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Original Article

Practical scaling-up of a four reactants multicomponent reaction (4-MCR)

[Ampliación práctica de una reacción multicomponente de cuatro reactivos (4-MCR)]

Abel Mondelo Rodríguez[1,3*], Denia González León[3], Yamila Verdecia Reyes[4], Rionaldo Forte Mesa[1], Yanier Nuñez Figueredo[2], Estael Ochoa Rodríguez[4]

[1]Laboratory of Chemical Synthesis, Center for Pharmaceutical Research and Development (CIDEM), Havana, 10600, Cuba.
[2]Experimental Pharmacology Laboratory, Center for Pharmaceutical Research and Development (CIDEM), Havana, 10600, Cuba.
[3]Center for Process Engineering Studies (CIPRO), Faculty of Chemical Engineering, Technological University of Havana, Havana, Cuba.
[4]Laboratory of Organic Synthesis (LSO), Faculty of Chemistry, University of Havana, Havana, Cuba.
*E-mail: abel.mondelo@cidem.cu

Abstract

Context: Translation of any chemical process from laboratory to pilot or commercial scale is by no means a simple linear process. This process is influenced by several parameters, such as temperature, agitation speed, the concentration of reactants, and their interactions. In addition, temperature and agitation speed are very related to heat and movement transfers, which are two critical unit operations that affect the scale-up process and reaction yield. A multicomponent reaction (MCR) is a process where more than three reagents react through two or more reactions, producing one biggest molecule. Its scale-up is complex considering the complex nature of the reaction pathway. Our project involves a 4-MCR for obtaining a 3,4-pyridone derivative, a very important product for obtaining a neuroprotective compound. Despite its importance in medicinal chemistry, no report about the scale-up of MCRs was found.

Aims: To develop a practical way to scale up a multicomponent reaction with four components, from laboratory to pilot scales.

Methods: A combination of the brute force method and dimensionless numbers was applied for the scale-up process based on the significant physical forces governing the process.

Results: Experimentally, it was previously demonstrated that temperature and fluid agitation and their interaction are the most significant parameters affecting reaction yield. Its related unit operations, heat and movement transport, were selected as the critical physical forces for the scaling-up process. The dimensionless numbers related to those unit operations are the Nusselt and Reynold numbers. In addition to other criteria applied, those dimensionless numbers were used to calculate the agitation speed for the upper scale. Likewise, as the reaction product precipitated through the cooling process, the obstruction of the reactor discharge valve was observed. Thus, a critical agitation speed was determined at the laboratory scale by applying the Zwietering rule through direct observation of solid sedimentation in the reactor.

Conclusions: The process showed high reproducibility at all scales, reaching good yields of around 73%. Product purity was higher than 99% for all batches. The method applied allows the successful scalability of the process to increase the scale to commercial.

Keywords: brute force method; dimensionless numbers; organic synthesis; physical forces; scale-up; Zwietering rule.

Resumen

Contexto: La traslación de cualquier proceso químico del laboratorio a la escala piloto o comercial no es en absoluto un proceso lineal sencillo. En este proceso influyen varios parámetros, como la temperatura, la velocidad de agitación, la concentración de reactivos y sus interacciones. Además, la temperatura y la velocidad de agitación están muy relacionadas con las transferencias de calor y movimiento, que son dos operaciones unitarias críticas que afectan al proceso de escalado y al rendimiento de la reacción. Una reacción multicomponente (MCR) es un proceso en el que más de tres reactivos reaccionan a través de dos o más reacciones, produciendo una molécula mayor. Su escalado es complejo si se tiene en cuenta la naturaleza compleja de la vía de reacción. Nuestro proyecto consiste en una 4-MCR para obtener un derivado de 3,4-piridona, un producto muy importante para obtener un compuesto neuroprotector. A pesar de su importancia en química médica, no se ha encontrado ningún informe sobre el escalado de MCRs.

Objetivos: Desarrollar una forma práctica de escalar una reacción multicomponente con cuatro componentes, desde el laboratorio hasta escalas piloto.

Métodos: Se aplicó una combinación del método de fuerza bruta y números adimensionales para el proceso de escalado basado en las fuerzas físicas significativas que gobiernan el proceso.

