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Environmental and Sustainability Analysis of a Supercritical Carbon Dioxide-Assisted Process for Pharmaceutical Applications

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Abstract: Drug delivery systems (DDS) are artificial devices employed to enhance drug bioavailability during administration to a human body. Among DDS, liposomes are spherical vesicles made of an aqueous core surrounded by phospholipids. Conventional production methods are characterized by several drawbacks; therefore, Supercritical assisted Liposome formation (SuperLip) has been developed to overcome these problems. Considering that the use of high pressures involves high energy cost, in this paper, sustainability indicators were calculated to quantitatively evaluate the emissions related to the attainment of liposomes containing daunorubicin (a model antibiotic drug) using the SuperLip process. The indicators were depicted using a spider diagram to raise the actual weaknesses of this technique; some variations were proposed in the process layout to solve the critical issues. According to the literature, many studies related to the pharmaceutical industry are expressed in terms of solid, liquid waste, and toxic emissions; however, liposomes have never explicitly been considered for an analysis of environmental sustainability.

Keywords: supercritical fluids; liposomes; pharmaceutical applications; biomedical

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1. Introduction

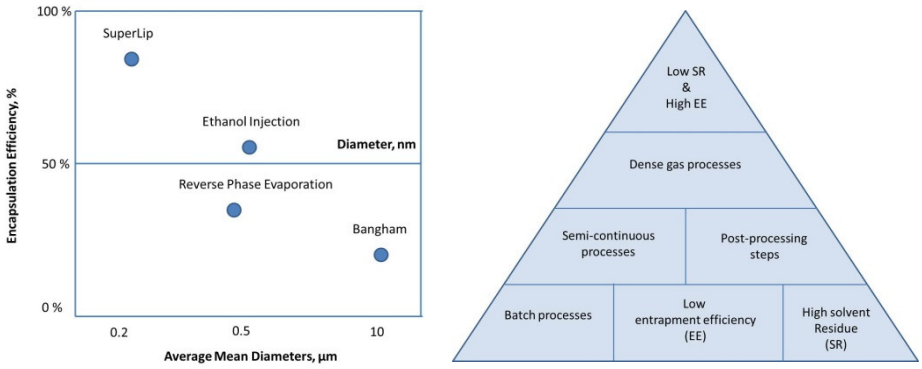
Drug delivery systems (DDS) are artificial devices employed to enhance drug bioavailability during topical delivery [1]. Several systems and complexes have been developed at micro and nano levels to achieve high entrapment efficiency of therapeutic agents [2], targeted delivery to specific human tissues, and improved protection of the entrapped drug from degradation phenomena [3].

Among DDS, liposomes are spherical vesicles made of an aqueous core surrounded by one or more layers of phospholipids [4], generally employed for pharmaceutical [5], cosmetic [6], and nutraceutical purposes [7]. Currently, the main liposomes producing countries are the United States of America, Republic of China, Japan, and the western countries of Europe [8], with the following market share: pharmaceutic industries (61.7%), cosmetics (22.8%), and nutraceutical industries (15.6%) [9–12].

The well-known conventional methods for liposome production are generally characterized by low entrapment efficiencies of active principles and difficult replicability of Particle Size Distribution (PSD), due to discontinuous process layouts[13]. The Supercritical assisted Liposome formation (SuperLip) technique has been recently developed to overcome these problems, consisting of the inversion of the traditional pro-

duction steps of production [14] through an atomization step directly into a supercritical medium containing the phospholipids. This process has been successfully tested for the entrapment of proteins, antioxidants, dietary supplements, dyes, and antibiotics [14,15].

The SuperLip process has been developed primarily at a lab scale; however, its configuration layout is continuous, which guarantees its replicability on a larger scale [16,17]. Comparing to other processes proposed in the literature, SuperLip has several advantages, as described in the sketches reported in Figure 1, where SR represents the Solvent Residue and EE the Encapsulation Efficiency (in particular, see Figure 1b).



(**a**) (**b**)

Figure 1. (**a**) A features map of encapsulation efficiencies and mean size of liposomes produced using different techniques. (**b**) Pyramid sketch of the main advantages and disadvantages of liposomes production (SR: Solvent Residue, EE: Encapsulation Efficiency).

