion potential. Methyl chloride is recognized as the largest source of stratospheric chlorine, significantly impacting the depletion of the ozone layer[40]. Consequently, HPMC K Grade, with an average DS of 1.4, exhibited the lowest ozone depletion potential, categorizing it within the 'moderate impact group' (see below). In comparison, HPMC E (DS = 1.9) and MC (DS = 1.8) showed higher ozone depletion potential, highlighting the role of methylation in increasing environmental impact.

For HPC, the environmental impact was significantly influenced by process-related smog formation emissions, making this the primary driver behind HPC's comparatively less favorable environmental performance. For smog formation, HPC exhibited the highest process-related smog emissions (around 84%), mainly due to chlorine emissions from the hypochlorous acid production used in the propylene oxide manufacturing process.

3.2.2 Low (0-5) and moderate (5-10) impact excipients

Excipients categorized within the 'moderate' impact group included the cellulose derivatives HEC, EC, CMC, the HPMC K grade derivatives, and MCC. Among the cellulose derivatives, HPMC K grade exhibited the highest impact (normalized value ~ 8.6), primarily due to its significant ozone depletion potential, which is attributed to the use of methyl chloride in its production as discussed earlier. Further, this group included the primarily petroleum-based (as reflected in their NREm values) excipients PVA and the Eudragit derivatives, as well as the bio-based excipients SSG, citric acid, and arginine. The Eudragit derivatives remained below the excipient average (< 1) across all impact categories, except for Eudragit EPO, which had an

elevated normalized NREm value of 1.4. PVA also exceeded the average of the excipients with an NREm value of 1.5. Further, PVA exhibited a comparatively higher smog impact (1.2) with a strong process-related contribution, accounting for 73 % of emissions in this category, primarily due to air emissions of vinyl acetate and ethylene. The environmental impact of citric acid and SSG was primarily driven by their substantial irrigation water consumption.

The low environmental impact group included the following excipients, listed in order of decreasing environmental impact: sucrose, SLS, SSF, tartaric acid, MgSt, PEG, mannitol, sorbitol, dextrose, starch, and glycerol. For the agricultural-based excipients, starch 1500, sorbitol, dextrose, mannitol, and sucrose, the highest contribution to overall environmental impact came from blue water consumption resulting from irrigation. This accounted for 69 % of the total impact for starch, 38 % for sorbitol, 49 % for dextrose, 39 % for mannitol, and 73 % for sucrose. In comparison to the corn starch-based excipients (starch, sorbitol, dextrose, mannitol), the sugar cane-based excipient sucrose had higher blue water consumption, as producing 1000 kg of sucrose required significantly more sugarcane than the amount of corn starch needed for the other excipients. MgSt and SSF were particularly notable for their high eutrophication potential, with nearly all emissions (99 %) being process-related. This was primarily caused by nitrate and phosphate emissions to water sources, by fertilizer use during the plantation phase of fresh fruit bunch production for crude palm oil. Palm oil cultivation is well-known for its extensive fertilizer consumption, which leads to the release of excess nitrogen and phosphorus into aquatic ecosystems and causes eutrophication[41, 42]. Tartaric acid exhibited a higher-than-average acidification potential, with a value of 32 kg SO_2 compared to the average of 28 kg SO_2. Approximately 47% of these emissions were process-related, primarily due to SOx emissions from sulfuric acid production. For the excipients Polysorbat 80, PEG 6000, and SLS, these showed the highest NREm values within this group, reflecting the total energy value of fossil fuels extracted from the ground for the materials itself. Notably, glycerol had almost no environmental impact, as it is a byproduct of the chemical industry (crude glycerol). Crude glycerol is the main byproduct produced during the transesterification process in the biodiesel plant[43].

3.3 Environmental biodegradability data assessment

The excipients were categorized into the following subgroups based on the biodegradation levels: 'readily biodegradable' (≥ 60 %), 'moderately biodegradable' (20–59 %), 'slightly/weakly biodegradable' (10–19 %), and ‘non-biodegradable' (< 10 %) %), as defined by the classification system of the OECD and slightly modified by Bading et al.[11] (Fig. 2). The

biodegradation behavior of the polymeric excipients is detailed in two previous studies; this discussion will focus exclusively on the monomeric excipients[10,11]. Among the 25 assessed polymeric excipients, 12 were classified as non-biodegradable, 5 as slightly biodegradable, 3 as moderately biodegradable, and 5 as readily biodegradable. In contrast, of the 13 monomeric excipients, 12 were categorized as readily biodegradable, and only 1 as non-biodegradable. This demonstrates a broader distribution of polymeric excipients across all biodegradation categories, with nearly half exhibiting persistence potential in the aquatic environment.

