GREEN CIRCULAR ECONOMY APPLIED TO PEPTIDE SYNTHESIS

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ABSTRACT While the focus of industry was once to cover people's needs, it is now imperative to address how to minimize or even stop generating waste, either by decreasing the amount produced or by recycling and reusing it in the same or another synthetic flow. The closed-loop strategy has gained ground in the context of global resource depletion. In this regard, renewable and sustainable processes are required to benefit both the industrial economy and environment. Here we discuss several aspects related to the circular economy, and how this economic system correlates to green chemistry and its application to the peptide science field.

KEYWORDS: Circular economy, green chemistry, recycling, solid-phase peptide synthesis, sustainability, redesigning, oligonucleotides.

INTRODUCTION

ORIGIN, definition & Perspective

The modern concept of circular economy (CE) arose in China in response to China's unrestrained economic growth and limited resources (1). However, its roots can be found in the XIX century in two books written by Simmonds, who was the editor of *The Journal of the Applied Science*, both entitled '*Waste Products and Undeveloped Substances*', published in 1873 (2). Simmonds called to reusing of the waste either that we generate or that lie around us in abundance. He said, "this book has given various hints that lead to the establishment of profitable economic industries and useful applications of several formerly neglected natural resources".

Later, in 1966, Boulding's book entitled '*The Economics of the Coming Spaceship Earth*' introduced and shaped the concept of sustainability in the economy (3). In 1976, Stahel and Reday introduced the research report entitled '*The Potential for Substituting Manpower for Energy*' into the European Commission and framed the economy in loops, studying its impact on job creation and economic competitiveness, among others (4).

At the beginning of this century, McDonough and Braungart, architect and chemist respectively, published the seminal book '*Cradle To Cradle: Remaking the Way We Make Things*' (5). The main message of this book is that everything can be recycled, a product can feed another product or become a fuel. Thus, such a closed loop means that products are continually circulating as valuable nutrients for industry. Overall, this is equivalent to true sustainability, a situation in which nothing is wasted. The book designs and describes a framework based on three principles: everything is a resource for something else, use clean and renewable energy, and celebrate diversity.

Development of CE has differed between countries and cultures. In this regard, each culture has shaped CE to its vision and needs. Indeed, the term CE has a very broad meaning and can be considered a sustainable development strategy that aims to tackle various concerns (6, 7). The main idea underlying CE is the maximization of the use of resources and minimization of unrecoverable waste, with the end goal of achieving the so-called "zero-waste" (8), in other words, moving away from the linear concept (take-make-use-dispose) to the circular/closed model (9). CE includes the 3Rs (reduce, reuse, recycle) (10), which have been transformed into the 6Rs (reuse, recycle, redesign, remanufacture, reduce, recover) concept for improved product sustainability (11). Analysis of CE demonstrates its vital role in achieving several goals of sustainable development, such as (but not limited to) affordable and clean energy, economic growth, and reasonable production and consumption, among others (6) (Figure 1).

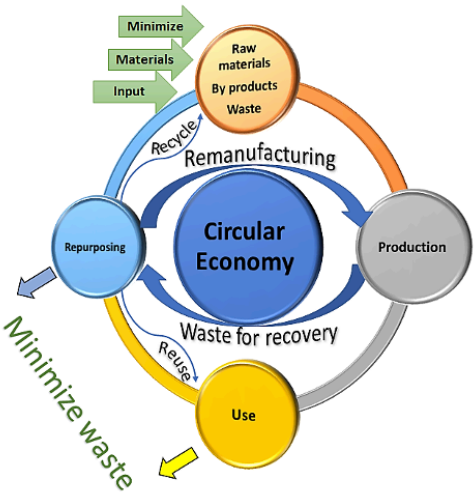


Figure 1. Simplified scheme of the circular economy closed loop. Adapted from (12, 13).

Several limitations associated with the linear concept, including resource scarcity, waste disposal cost, and loss of the residual energy of waste, drove the switch to the circular model. However, the move to the CE concept is not without cost. Given that CE goes beyond the production and consumption ideology, a well-designed process is needed that can drive materials into the circular flow. Furthermore, a complete vision of how to best use the resources and the waste produced afterwards is crucial (14). Furthermore, various processes, such as the reuse of goods, energy recovery, and product refurbishment, among others, must be considered throughout the CE process.

Extra care must be taken to facilitate the implementation of CE in chemical processes. In this context, the complexity of the molecules used in the synthetic process must be kept to a minimum to facilitate their recycling (13, 15). Some materials that support recycling can be added, whereas those that hinder this process should be excluded. A well-designed protocol must be available to ensure the recovery of unused substrates or auxiliary products, in addition to any unexpected side-products (15). The use of renewable resources would tackle resource scarcity, which is one of the challenges that CE strives to solve (15). The validity of CE can be assessed by conducting a circularity metric measurement versus a set of requirements that measure value-added versus the burden of adopting a particular CE protocol, and determining which protocol is most suitable for the purpose (7).