Resultados: Experimentalmente, se demostró previamente que la temperatura y la agitación del fluido y su interacción son los parámetros más significativos que afectan al rendimiento de la reacción. Sus operaciones unitarias relacionadas, transporte de calor y movimiento, se seleccionaron como las fuerzas físicas críticas para el proceso de escalado. Los números adimensionales relacionados con esas operaciones unitarias son los números de Nusselt y Reynold. Además de otros criterios aplicados, esos números adimensionales se utilizaron para calcular la velocidad de agitación para la escala superior. Asimismo, al precipitarse el producto de reacción por el proceso de enfriamiento, se observó la obstrucción de la válvula de descarga del reactor. Así, se determinó una velocidad de agitación crítica a escala de laboratorio aplicando la regla de Zwietering mediante la observación directa de la sedimentación de sólidos en el reactor.

Conclusiones: El proceso mostró una alta reproducibilidad a todas las escalas, alcanzando buenos rendimientos en torno al 73%. La pureza del producto fue superior al 99% en todos los lotes. El método aplicado permite la escalabilidad exitosa del proceso para aumentar la escala a comercial.

Palabras Clave: escalamiento; fuerzas físicas; método de fuerza bruta; números adimensionales; regla de Zwietering; síntesis orgánica.

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AUTHOR INFO
ORCID: 0000-0003-3318-1787 (AMR) 0000-0002-0541-1816 (EOR)
0000-0001-5633-4518 (YNF)

INTRODUCTION

The syntheses of organic compounds have reached a high level of sophistication and excellence, which permits to synthetize a structurally complex compound. In chemical synthesis processes, it not only seeks to generate efficiently new bond formations but else it is desirable a regio- and chemo-selective control too, as well as a stereo-chemical control and reducing possible the stage number of the process (Ocampo Conde, 2013). The discovery of new reactions capable of forming multiple bonds in just one stage, with good yields and respect for the environment, has become a great challenge for organic chemists. In this sense, multicomponent reactions (MCRs) comply with all these criteria and play an innovative role (Akalai, 2016). These reactions incorporate all starting materials (three or more) in the product structure in just one stage, where basically all or most of the atoms contribute to the newly formed product (Ameta and Dandia, 2017; Van der Eycken and Sharma, 2022; Zhu et al., 2015). MCRs provide a new approach to the efficient synthesis of diverse compounds and the generation of a library of compounds. Likewise, MCRs are considered one of the more efficient strategies for synthesizing complex organic small molecules and peptides (Ameta and Dandia, 2017; Jieping et al., 2015; Kielland, 2011; Van der Eycken and Sharma, 2022; Zhu and Bienayme, 2005). Besides, MCRs have often demonstrated higher yields than the classic chemistry. A milestone in this field is the 7-component MCR (Fig. 1) reported by Dömling and Ugi (1993).

Particularly, asymmetric multicomponent reactions (AMCRs) involve the obtaining of chiral compounds through the reaction of three or more reagents simultaneously added (Ocampo Conde, 2013). This kind of strategy shows some advantages over the classic one, such as cost, time, and energy saving, as well as a low environmental assessment (impact) and drastically reduced effort (Ocampo Conde, 2013; Van der Eycken and Sharma, 2022; Zhu et al., 2015). All these advantages, in addition to the high stereoselective level reached in some reactions, have convinced the chemists to adopt this new synthetic strategy or, at least, to consider it as a viable option. Torres et al. (2017) reported the synthesis of 3,4-pyridone derivatives (Fig. 2) through a 4-MCR in the synthetic pathway for obtaining a new neuroprotective compound developed by Verdecia Reyes et al. (2016).

All MCRs, including the AMCRs, hold a privileged position in organic synthesis and are currently gaining momentum in the fields where fast access to high levels of structural diversity is needed. The majority of work has been devoted to the synthesis of heterocyclic adducts from non-heterocyclic reactants (Van der Eycken and Sharma, 2022). This is especially important in medicinal chemistry and key to drug discovery. Merging the synthetic power of MCRs with the particular reactivity of heterocycles leads to an impressive array of new transformations and unprecedented connectivity patterns. These new scaffolds are produced in a straightforward manner, often by simply mixing the reactants, and, due to their combinatorial nature, are amenable to parallelization. Furthermore, the highlighted processes show high levels of structural variability. The reaction discovery based on these heterocycle-based MCRs is already very fruitful, and although it is in its infancy, the description of this uncharted reactivity is paving the way to systematically using these processes in synthetic chemistry (Van der Eycken and Sharma, 2022).

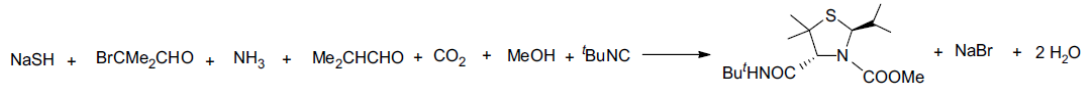

Figure 1. Deömling and Ugi condensation of seven different starting materials (7CC) MCR.