The results for the monomeric excipients align with expectations, as this group includes naturally occurring compounds such as sugars, sugar alcohols, fatty acids, amino acids, and acids like citric- and ascorbic acid, all of which have well-established natural degradation pathways. The only monomeric excipient classified as non-biodegradable and therefore potentially persistent is sucralose. This finding for sucralose aligns with the results of a previous study, which observed no detectable biodegradation in an OECD 301 F test using activated sludge[44].

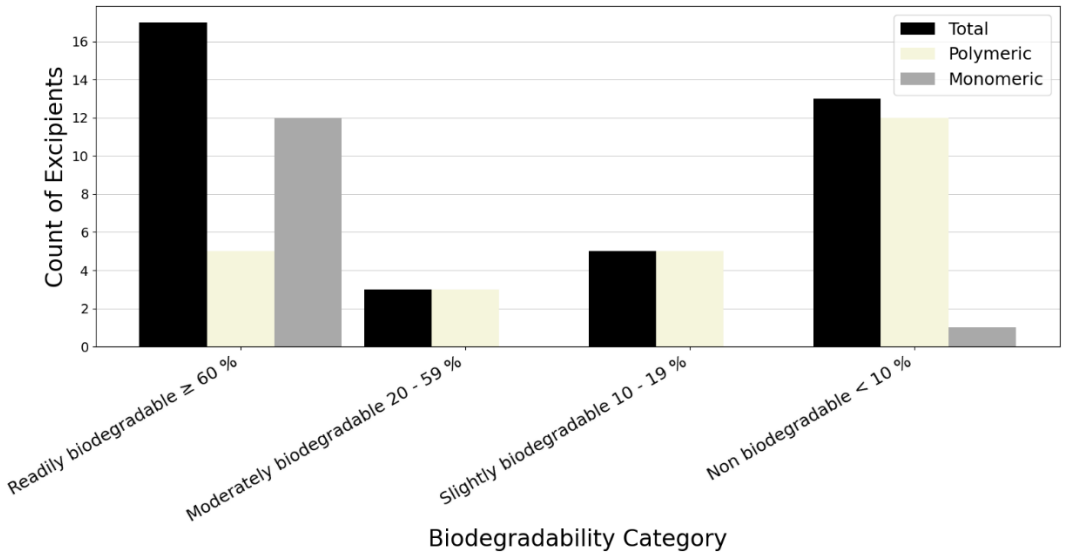


Fig. 2 Biodegradability Distribution of 25 Polymeric and 13 Monomeric Excipients (Total: 38). Classification based on Bading et al.11 into readily biodegradable (≥ 60%), moderately biodegradable (20–59%), slightly/weakly biodegradable (10–19%), and non-biodegradable (< 10%).

3.4 Overall Features of Excipient Selection Guide - ESG

The Excipient Selection Guide (ESG), developed according to the methodology outlined in Section 2.3, is presented as a heatmap that visually displays the normalized scores ranging from 0-1 for each impact category and environmental biodegradability as end-of-life option (Fig. 3). The far-right side of the heatmap displays the aggregated score, which ranges from 0 (most preferred excipient) to 8 (least preferred excipient). This aggregated score is the sum of the normalized environmental biodegradability and the seven LCA impact categories. NREc is not included in the aggregated score to avoid double counting, as it is incorporated into NREt. By presenting each impact category separately before the aggregated score, the guide provides a transparent insight into the environmental performance of each excipient across different indicators, which have been detailed in previous sections. This breakdown allows users to clearly see the contribution of individual categories. The non-normalized values for each excipient, as detailed in the methodology, are provided in the accompanying Excel spreadsheet, which lists the raw data for each impact category and biodegradability percentage. The spreadsheet serves as the detailed data source, while the heat map provides an intuitive color-coded summary for easier interpretation and decision making. This transparency is important for the selection but also for its use as a tool for more sustainable excipients by exchanging respective data.

Most existing studies have concentrated on specific excipients, such as lubricants or formulations with model active pharmaceutical ingredients (APIs)[19,20,26,45]. However, the ability to make robust comparisons across excipients is constrained by the limited availability of studies, which primarily focus on case studies or specific applications, such as the evaluation of lubricants used in pharmaceutical formulations. Additionally, these studies often fail to fully disclose their LCIs and instead rely on heterogeneous LCI data sourced from common databases like ecoinvent[19,20,26,45]. Although ecoinvent is widely used, it often contains proxy data, which is characterized by a lack of transparency and reliance on aggregated information, rather than on measured data for key inventory flows[27]. This reliance on proxy data can lead to inaccurate results, undermine the reliability of environmental assessments, and make it difficult to draw meaningful comparisons across different studies.