According to the literature published on SuperLip, it is possible to affirm that other well-known processes, such as ethanol injection [18–20] or reverse-phase evaporation [21,22], resulted in the production of larger liposomes (around 500 nm) and encapsulation efficiencies between 40% and 60%. In particular, the conventional Bangham method [23,24] results in vesicles' mean dimensions highly variable, from 1 to 100 µm, and encapsulation efficiencies are generally lower than 30%. Concerning Figure 1b, the bottom of the pyramid is characterized by the worst operating conditions. These processes are characterized by low entrapment efficiencies, and amounts of solvent residues above the Food and Drug Administration (FDA) imposed limits [25]. Therefore, a high solvent residue also means that these processes create liposomes formulations with a high level of toxicity [26]. In the second level of the pyramid, semi-continuous processes and post-processing steps are reported, such as Reverse Phase Evaporation and Microfluidic channel techniques [27], that result in the production of quasi-homogeneous samples. Due to this not optimal homogeneity, vesicles mean dimensions are reduced after extrusion or sonication [28]. At the third level of this pyramid, dense gas processes find a good location, also in terms of reduced solvent residue, thanks to the use of carbon dioxide in supercritical conditions. These processes, such as Supercritical AntiSolvent (SAS) [29], Depressurization of an Expanded Solution into Aqueous Media (DESAM) [30], Depressurization of a CO_2-expanded liquid organic solution (DELOS) [31], and Supercritical Reverse Phase Evaporation (SRPE) [32], were developed to avoid the high cost of post-processing steps, avoiding loss of expensive molecules. These methods were obtained after great technical efforts and encountered a success after proposal in the academic community. Some improvements were still needed to produce liposomes available to be sold in the market with a good balance among profitability, environmental impact, and energy consumption [21,33]. SuperLip process was demonstrated to provide all these advantages.

For the reasons listed above, it was considered attractive to focus on the main advantage of SuperLip: the low solvent residue, as indicated in previous work [17]. To better explain this advantage, a working map has been proposed in Figure 2, creating a strict

correspondence among two important operating parameters (Gas to Liquid Ratio, i.e., feeding ratio, calculated as carbon dioxide over ethanol flow rates on a mass basis, and mean diameter of the liposomes produced). In this diagram, the surface of each circled area represents the concentration of solvent residue obtained in different operating conditions; whereas, the center of each circle is related to a specific Encapsulation Efficiency and a specific Gas To Liquid Ratio. As indicated in Figure 2, vesicles produced with the conventional technique are characterized by a high level of solvent residue (20,000 ppm), measured after evaporation; whereas, small circles are related to liposomes produced with an ethanol residue lower than 150 ppm.

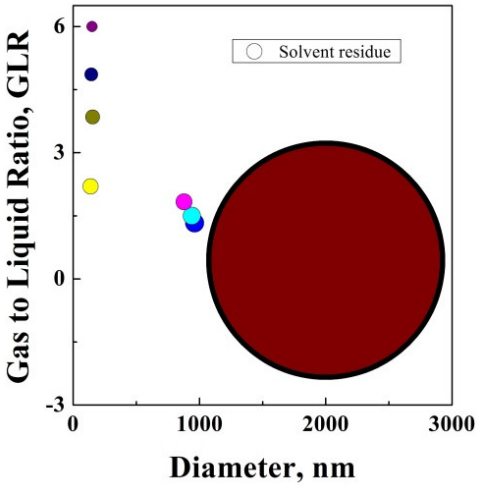


Figure 2. Bubble diagrams: comparison of solvent residue amount among SuperLip process (small circles) and conventional method (large circle). The surface of circles express the concentration of ethanol in ppm, in the final aqueous suspension.

After these considerations, the elimination of solvent residue becomes fundamental in pharmaceutical processes [34]; in particular, SuperLip eliminates large parts of its solvent from the top of the main process unit. The remaining amount can be eliminated using rotary evaporation performed on the recovered liquid suspension. This step can avoid the pharmaceutical formulations to be toxic for cells [18,34]. Moreover, the commercial profitability of SuperLip has been already demonstrated, in terms of economic and financial analysis [35].