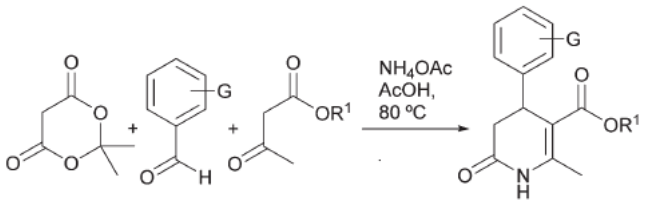

Figure 2. MCR for the synthesis of 3,4 piridone derivatives.

On the other hand, a chemical reaction involves several factors that are specific to the system under study and affect the yield and purity of the product (Hitchin, 2022). Sometimes, the variation of some of those factors leads to undesirable reactions and by-products. In theory, the scale-up of a chemical reaction must consider all factors affecting the process, which change from one reaction to another. For a successful approach, it is important to evaluate and understand the more significant factors due to numerous influential factors in the reaction; only a few numbers of them will control and determine the scale-up behavior. The activities must be directed to identify the key parameters for the scale-up and its understanding (Houson, 2011). Successful scale-up means that larger scale operations keep or improve the results at laboratory or bench scale. As a common practice, chemists perform the scale-up only with an increase in reaction volume. Nevertheless, the performance could completely fail or be poorer than witnessed on a smaller scale. Scale-up must consider several interdependent, flow-sensitive physical processes occurring simultaneously (Paul et al., 2004).

However, it is very complex to determine and evaluate each parameter involved in the chemical reaction. It is important to focus on the main factors or the more significant ones to reach a desirable product. Commonly, reactor scale-up is based on the reactor mode, mixing, and heat transfer (Bonem, 2018). According to Harmsen (2019), there are four major proven scale-up methods nowadays used in industry: Brute force, Model-based, Empirical, and Empirical-model hybrid. Other scale-up methods applied are dimensionless numbers and direct methods (Harmsen, 2019; Zlokarnik, 2002).

In the brute force method, all critical factors and conditions for scale-up are kept the same. In practice, this is a complex process because, typically, at least 1 or 2 key factors will inevitably change upon scale-up. The model-based scale-up is focused on numerical models for predicting the unit operation performance. The models must contain all physical, chemical, thermo-dynamical, and hydrodynamic parameters. An important aspect of a robust result in this method is the validation of the models through experimental data. The empirical scale-up method is based on the experimentation at several scales, often three or four scales. Besides, at each scale, certain parameters must commonly be adjusted to get the desired performance. Thus, it could be considered a trial-and-error method because this method is not very reliable, in addition to being expensive and time-consuming. Also, the real critical scale-up parameters could be unknown. The dimensionless numbers scale-up method involves all dimensionless numbers for a specific process and keeps its value the same at scale-up from the laboratory or bench scale to higher scales (Harmsen, 2019; Zlokarnik, 2002).

The direct scale-up method does not include experimentation and process development. In this approach, a process is directly designed, constructed, and started up at a novel commercial scale. In fact, the critical parameters of the process are unknown, and the process development has a very high uncertainty regarding the results. Thus, it could be considered a blind scale-up. In addition, according to Merrow (2011), this method always fails.

The 5-ethoxycarbonyl-6-methyl-4-(2-nitro-phenyl)-3,4-dihydro-2(1H)pyridone is a small molecule with a chiral carbon atom, which is obtained through an asymmetric 4-MCR synthesis (Fig. 2), developed by Verdecia Reyes et al. (2016). This compound is the first intermediary product in the synthetic route of a new benzodiazepine, a potential neuroprotective compound, which is under pharmacological research (Nuñez-Figueredo et al. 2018; Wong-Guerra et al., 2019; 2021). Thus, the large-scale synthesis of this product will ensure the release of enough quantity of the active pharmaceutical ingredient (API) to carry out all regulatory preclinical assays, stability studies, and phase I clinical trials for two high-incidence diseases: Parkinson's disease and cognitive impairment and Alzheimer's disease. Therefore, the objective of this work is to obtain enough 5-ethoxycarbonyl-6-methyl-4-(2-nitro-phenyl)-3,4-dihydro-2(1H)pyridone to ensure the translation of the new benzodiazepine to clinical trials.