High-quality LCI data, however, is essential for conducting comprehensive LCAs of chemicals, including pharmaceutical excipients[46]. In our study, therefore, the strength of the ESG is built

554 on high-quality data developed with standardized methodologies to allow for a high level of
555 transparency and consistency. The biodegradability data herein used are generated in
556 accordance with OECD 301 guidelines, aligning with regulatory requirements and EU
557 taxonomy criteria for environmental sustainability[8]. This standardization enhances the
558 comparability of biodegradability results across different excipients, providing a robust
559 foundation for environmental assessment.

560 To facilitate robust environmental assessments of excipients and to provide reliable decision
561 support, it is necessary to minimize the use of non-specific proxy data in key data sources and
562 instead prioritize specific industry-sourced data whenever possible[27]. However, significant
563 reservations exist regarding access to detailed plant data for every manufactured chemical[47].
564 This study therefore employs a unified dataset developed on literature and patents that reflect
565 real industrial manufacturing processes, addressing concerns and aligning with previously
566 reported findings to most accurately represent real-world plant operations[47]. This design-based
567 alternative to existing heterogeneous LCI data in databases provides very comparable and
568 readily available LCI data. It ensures consistent data collection across excipients, provides
569 repeatable results for improved comparability, and includes all processes and flows without cut-
570 offs. By being consistent in LCI data collection and LCIA method (TRACI), the presented tool
571 ensures comparability and consistency of LCA results providing a solid foundation for
572 developing this ESG (Fig. 3). Using their own specific data would allow ESG users to adapt
573 their evaluation accordingly and conduct a sensitivity analysis in both selection and design
574 applications to identify specific areas for improvement in procurement and design.

575 The reduction of comprehensive data collection into a single aggregated score should not be
576 viewed merely as information aggregation. Instead, it offers an easier way to interpret the LCA
577 results, making these more accessible and actionable for decision-making in pharmaceutical
578 manufacturing and identify weaknesses and the potential for improvements. This aligns with
579 the European Commission's estimation that more than 80 % of the environmental impact of a
580 final product is determined by decisions made at the design stage[21] and the SSbD approach.
581 Therefore, integrating environmental considerations early in the product development process
582 is essential for achieving more sustainable pharmaceutical manufacturing. The ESG is a
583 versatile tool for this.

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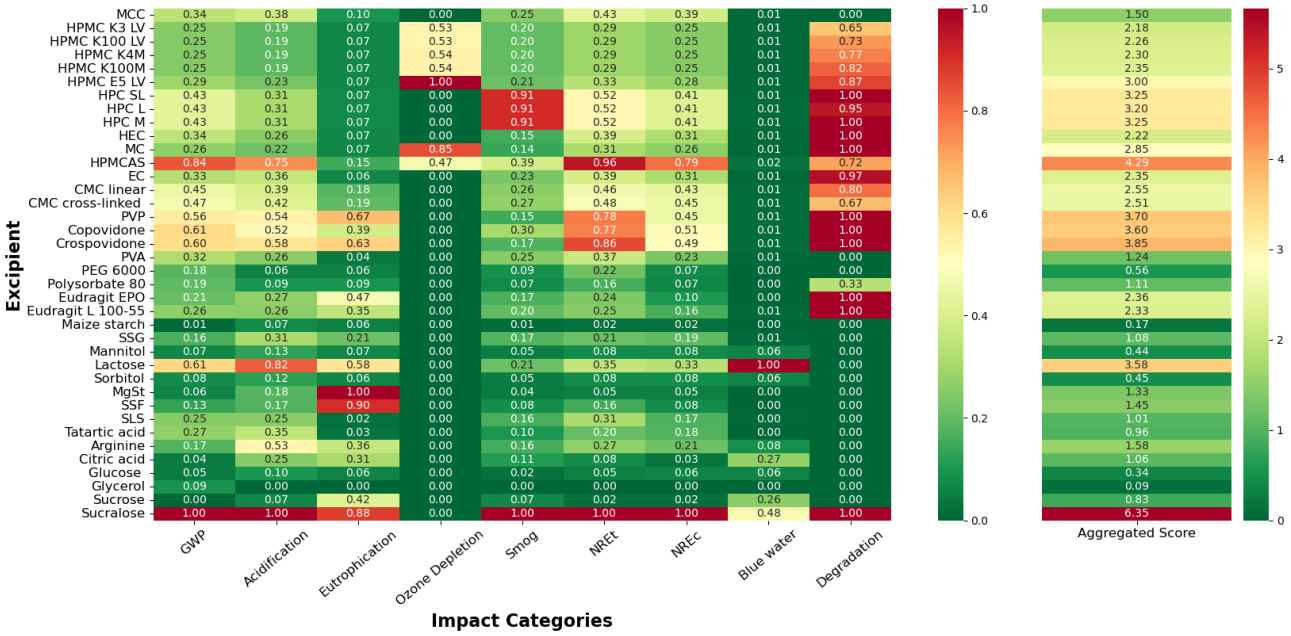