Solvent residue causes a significant environmental impact during the production of drug carriers. Therefore, the most common way to calculate the environmental impact is represented by the analysis of sustainability indicators, or the Life Cycle Assessment, largely used in many fields, such as energy [36], beverages and foods [37–41], pharmaceutical delivery [42–44] systems, cosmetics [45], and wastewater treatments [46]. Concerning pharmaceutical industry, a few papers [43,47–49] are related to the management of solid waste and solvent treatment; moreover, liposomes have never been considered for a sustainability evaluation.

Therefore, the aim of this work is the assessment of the environmental impact of the SuperLip process. The eco-balance of this technique will be evaluated to study the effects of liposomes production using this supercritical assisted technique, from the acquiring of raw materials and reagents to the manufacture of the final produced vesicles. An inventory of materials employed in this process and energy consumption will be provided, evaluating the inputs and the outputs of the process, and making a final analysis on the results, according to market profitability reference. A model drug such as daunorubicin, which is generally employed against leukemia [50], will be considered for this analysis. The results of this study will also improve the proposed technique and certify its quality,

with the final aim to assess the profitability of a scale-up for this process, to achieve high volumes of commercialization of this liposome-based products.

2. Process Description

2.1. Apparatus

SuperLip process consists of three feeding lines: carbon dioxide is pumped at the flow rate of 6.5 g/min using an Ecoflow pump (mod LDC-M-2, Lewa, Germany), until reaching the pressure of 100 bar; an ethanol/phospholipids solution is fed at the flow rate of 3.5 mL/min, using a high-pressure precision pump (Model 305, Gilson, France). Ethanol and carbon dioxide are first mixed and then heated up to 40 °C, using thin Band Heaters (3 × 120 W, Watlow Italy, Milano, Italy). The carbon dioxide over ethanol feeding ratio is called Gas to Liquid Ratio of the Expanded Liquid (GLR-EL), and it has been set at 2.4. The ethanol+lipids+carbon dioxide mixture is sent to a stainless-steel vessel (500 cm^3) that works at the pressure of 100 bar and temperature of 40 °C, heated using Band Heaters (2 × 400 W, Watlow Italy, Milan, Italy).

A third feeding line sends water (plus a dissolved hydrophilic drug) to the system; another high-pressure precision pump supports this feeding line at the flow rate of 10 mL/min (Model 305, Gilson, France). The water flow rate is atomized in droplets in the formation vessel, using an 80 μm nozzle.

The production of liposomes occurs in the vessel of SuperLip, by first creating water droplets and then the lipid layer around. Liposomes are collected from the bottom of the vessel using an on/off valve. The separation of the ethanol/carbon dioxide expanded liquid occurs from the top of the vessel, where an exit line has been designed. This line is heated at 30 °C using a tubular resistance (275 W, Watlow Italy, Milan, Italy). A stainless-steel separator (300 cm^3) is employed to separate ethanol and carbon dioxide at the pressure of 10 bar. A rotameter (mod. N.5–2500, Serval 115022, ASA, Italy) is used to measure carbon dioxide flow rate.

Liposomes are produced from SuperLip in aqueous suspension. However, a reduced amount of ethanol is still present in the final solution; therefore, liposomes suspensions are sent to rotary evaporation, operating at 30 °C under vacuum at a stirring rate of 120 rpm (for 30 min), in order to eliminate solvent residue without damaging vesicles produced.

2.2. Materials and Procedures

The raw materials for the production of liposomes production are essentially phospholipids, that are provided by several companies such as Sigma Aldrich (Milan, Italy) or Lipoid (Ludwigshafen, Germany). Daunorubicin has been purchased from Sigma Aldrich, Milan, Italy; whereas, distilled water was self-produced using a lab-scale distillation column, separated from the SuperLip plant. Carbon dioxide is provided by Morlando Group, Naples, Italy, and it is stocked into an external tank with a volume of 800 L. The carbon dioxide needs to be cooled using a cooling bath at the temperature of −10 °C; once that carbon dioxide is in liquid state, it is pumped to the system, where it is again heated up to 40 °C. The pumps guarantee the pressure of 100 bar constant to achieve supercritical conditions for carbon dioxide. Ethanol and water are pumped into the system as well. The heart of the production is characterized by ethanol and carbon dioxide pre-mixing and heating, followed by the mixture in the formation vessel, together with the atomized droplets of water + drug. The final product is the liposomes suspension, which is subjected to solvent elimination post-treatment. Ethanol and carbon dioxide are separated from the formation vessel and sent to depressurization and splitting. In Table 1, the process details and main activities are described.