MATERIAL AND METHODS

Materials and equipment

Common Pyrex, Quickfit, and Pobel glassware and a 500 mL glass jacketed cylindrical reactor were used for all laboratory scale experiments. For bench and pilot scales, a 500 mL and a 2 L glass jacketed cylindrical reactor with an anchor agitator at both scales and similar impeller diameter to reactor diameter (d/D) were used to maintain geometrical similarity. Other types of equipment used were laboratory rotavapor Büchi R205, heater bath Büchi B-490, vacuum controller Büchi V-800, vacuum pump Büchi Vac V-500, chiller Büchi B-741; overhead agitator Witeg HS100D, a Pobel magnetic stirrer with a heating plate, Edward 3 vacuum pump, MLW thermostat, VWR Scientific 1162 Poly Science chiller, Sartorius analytical balance, Shimadzu UX620H semi-analytical balance, HPLC – conventional Azura Knauer Technology with autosampler and a Salvis vacuum oven. Chemical reagents were supplied by Sigma-Aldrich. Solvents were pure for analysis from Merck. Distilled

water was obtained at CIDEM with Pobel Desatank 8 distillatory.

Synthesis of 5-ethoxycarbonyl-6-methyl-4-(2-nitrophenyl)-3,4-dihydro-2(1H)pyridone at laboratory scale

A 500 mL glass jacketed cylindrical reactor with a reflux condenser was added 122 mL of acetic acid, and it was connected to the stirrer. An equal amount of Meldrum acid, 2-nitrobenzaldehyde and ethyl acetoacetate, and 1.2 equivalent of ammonium acetate were added to the reactor. The reaction mixture was heated and refluxed for 7 h, and the reaction progress was followed through TLC, 20 × 20 cm Merck silica gel TLC plate 60G F254 over the aluminum plate with n-hexane/ethyl acetate (1/1) and revealed under UV Spectroline® ENF-240C/F lamp operating at 254 nm. After this time, the resulting mixture was cooled at room temperature and filtered by a vacuum. The solid was washed with di-ethyl-ether (Verdecia Reyes et al., 2013). The product was dried at 60°C and 26.7 kPa for 4 h.

Determination of operating conditions for bench and pilot scale

Scale-up experimentation was defined by applying a scale-up factor of 5 regarding mols of the limiting starting reagent Meldrum acid, corresponding to 100 mmol at the laboratory scale, 500 mmol at the bench scale, and 2500 mmol at the pilot scale. For bench and pilot scales, operating conditions kept the best values of the most significant parameters obtained at the laboratory scale in previous work (Mondelo Rodríguez et al., 2017). The scale-up criteria applied to estimate the agitation speed were correlated with the heat and momentum transport unit operation. The dimensionless numbers related to those unit operations were calculated according to equation [1] (Green and Southard, 2019; Kresta et al., 2016).

$$Nu = \frac{h * D}{k} = 0.36 * \left(\frac{d^2 * N * \rho}{\mu}\right)^{2/3} * \left(\frac{Cp * \mu}{k}\right)^{1/3} * \left(\frac{\mu b}{\mu w}\right)^{0.18} \quad [1]$$

Where Nu: Nusselt number (anchor impeller); h: Heat-transfer coefficient; N: agitation speed; D: inside diameter of the vessel; d: impeller diameter; ρ: density; μ: viscosity; Cp: heat capacity at constant p; k: thermal conductivity of the fluid; $\frac{\mu b}{\mu w}$: bulk-to-wall viscosity ratio.

Power per volume unit ($\frac{P}{V}$) (Green and Southard, 2019; Kresta et al., 2016), according to equation [2].

$$\frac{P}{V} \approx N^3 \times d^2 \quad [2]$$

Where P is power; V: volume; N: agitation speed; d: impeller diameter.

Reynold number (Re) (Green and Southard, 2019; ; Kresta et al., 2016; McConville, 2002), according to equation [3].

$$Re = \frac{N \times d^2 \times \rho}{\mu} \quad [3]$$

Where N: agitation speed (min^{-1}); d: impeller diameter; ρ: density; μ: viscosity.

Impeller tip speed (Green and Southard, 2019), according to equation [4].

$$v = \pi \times N \times d \quad [4]$$

Where v: impeller tip speed; N: agitation speed (min^{-1}); d: impeller diameter; π: constant.

Likewise, the Froude number and Zwietering criteria were selected due to the vortex's formation due to the lack of baffles and the evidence of solid precipitation. These two additional criteria were used to determine the critical speed for avoiding the spill of the reaction mixture and keeping 100% of solids in suspension, respectively.