Fig. 3 Excipient selection guide represented as a heatmap, showing normalized values for the assessed impact categories: GWP, Acidification, Eutrophication, Smog, Total NRE, Blue Water NREc, and Environmental Biodegradation. The far-right side displays the aggregated score (0 = most preferred, 8 = least preferred). NREc is excluded from the score to avoid double counting, as it is included in

3.4.1 ESG as Tool for Selection

The ESG serves two key purposes: it provides a comprehensive framework for selecting more environmentally sustainable excipients and supports the development of greener (environmentally benign by design) and more sustainable by design alternatives of the future. One of the ESGs main applications is ranking pharmaceutical excipients based on their aggregated environmental scores, allowing for potential informed substitution decisions. For instance, in the group of disintegrants, the rankings were as follows: SSG with a score of 1.08, CMC crosslinked with 2.51, and Crospovidone with 3.85. This shows that Crospovidone had the least favorable environmental profile, while SSG was the most environmentally friendly (Fig. 3). This ranking approach can be applied to other excipient groups such as fillers, enabling users to compare and distinguish alternatives that may appear generally equivalent when viewed only through the lens of toxicity or performance. This is particularly relevant as excipients are generally considered pharmacologically and toxicologically inert[48]. For example, within the filler group, maize starch emerged as the most environmentally favorable excipient, with an aggregated score of 0.17, while lactose ranked the least favorable with a score of 3.58. These rankings can guide excipient selection by providing additional information on

environmental sustainability, not just functional properties. A striking example highlighting the importance of excipient selection is sucralose. It would be expected to be sustainable, or at least green, including good biodegradability in the natural environment, as it is a natural-based compound. However, according to our findings, it should preferably be replaced in the future due to its high environmental impact during production and its classification as 'non-biodegradable' (Fig. 3). The well documented presence of sucralose in aquatic environments has been shown to affect the physiology and locomotion behavior of *Daphnia magna*, potentially leading to ecological consequences[49]. This further highlights the urgency of considering sucralose's environmental impact and the need for its reconsideration as a future excipient.

3.4.2 ESG as a Tool for Process Improvements

Beyond its role as a selection tool, the ESG also serves as a valuable resource for excipient development and process optimization. By analyzing process-related emissions, excipient suppliers can target specific GTG stages for impact reduction and improved environmental performance. The group of pharmaceutical binders exemplifies this need, as none of the assessed excipients fell within the "green" environmentally favorable range. For example, controlling ammonia emissions (for PVP and Copovidone), chlorine emissions (for HPC), and methyl chloride emissions (for HPMC) could reduce the environmental burdens associated with these excipients. However, the feasibility of implementing technical measures to mitigate such emissions is beyond the scope of this study, as these emissions are inherent to the production processes. Further, a broader driver of environmental impact across excipients identified in this study was NREc, a measure of fossil fuel combustion. Among binders, HPMC derivatives showed better NREc values compared to other binders. However, they exhibited high ozone depletion potential due to the use of methyl chloride in production. This recalls historical issues with regrettable substitutions, particularly with ozone-depleting chlorofluorocarbons[13]. This underscores the necessity of careful excipient selection to mitigate trade-offs across various environmental impact categories. Nevertheless, emissions from fossil fuels remain a substantial challenge in excipient production. In the bigger picture, reducing NREc requires transformative approaches, including the accelerated global deployment of renewable energy and the reduction of fossil fuel combustion in production facilities. However, despite the crucial role of renewable energy in decarbonization, corporate commitments to its adoption and investment remain limited[50]. Therefore, until renewable energy solutions are more widely available and integrated

into industrial processes, selecting excipients with lower NREc values remains a key principle and an essential strategy for improving environmental sustainability.