Table 1. Process details and assumptions.

Process	Characteristics and Details
Energy supply to facility	Italian energy mix medium voltage
Production	
Pressurization	t_1 = 0.25 h; carbon dioxide and ethanol supply; energy supply
Operating conditions stabilization	T = 40 °C; P = 100 bar; t_2 = 0.2 h; carbon dioxide and ethanol supply; energy supply
Injection	T = 40 °C; P = 100 bar; t_3 = 1 h; carbon dioxide and ethanol supply; water solution; energy supply
Separation	T = 30 °C; P = 10 bar; t_4 = 1 h
Stocking	T = 4 °C; P = 1 bar; t_5 = 0.5 h
Carbon dioxide supply to facility	Transport by truck, 28 t from Sant'Antimo (Italy) To the University of Salerno (Italy), distance = 67 km
Reagents supply to facility	Transport by truck, 28 t from Milan to the University of Salerno (Italy), distance = 800 km

3. Methodology

As indicated in similar studies [51], this work aims to evaluate the emissions related to the use of the SuperLip technique to produce a liposomal formulation containing an active principle (daunorubicin). The system boundaries are characterized by the operating parameters described in this section and are highlighted in Figure 3. Equipment impacts were not included.

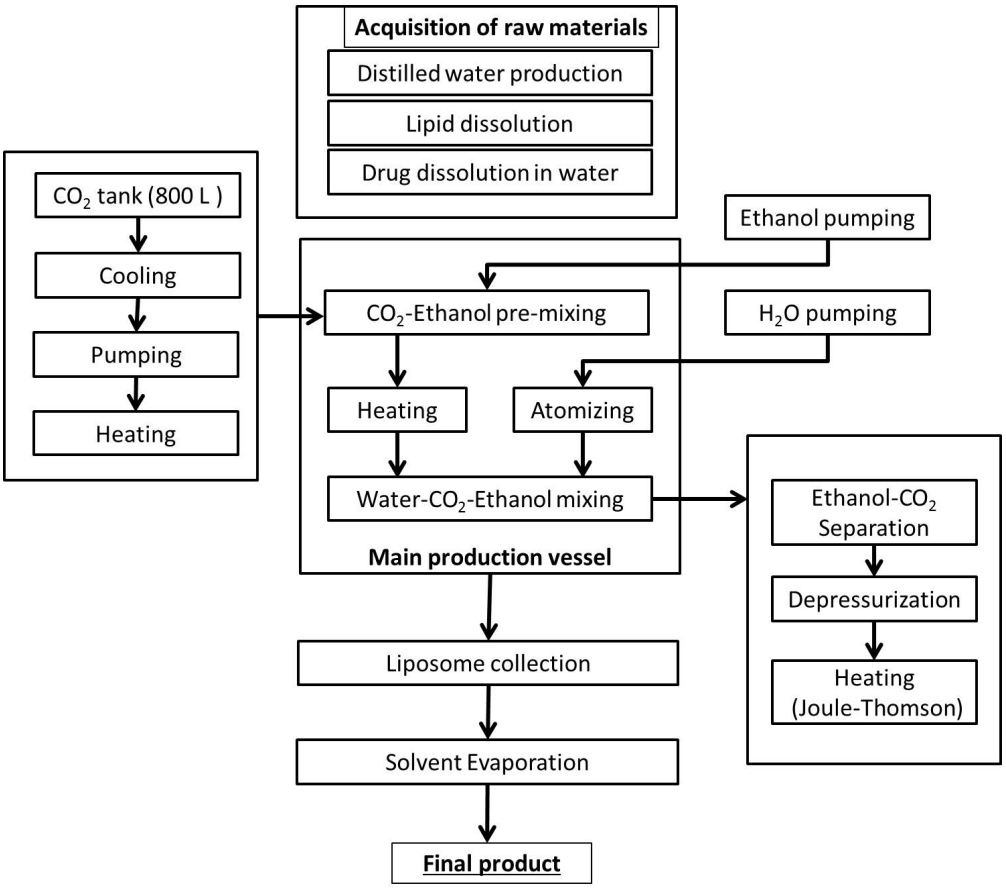


Figure 3. SuperLip system boundaries.