Froude number (Green and Southard, 2019), according to equation [5].

$$Fr = \frac{N^2 \times d}{g} \quad [5]$$

Where N: agitation speed; d: impeller diameter; g: gravitational acceleration.

Speed for just suspension (Zwietering rule) (Green and Southard, 2019; Zwietering, 1958), according to equation [6].

$$N_{JS} \times d^{0.85} \quad [6]$$

Where N_{JS}: agitation speed for just suspension; d: impeller diameter.

Process validation

Process validation was carried out by a continuous verification of the synthetic process (EMA, 2016; ICH Q8, 2017). The temperature of the chemical reaction was monitored every 30 min to determine the starting point of the refluxing time at 90-100°C. After 7.5 h of reflux, the progress of the reaction was checked by thin layer chromatography (TLC) with n-hexane – ethyl acetate (3-1) as mobile phase as acceptance criteria for ending the reaction, the Disappearance of Meldrum acid and 2-nitro-benzaldehyde spots. Product purity was verified by HPLC with diode array detection and gradient elution (Fig. 3). The reaction yield was also considered an indicator of the process validation. The reaction process was replicated eighteen times at each scale, applying the conditions that afford better yields, as reported by Mondelo Rodriguez et al. (2017). The result data for reaction yield and

purity was processed through the statistical software Statgraphics Centurion version 16.1.03. The means and standard deviations were determined.

Determination of agitation speed just for suspension

Five screening experiments were performed at bench scale, changing the agitation speed between 300-500 min^{-1}, increasing it to 50 min^{-1} each time. The reaction mixture was checked, every 15 min by direct observation to determine at which agitation speed 100% of the solid was kept suspended.

Statistical analysis

The variation of data should be expressed in terms of mean ± standard deviation (SD), along with 18 observations. Statistical software Statgraphics Centurion version 16.1.03 was used with 0.05 confidence. The analysis of variance between groups of data of the reaction yields and purity at each scale was performed for a 0.05 p-value. No other additional test was applied.

RESULTS

System classification

The reaction system is completely homogeneous while the mixture is refluxing. Once it is cooling, the product precipitates, becoming a heterogenic mixture, and the sedimentation of solids is observed. From this result, an anchor impeller was selected to provide a tangential flow that drags solids from the wall and bottom of the reactor. In addition, it was necessary to have higher agitation speeds to keep them in suspension. Thus, it was selected a direct observation method, at a laboratory scale, changing the agitation speed from 300 min^{-1} to 500 min^{-1} to determine the critical speed value at which solids sediment.

Synthetic pathway scale-up analysis

Commonly chemists consider the scale-up process as a simple increase in the reagents involved and the reaction volume (Worstell, 2016). They do not think about technological parameters such as agitation speed or some interactions not observed at the laboratory scale. The lack of a theoretical approach and/or an incorrect consideration of it leads to a failed scale-up, including fatal accidents. Observations in bench- or pilot-scale experiments permit the acquisition of a set of data for defining critical parameters and risks for a successful scale-up. Successful implementation means reaching a commercial-scale process with similar results to those obtained at the pilot plant (Harmsen, 2019).

Once demonstrated in previous work, at the laboratory scale, the optimization of the synthetic pathway (Mondelo Rodriguez et al., 2017), through the procedure reported by Verdecia Reyes et al. (2016), showed the temperature and agitation speed as the more significant parameters governing the reaction process. Thus, heat transfer and momentum transport were identified as the critical unit operations for the process scale-up, according to the brute force method (Harmsen, 2013; 2019; Harmsen et al., 2018). From here, Nusselt, Reynold, power per unit of volume, and tip speed dimensionless numbers (Equations 1 to 4) were selected to comply with the critical physical forces of the scale-up. For the scale-up criteria, each dimensionless number was kept equal at both scales affording the Equations 7 to 13. The reaction mixture does not change with the scale increasing; thus, physical parameters such as viscosity, density, or heat capacity at constant pressure remain constant and can be removed from the equations. In addition, sedimentation of the precipitated product was observed at the cooling stage. Thus, it was necessary to implement the Zwietering rule (Equation 6) as additional scale-up criteria for keeping the solids in suspension. That last criteria afford an estimation of the critical agitation speed value at the upper scale, under which the solid sediments obstruct the reactor discharges. Besides, a lot of solids are kept in the vessel, and large volumes of solvent are needed to extract them. Likewise, vortices formation was observed due to the lack of baffles, and it was necessary to introduce the Froude number (Equation 5) to estimate the top critical agitation speed and prevent the spill of the reaction mixture. The geometric similarity was fulfilled using glass-jacketed cylindrical reactors of 500 mL and 2000 mL for laboratory, bench, and pilot scales, respectively. The reaction parameters were selected from the best values determined in previous work (Mondelo Rodríguez et al., 2017). The reaction mixtures at each scale were the same. Thus, the physical properties were fulfilled, too.