In the context of the field of peptide chemistry, in particular the green peptide synthetic protocol, and taking into account the limitations associated with the synthesis of these complex molecules, we would like to introduce the term 'Green Circular Economy' (GCE) and define it as *'a previously designed closed-loop protocol that aims to utilize minimum amounts of renewable, sustainable, and biodegradable resources, and in which waste will be minimized/recycled, the side products repurposed and the materials regenerated, with the overall aim to benefit the environment, humankind, and to reducecost.'*

CIRCULAR ECONOMY AND GREEN CHEMISTRY

At the time the CE concept was developing, green chemistry was coined by Anastas (16). It is governed by the 12 green principles (17), which can be determined via environmental health and safety (EHS) assessment or life-cycle assessment (LCA) methods. Such approaches focus on sustainability, biodegradability, and recyclability (18). Accordingly, CE overlaps with various principles of green chemistry.

In this regard, Chiang and co-workers did a comprehensive study in which they explained strategies for implementing the 12 green chemistry principles and how they could be integrated with the CE (19). Interestingly, they developed a new categorizing system (prevention, assurance, and sustainability), which correlates with CE in a closed-loop pattern, and then classified the 12 green chemistry principles under each category accordingly (Table 1).

no.	Circular Economy	Green Chemistry
1	Collect and use waste	Prevent waste
2	Maximize atom circulation	Maximize atom economy
3	Optimize resource efficiency	Less hazardous synthesis
4	Strive for energy persistence	Design benign chemicals
5	Enhance process efficiency	Use safer solvents and auxiliaries
6	No out-of-plant toxicity	Increase energy efficiency
7	Target optimal design	Use renewable feedstock
8	Assess sustainability	Reduce chemical derivatives
9	Apply ladder of circularity	Use catalytic (versus stoichiometric) conditions
10	Self-service, not product	Design for degradation
11	Reject lock-in	Real-time analysis of pollution
12	Unify industry and provide coherent policy framework	Minimize potential for accidents

Table 1. The 12 principles of the circular economy versus green chemistry. Adapted from (19).

Orange-prevention; blue-assurance; yellow-sustainability; green-overlaps with assurance and prevention; grey-overlaps with sustainability and assurance; purple-overlaps with prevention and sustainability

It can be appreciated that all 12 green chemistry principles promote the transition to the CE concept. For example, the 'prevent waste' principle encourages the non-generation of waste rather than treating/cleaning it, the 'atom economy and the E-factor' describe process efficiency, the 'catalysis principle' calls for avoidance of stoichiometric amounts of reagents and the 'use of renewable resources'. Together, these principles endorse the limitation of unwanted waste and actively contribute to a CE strategy. Furthermore, comparison of the 12 principles of green chemistry (17) with those of the CE proposed by Slootweg *et al.*(20) shows a clear correlation (Figure 2).

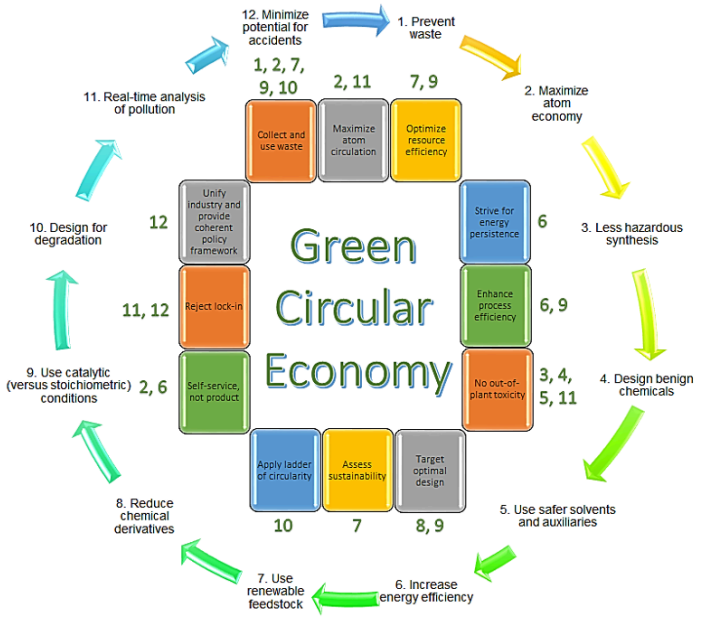


Figure 2. Correlation between the "24 principles" of green chemistry and the circular economy. Numbers refer to each green chemistry principle. Adapted from (17, 20).

CIRCULAR ECONOMY IN CHEMICAL PROCESSES

The chemical manufacturing sector has witnessed various efforts to adopt the CE strategy in synthetic approaches. Helms' group recovered monomers from common additives and even the waste stream, in which, it was used to remanufacture the same polymer without any loss in the performance (21). García's group used polycarbonate waste by taking advantage of its inherent decomposability (under alkaline conditions) and converting it (in one step) to a value-added poly(aryl ether sulfone) (22). CE has also been applied in the photo-flow chemical synthesis of vitamin D_3, where unreacted (7-dehydrocholesterol) was recycled after photo-flow irradiation by dissolving it and vitamin D_3 together (to simulate the real reaction). 90% of vitamin D_3 was extracted from the first run and a total of 99% was achieved after the second run (23).