An indicator is an index used to define the sustainability conditions of a working process, giving a practical sense and perception of the system. It generally does not work as a preliminary index, but it contains information about an already developed phenomenon or process. It is a way to give a precise meaning to the raw data of the process.

Due to the large number of collected values related to a process plant and several existing indexes, the application of this methodology to different processes could be difficult. For this reason, a sustainability scale can be defined by enclosing two scenarios representing the best case (100% sustainable process) and the worst case (0% sustainable). The final score related to each indicator is represented by a combination of worst, best case, and actual value, i.e., the real value of the parameters measured in the process. The general formula is the following:

$$\text{Percent Score} = \frac{\text{Actual- Worst}}{\text{Best - Worst}} \times 100\%$$

Sustainability indicators were studied according to the calculation of the following percent scores [52,53] defined in Table 2.

Table 2. Sustainability indicators calculation formula.

Formula	Best	Worst
$\text{Global warming potential} = \frac{\text{Total mass of } CO_2 \text{ released}}{\text{Mass of product}}$	No CO_2 released	All CO_2 released
$\text{Global warming intensity} = \frac{\text{Total mass of } CO_2 \text{ released}}{\text{Sales revenue}}$	No CO_2 released	All CO_2 released
$\text{Specific energy intensity} = \frac{\text{Total energy of the process}}{\text{Mass of product}}$	Min. theoretical energy (Gibbs)	5.85×10^{11} KJ/Kg [54]
$\text{Energy intensity} = \frac{\text{Total energy of the process}}{\text{Sales revenue}}$	0	2.294×10^{9} KJ/EUR [55]
$\text{Specific liquid waste volume} = \frac{\text{Total liquid volume rated as waste}}{\text{Mass of product}}$	0	100%
$\text{Reaction mass efficiency} = \frac{\text{Mass of product}}{\text{Total mass of reagents}}$	100%	0%
Total material consumption= Total mass input *	2.5×10^{-2} Kg	1 Kg
$\text{Mass intensity} = \frac{\text{Total mass input}}{\text{Mass of products}}$	1	40 Kg/Kg [56]
$\text{Value mass intensity} = \frac{\text{Total mass input}}{\text{Sales revenue}}$	0	52 Kg/EUR [57]
$\text{Fractional water consumption} = \frac{\text{Volume of fresh water consumed}}{\text{Mass of product}}$	0	2.95 m^3/kg [58]
$\text{Water intensity} = \frac{\text{Volume of fresh water consumed}}{\text{Sales revenue}}$	0	1.55 m^3/EUR [58]
$\text{Recycled material fraction} = \frac{\text{Recycled mass input}}{\text{Total mass input}}$	1	0 Kg/Kg

* Total material consumption was calculated considering the mass of a unit of product, equal to 0.025 Kg (in this study) in the best conditions and 40 times that value in the worst condition [56].

4. Results and Discussion

The sustainability indicators, whose formulas were reported in the Methods Section, were calculated taking into account the actual values of SuperLip working conditions, considering the best and worst values indicated for each specific situation. Environmental and economic indicators such as Global Warming Potential and Global Warming Intensity were considered. These two indicators need to be shown together; indeed, the

first one correlates the emissions of carbon dioxide (on mass basis) to the mass of product obtained. The second one correlates the emissions of carbon dioxide to the economic value of the products sold. In other words, these two indicators compare the environmental impact and the profitability of the process, in order to understand if the process is lacking in both areas, or lacking in just one of the two. This comparison has the potential to indicate the points of strength of the process and the main weaknesses.

More indicators about energy consumption were also evaluated, providing correlations among power consumption and the mass of products or the sales revenue generated by those products. Another environmental indicator was determined by liquid waste volume and recycled material fraction. The system's productivity was also evaluated in terms of reaction efficiency, i.e., the transformation of raw materials into products through the process. Total mass consumption was put in correlation with the mass of product and also to the sales revenue. Actual values shown in Table 3 represent the real situation of SuperLip working in standard conditions.

Table 3. List of actual values calculated for each sustainability indicator.