Equal Nusselt number at both scales, according to equation [7].

$$\frac{Nu_2}{Nu_1} = \left(\frac{\left(D_2{}^2 * N_2\right)^{2/3}}{\left(D_1{}^2 * N_1\right)^{2/3}}\right)\left(\frac{\mu b}{\mu w}\right)^{-0.03} = 1 \qquad [7]$$

Equal heat transfer coefficient at both scales, according to equation [8].

$$\frac{h_2}{h_1} = \left(\frac{\left(D_2{}^2 * N_2\right)^{2/3}}{\left(D_1{}^2 * N_1\right)^{2/3}}\right) * \frac{D_1}{D_2} * \left(\frac{\mu b}{\mu w}\right)^{-0.03} = 1 \qquad [8]$$

Equal power per volume unit at both scales, according to equation [9].

$$\frac{\left(\frac{P}{V}\right)_2}{\left(\frac{P}{V}\right)_1} = \frac{N_2{}^3 * D_2{}^2}{N_1{}^3 * D_1{}^2} = 1 \quad [9]$$

Equal Reynold number at both scales, according to equation [10].

$$\frac{Re_2}{Re_1} = \frac{N_2 \times D_2{}^2}{N_1 \times D_1{}^2} = 1 \quad [10]$$

Equal tip speed at both scales, according to equation [11].

$$\frac{v_2}{v_1} = \frac{N_2 * D_2}{N_1 * D_1} = 1 \quad [11]$$

Equal Froude number at both scales, according to equation [12].

$$\frac{Fr_2}{Fr_1} = \frac{N_2{}^2 * D_2}{N_1{}^2 * D_1} = 1 \quad [12]$$

Equal Zwietering rule at both scales, according to equation [13].

$$\frac{N_{JS2} * D_2{}^{0.85}}{N_{JS1} * D_1{}^{0.85}} = 1 \quad [13]$$

Chemical reaction scale-up

Experiments were performed, setting the conditions at which better yields were reached in previous optimization studies (Mondelo Rodriguez et al., 2017). Bench and pilot scales were conducted at 410 min^{-1} as was estimated through the selected scale-up criteria. The process was replicated eighteen times at each scale, showing a high reproducibility regarding the reaction yield and purity in all scales studied (Table 1).

The comparative ANOVA analysis of the yields at all scales (Table 2) showed a p-value higher than 0.05; thus, there is no statistically significant difference between the means of the three scales with a 95.0% confidence level, demonstrating the reproducibility of the results. This result suggests that the applied reaction conditions could be successfully used at higher scales, including the commercialization scale. Laboratory and bench scales were performed in the same 500 mL glass reactor because this reaction vessel admits the nominal volume of the reaction for both scales. The pilot scale was performed at the 2000 mL reactor.

The data of the yields at each scale fits a Normal Probability Distribution. The density function (Fig. 4) shows an almost overlapping Gaussian bell for the bench and pilot scale data sets, evidencing a higher reproducibility in its results.

Agitation speed estimation

The agitation speed at the upper scale (bench or pilot) was cleared from each scale-up criteria applied (Equations 8-13). In Table 3, all calculated values of the agitation speed are reported by all criteria considered. Screening experiments, at bench scale, changing the agitation speed between 300-500 min^{-1} showed a range for the agitation speed for keeping the 100% of solid suspended between 350-400 min^{-1}. Six other experiments were additionally performed in that agitation speed range to determine the exact value for avoiding sedimentation in the reactor. This value was applied in the Zwietering criteria (Equation 13) to determine the speed for just suspension at higher scales (both bench and pilot). The critical values of 419 min^{-1}, calculated through the Froude number, and 399 min^{-1}, calculated by Zwietering criteria, established the higher and lower limits for the agitation speed limit. Nevertheless, no one of the criteria analyzed reported a speed value between those limits. Thus, the experimental agitation speed was selected empirically. In addition to the scale-up criteria defined, the heat transfer coefficient scale-up criteria were considered by clearing it from the Nusselt number (Equation 8), reporting 472 min^{-1}, excessively high related to the upper limit speed defined (Table 3).