CIRCULAR ECONOMY IN PEPTIDE SYNTHESIS

The presence of peptides in the pharmaceutical arena is gaining momentum (24). Each year, a number of peptides are authorized by the FDA, with a total of 14 peptides having gained FDA approval over the last 5 years (25). Given the increased demand for peptide entities, a closer look at the application of the CE concept to peptide synthesis is timely, particularly since the "method of choice" for peptide production is solid-phase peptide synthesis (SPPS), a protocol characterized by high use of solvents, mainly in multiple washing steps.

In 1985, Houghten introduced the tea-bag simultaneous multiple-peptide synthesis concept initially for the *t*-butyloxycarbonyl (Boc)/Benzyl (Bzl) strategy(26). Later, Guzman and co-workers extended its application to the fluorenylmethoxycarbonyl (Fmoc)/*t*-Butyl (*t*Bu) strategy (27). The main idea of this approach is that each peptide is synthesized separately in a tea-bag. The deprotection step is carried out simultaneously for all the tea-bags in a bottle that contains the deprotection solution. All the tea-bags are then transferred to successive bottles that hold the washing solutions. Finally, the coupling reaction is carried out for each incoming amino acid simultaneously. If only proteinogenic amino acids are used, then 20 bottles (one for each amino acid) are needed (Figure 3). Once the coupling has reached completion, all the tea-bags are placed in the container with the deprotection solvent to continue the cycle. The strategy complies with the CE concept as the same washing and deprotection solutions, and even the coupling solutions, can be used several times.

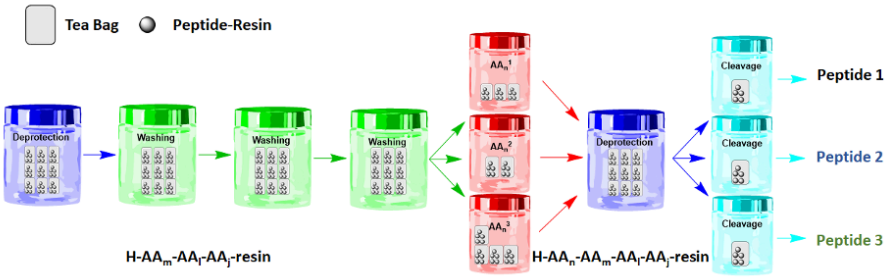


Figure 3. Schematic representation of "tea-bag" simultaneous multiple-peptide synthesis concept. Adapted from (26, 27).

In 2019, during our group's efforts to green SPPS, we successfully reused 2-chlorotrityl chloride (CTC) resin (after proper activation and ensuring that the beads are intact from a morphological point of view (28)) in additional syntheses. The work was exemplified by synthesizing several dipeptides with purities comparable to those achieved in the first synthesis (29).

Pawlas and Rasmussen successfully recycled 86% of the solvent and OxymaPure, which is used as a coupling additive, from the SPPS stream and synthesized a peptide with purity comparable to that obtained with virgin reagents (30).

In 2020, Mainkar and co-workers used a completely recyclable benzoisothiazolone (BIT) redox coupling reagent. Their work was exemplified by the successful recycling of 87% of the BIT activator, giving the final product in 88% yield (31).

It is worth highlighting that Dev and co-workers described the recycling protocol for the coupling reagent ethyl 2-cyano-2-(2-nitrobenzenesulfonyloxyimino)acetate (oNosylOXY). However, no data regarding the performance of the regenerated coupling reagent was reported (32).

Recyclability can be also applied in solution-phase peptide synthesis. In 2015, Zhang and co-workers used 6-(3,5-bis(trifluoromethyl) phenyl)-1H,4H-2aλ^3-ioda-2,3-dioxacyclopenta(hi)indene-1,4-dione (p-BTFP-iodosodilactone) as a hypervalent iodine(III) coupling reagent for the synthesis of dipeptides and also Leu-enkephalin pentapeptide. This coupling reagent can be easily regenerated after completion of the coupling reaction. However, no data from the regenerated coupling reagent were reported in their study. In terms of CE, the use of recyclable reagents is considered advantageous over traditional methodologies (33).

CONCLUSION

CE emerges as a cornerstone to tackle global resource scarcity and pollution while simultaneously boosting economies and maintaining their growth rates. However, success in this endeavor calls for collaboration between different sectors, including academia, industry, and governments. The implementation of GCE in SPPS is still in its infancy, as reflected by only a few reports in the field. However, given the increasing demand for peptide entities, it is expected that further work will allow the transfer of the various synthetic steps of SPPS to the closed-loop model.

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