Indicator	Description of the Parameter	Value	Unit
Global warming potential	Total mass of CO_2 released	1.06×10^{-2}	Kg
	Mass of product	2.50×10^{-2}	Kg
	Ratio	43%	Kg/Kg
Global warming intensity	total mass of CO_2 released	1.06×10^{-2}	Kg
	sales revenue	27.5	EUR
	Ratio	3.9×10^{-4}	Kg/EUR
Specific energy intensity	total energy consumed in the process	34.7	KJ
	mass of product	2.50×10^{-2}	Kg
	Ratio	1 389.20	KJ/Kg
Energy intensity	total energy consumed in the process	34.7	KJ
	sales revenue	27.5	EUR
	Ratio	1.2629	KJ/EUR
Specific liquid waste volume	total volume of liquid rated as waste (ethanol)	6.58×10^{-3}	Kg
	Mass of product	2.50×10^{-2}	Kg
	Ratio	26%	
Reaction mass efficiency	Mass of product	2.50×10^{-2}	Kg
	Total mass of reagents	3.42×10^{-2}	Kg
	Ratio	73%	
Total material consumption	total mass input	3.424×10^{-2}	Kg
Mass intensity	total mass input	3.42×10^{-2}	Kg
	mass of product	2.50×10^{-2}	Kg
	Ratio	1.370	Kg/Kg
Value mass intensity	Total mass input	3.42×10^{-2}	Kg
	sales venue	27.5	EUR
	Ratio	1.25×10^{-3}	Kg/EUR
Fractional water consumption	volume of fresh water consumed	2.50×10^{-2}	m3
	mass of product	2.50×10^{-2}	Kg
	Ratio	1.0000	m3/Kg
water intensity	volume of fresh water consumed	2.50×10^{-2}	m3
	sales venue	27.5	EUR
	Ratio	9.09×10^{-4}	m3/EUR
Recycled material fraction	Recycled mass input	0	Kg
	total mass input	3.42×10^{-2}	Kg
	Ratio	0	Kg/Kg

Actual values were inserted in the score calculation formula, and the scores for each indicator were obtained in terms of percentage (see Table 4 under the column "before optimization"). In this context, 0% represents "totally not sustainable" and 100% "totally sustainable".

To achieve better control of parameters and increase the previously calculated scores, some modifications could be proposed to the layout of the process. In particular, energy does not require specific intervention. Therefore, no problems were registered in terms of the operating cost of the process.

The weakest points of the process SuperLip emerged as the feeding of CO_2, ethanol, and water. To improve these pumping steps and increase the related sustainability scores, 90% recirculation of ethanol has been proposed via rotary evaporation followed by condensation. The additional instrument energy required is negligible, according to the volumes of production. In this manner, the specific liquid waste volume will be 10% of the previously calculated one.

Another possible modification is the 90% recirculation of carbon dioxide employed in the process. In this manner, global warming potential will be calculated considering only 10% of the carbon dioxide releasing mass.

Consequently, reaction mass efficiency will be positively increased to 85%, and recycled material fraction will become the sum of 90% of ethanol recirculated plus 90% of recirculated carbon dioxide. Moreover, a 50% water recirculation has been proposed to the process after recovering the processing water. The effect of these new calculations and process layout brought to the definition of a new scenario, as expressed in Table 4.

Table 4. Scores calculated before and after optimization of the process.

	Before Optimization	**After Optimization**
Global warming potential	57%	96%
Global warming intensity	100%	100%
Specific energy intensity	100%	100%
Energy intensity	100%	100%
Specific liquid waste volume	74%	97%
Reaction mass efficiency	73%	85%
Total material consumption	99%	99%
Mass intensity	99%	99%
Value mass intensity	100%	100%
Fractional water consumption	66%	83%
Water intensity	100%	100%
Recycled material fraction	0%	45%

The sustainability analysis calculated and reported in Table 4 was translated into a spider diagram (shown in Figure 4), i.e., a visual tool used to organize scores and compare them logically and quickly.

In this diagram, the blue line represents the previous situation, while the orange line is related to the case in the new process configuration; it is possible to say that sustainability indicators significantly increased after process modification (see Figure 4 and Table 4).

According to results shown in Table 4, the differentiation among Global Warming potential and Global Warming Intensity results to be particularly important. Sustainability analysis generally embraces all three spheres of a process: environmental impact, economic convenience, and social impact. In this case, GWP and GWI represent the intersection between environment and economic impacts. In fact, after a careful analysis of this process, it appears very clear that the GWP, that is the environmental impact related to the product mass, has a score of only 57%; on the other hand, the GWI, that is the environmental impact correlated to the sales of the product, has a top score of 100%. In

other words, the economic value of SuperLip products is so high that almost justifies the process carbon dioxide emissions; however, the other indicator shows that the environmental impact is not negligible. Therefore, the idea of modifying the process layout adding a recirculation step, resolves the environmental problem while maintaining a high economic value of the products.