3.4.3 ESG as a Design Tool

While incremental improvements, as discussed in Section 3.4.2, are important for reducing the environmental impact of excipients, more disruptive innovations are essential to achieve significant sustainability gains. To truly transform the environmental footprint of the pharmaceutical industry, it is necessary to develop new value chains and processes that go beyond the optimization of existing excipients. These innovations may involve the creation of entirely new excipients with improved environmental profiles, particularly those with enhanced biodegradability and reduced environmental impact during production. Such disruptive changes would require a rethinking of current approaches to excipient development and production, with a focus on sustainable redesign and even the design of excipients from scratch.

3.4.4 Future Considerations for Environmental Trade-offs

The ESG developed in this study integrated LCA with environmental biodegradability, a key parameter in environmental risk assessment. This combined approach addressed distinct stages of an excipient's lifecycle, including all CTG manufacturing stages and end-of-life biodegradability. This aligns well with recommended approaches, as a shift from decoupled assessments of risk and sustainability for decision making is needed to identify and minimize risk-sustainability trade-offs through a more overarching and holistic approach (systems thinking)[51,52].

The results from our integrated approach highlighted critical trade-offs between environmental performance during production and post-use environmental biodegradation. For instance, lactose exhibited high environmental biodegradability, making it environmentally favorable at the end-of-life stage. However, its production posed significant environmental challenges, particularly in terms of blue water consumption, GWP and acidification impacts (Fig. 3). Conversely, Eudragit derivatives displayed comparatively lower impacts during production but raised concerns regarding environmental persistence post-use. These examples highlight the need to address such trade-offs and underline that a full life cycle-based assessment and SSbD thinking is needed. It also suggests that one should think about entering the hitherto unexplored

space of the chemical universe. History shows that failing to account for environmental trade-offs can shift burdens, increasing the risk of regrettable substitutions in the future[13,53,54]. This underscores the importance of systematically identifying and evaluating environmental trade-offs to support informed decision-making including new products and enhance thereby environmental sustainability. This approach consistently broadened the assessment scope to uncover environmental trade-offs across the full lifecycle of pharmaceutical excipients. Aligned with the SSbD framework, which advocates among other points for the integration of risk assessment with LCA, this approach provides a clearer understanding of both the direct and indirect environmental impacts associated with pharmaceutical excipients. A key factor in this process will be prioritization approaches that consider usage volumes and concentrations not only in pharmaceutical products but also in other industries. The recent prioritization study by Brunning et al. focused on polymers used across various industrial applications rather than being specifically tailored to the pharmaceutical sector. However, the findings from their study reinforce the broader relevance of our approach, as our assessment also serves multiple industries beyond pharmaceuticals[9].

4. Conclusions

This study provides, for the first time, a consistent and systematic assessment of critical environmental indicators of pharmaceutical excipients, addressing the need to monitor key interlinked planetary boundaries. Given the complexity of environmental systems, it is essential to evaluate in a precautionary manner multiple environmental impact categories, as distinct planetary boundaries are influenced by various interconnected environmental pressures. Specifically, our study provides an extensive collection of important data on 38 separate excipients which allows for a first ranking. Notably, pharmaceutical binders (N-vinylpyrrolidone polymers, HPC, and HPMC) emerged as a particularly critical group because the environmental profiles did not fall within the "green" or sustainable range.

Current frameworks for environmental sustainability are largely conceptual or theoretical, mainly due to a lack of comprehensive environmental data, particularly life cycle data. However, life cycle considerations are essential to fully understand the environmental impact of excipients throughout their life cycle. In their case, the poor data situation is exacerbated by the almost complete absence of environmental biodegradability data. Our study therefore makes a much-needed contribution by demonstrating the value of integrating LCA with environmental

biodegradation. This combined approach helps bridge these data gaps and enhances the ability to make well-informed and sustainable choices.

The results of this study provide a solid basis for identifying environmental trade-offs and improvement potential for existing compounds and processes (incremental improvements) and their new design (disruptive innovations) in the context of excipient substitution, including their manufacture. ESG as an assessment, decision-making and development tool will support the much-needed effort by non-pharmaceutical industries to prioritize excipients for substitution with more sustainable alternatives based on their environmental performance and to avoid trade-offs across industries. Ultimately, while this ESG was presented with a focus on the pharmaceutical sector, its application extends far beyond, offering a practical tool to support decision-making in industries reliant on excipients, including food, cosmetics, and personal care sectors. It can also serve as an example of how to combine multiple aspects of environmental sustainability in a systems-thinking way to avoid point solutions that lead to rebound effects, and to broaden the benign by design approach from its current focus on the environmental biodegradability of chemical compounds, including pharmaceuticals and many others.

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