The value obtained by Reynold's number is illogically low. It is less than half of the lower limit of 399 min^{-1}, established by the Zwietering rule. Nevertheless, Reynold's number is included in the Nusselt number equation. Thus, this has already been taken into account for scaling up (Equation 1). Experimentally, the agitation speed selected was 410 min^{-1}, corresponding with the average speed considering both constraint limits, although it is also low with respect to the value calculated by the heat transfer coefficient criteria.

Table 4 reports the average reaction yield at each scale with the standard deviation. The process was replicated eighteen times, showing high reaction yields and good reproducibility when increasing scales (Tables 1 and 3). Besides, the product showed a very high purity directly from the synthesis, upper than 99% (Fig. 1).

Table 1. Yield and purity of the product at each scale.

Exp. No.	Lab. scale		Bench scale		Pilot scale	
	Yield (%)	Purity (%)	Yield (%)	Purity (%)	Yield (%)	Purity (%)
1	72.5	99.4	73	99.6	71	99.5
2	70	99.4	70	99.4	70	99.6
3	72	99.6	74	99.5	72	99.7
4	76	99.3	71	99.5	74	99.5
5	75	99.8	73	99.7	75	99.5
6	72.2	99.9	73	99.6	74	99.4
7	73	99.5	75	99.5	73	99.6
8	75	99.6	72	99.6	75	99.5
9	70	99.7	71	99.7	70	99.6
10	76	99.5	70	99.5	72	99.7
11	76	99.5	74	99.5	72	99.5
12	72	99.4	76	99.4	76	99.4
13	71	99.3	73	99.6	75	99.5
14	74	99.6	75	99.5	71	99.5
15	74	99.5	70	99.6	70	99.4
16	70	99.6	75	99.7	75	99.6
17	73	99.7	76	99.5	76	99.5
18	75	99.5	76	99.4	75	99.6
Average	**73.15**	**99.54**	**73.17**	**99.54**	**73.11**	**99.53**
SD	**2.03422**	**0.15713**	**2.06155**	**0.09558**	**2.07870**	**0.08819**

Table 2. Analysis of variance for comparing the yields and purity between laboratory, bench and pilot scale.

Source	Sum of Squares	DF	Medium square	F-ratio	p-value
Between groups	5.8086	2	2.9043	0.68	0.5122
Intra groups	253.241	59	4.29222		
Total (Corr.)	259.05	61			

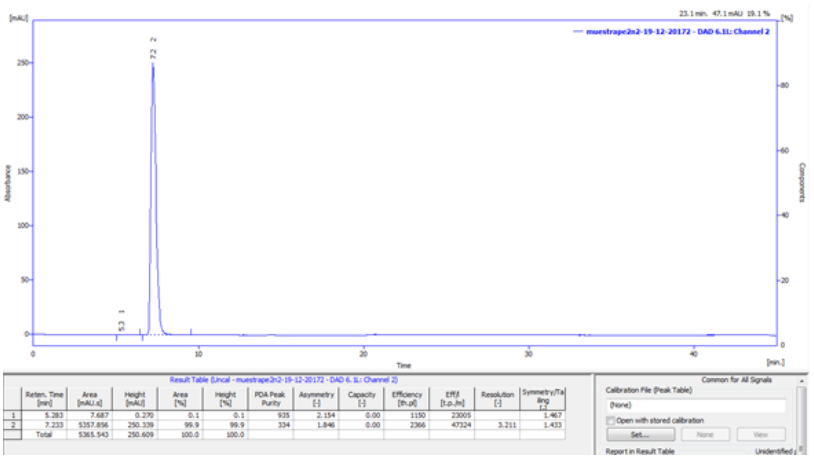

Figure 3. Reverse phase HPLC chromatogram of 5-ethoxycarbonyl-6-methyl-4-(2-nitro-phenyl)-3,4-dihydro-2(1H)pyridine, batch 23006-I (source: Mondelo Rodriguez et al. 2017).

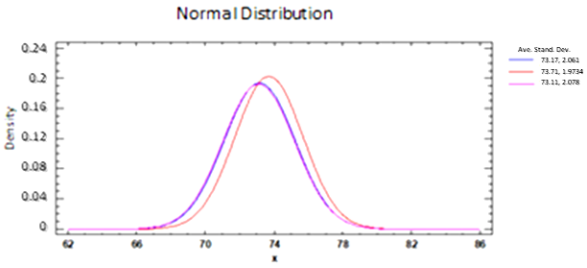

Figure 4. Density function of the yield data set for each scale.