This positive effect was also registered, after the introduction for recirculation, in terms of liquid waste and the fraction of water consumed in the process. The overall variation of the SuperLip layout resulted in an overall increase in the efficiency of the process.

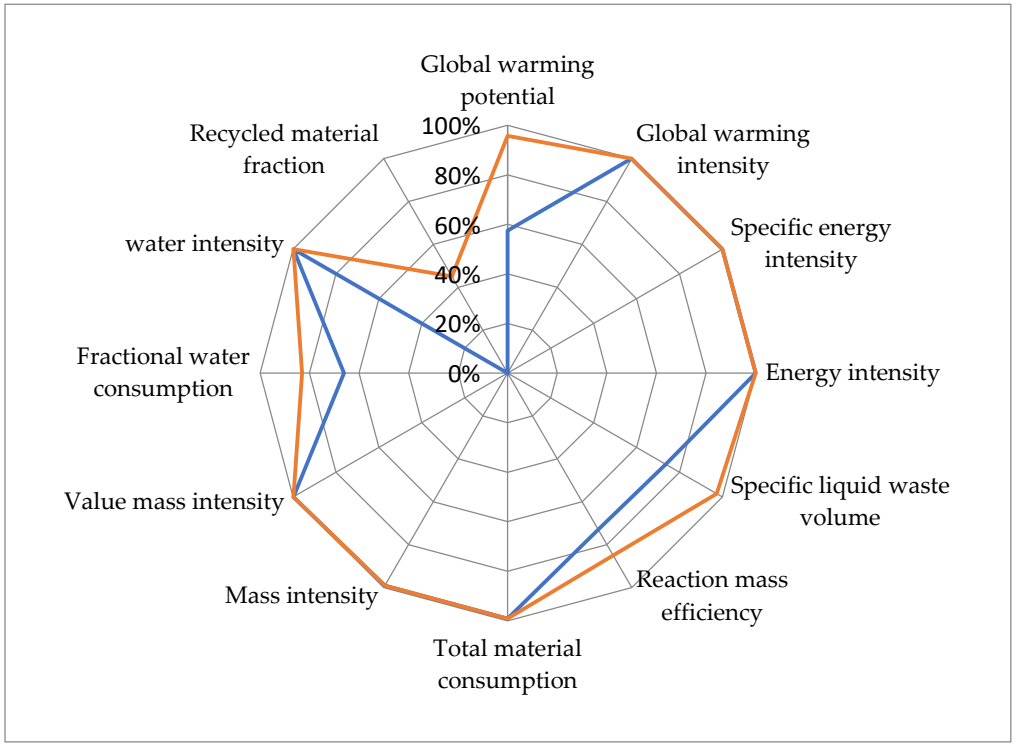


Figure 4. Spider diagram representing the sustainability analysis of the SuperLip process before (blue) and after (orange) optimization.

5. Conclusions

In this work, we started by analyzing a process employed to produce drug carriers at high working pressures. After establishing the process economic profitability, we realized that further analysis on sustainability was needed. Considering the concept of sustainable development, we studied the possibility of using resources without compromising their future availability; in our case, this resulted in a proper recirculation of the process input materials.

The SuperLip process was studied in terms of power consumption, sales revenue, and global warming to balance them simultaneously. The key points were characterized by waste management in terms of recycling, and energy recovery through process efficiency enhancement, reducing the impact of CO_2 emissions on the mass of products obtained from the process. Process indicators were calculated and analyzed in the SuperLip working process, from cradle to grave, not just considering it as a black box.

After proposing recirculation, several indicators improved significantly, such as global warming potential, from 57% to 96%; specific liquid waste volume, from 74% to 97%; reaction mass efficiency, from 73% to 85%; fractional water consumption, from 66% to 83%, and recycled material fraction, from 0% to 45%. The analysis resulted in being successful in demonstrating the sustainability potential of the SuperLip process. Further

studies will regard the possibility of scaling up this analysis to other industrial processes to produce polymeric drug carriers.

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