Table 3. Agitation speed calculated by different scale-up criteria.

No.	Criteria	$N_{calculated}$ (min^{-1})
1	$Nu_1 = Nu_2$	230
2	$h_1 = h_2$	472
3	$Re_1 = Re_2$	143
4	$Fr_1 = Fr_2$	419
5	$v_1 = v_2$	293
6	$(P/V)_1 = (P/V)_2$	372
7	Zwietering rule	399

Table 4. Experimental reaction yields at each scale.

Scale	Starting mols of Meldrum acid	Yield (%)
Laboratory	0.1	73.71 ± 1.9734
Bench	0.5	73.17 ± 2.0615
Pilot	2.5	73.11 ± 2.0787

DISCUSSION

Process scale-up was carried out at laboratory and bench scale in a 500 mL glass jacketed reactor and at pilot scale in a 2000 mL glass jacketed reactor. The study permitted the evaluation of the better reaction parameters obtained in previous work (Mondelo Rodríguez et al., 2017). In addition, a technological improvement was performed by introducing a purification step by adding ethyl ether to the reaction mixture once 30°C was reached inside the reactor. In addition, another 30 min of agitation was added to permit the di-ethyl ether to act. This additional step leads to the determination of a product with high purity, determined by HPLC with a diode array detector (Fig. 1), after filtration and washing with ethyl ether. Likewise, the first experiments at bench scale showed sedimentation of the product during the cooling process at relatively lower agitation speeds and the obstruction of the reactor discharge. This behavior was not seen at the laboratory scale due to the lower reaction volume. Thus, it was experimentally determined, by direct observation over agitation variation, the agitation speed just for suspension, which become a critical parameter for scaling-up. This last parameter was included in the scale-up criteria for a successful process (Equation 13).

The agitation speed calculated when the heat transfer coefficient scale-up criteria (Equation 8) were applied showed a higher value of 472 min^{-1}. Nevertheless, it could not be evaluated because of the restriction value introduced by Froude number criteria for avoiding reaction mixture spill from the reactor. Another lower agitation value has not been studied due to the lower agitation speed restriction established by the Zwietering criteria. The agitation speed of 410 min^{-1} was selected based on the average value for moving off the limit restriction values. The experimentation at laboratory, bench, and pilot scale affords a higher yield and purity of the product than the values reported by Verdecia Reyes et al. (2016). In addition, technological improvement allows for standardized results at a larger scale due to the higher amount of product obtained, affecting the filtration and purification process. Process validation permits us to establish process control to identify any deviation from the standard procedure. Continuous temperature control and thin layer chromatography (TLC) were the main process parameters for defining the time for refluxing, end of the reaction, and total reaction time. It is remarkable that the reaction´s time and time of reflux were 12 h and 7.5 h, respectively. Those values did not show variation between scales. In addition, the definition of the reaction ending by TLC allows us to reach a very high conversion at each scale of at least 99%, verified by HPLC. This result constitutes a milestone demonstrating the repeatability of the reaction process by different researchers and at different seasons of the year. The reaction yield and purity at all scales showed a very similar value with a standard deviation around 2.0 and 0.1, respectively. Besides, the analysis of variance, when comparing each scale result, showed that there is no difference between yield and purity between the laboratory scale with the bench scale and the pilot scale. It is important to highlight the importance of controlling

temperature and to check the reaction progress by TLC. Reaching yields higher than the reported yield by the inventors in the patent and keeping its high values and purity directly from the synthesis process is remarkable for the scale-up and manufacturing of the synthetic route for the new neuroprotective API in development. In addition, as an intermediary product of a new neuroprotective in development at CIDEM, the scale-up and process validation affords new experimental data for the registration of the new active pharmaceutical ingredient.

Study limitations

In this study, just one limitation related to the primary standard for HPLC purity determination was encountered. This product is a new heterocyclic compound involved in the synthesis of a new neuroprotective developed by research teams from the University of Havana and CIDEM. Thus, it was necessary to obtain and evaluate the standard.

CONCLUSION

The reaction process was successfully scaled up to a pilot scale using the brute force method combined with the dimensionless number method. The process was validated by a continuous control process through the experimental determination of temperature and the verification of the reaction progress by TLC. The reaction was replicated 18 times at each scale, reaching a reaction yield and purity of around 73% and 99%, respectively, without any complex purification process. The complete study was performed by three different researchers (a senior researcher and two juniors) at different seasons of the year and in two years. This result demonstrates the reproducibility of the process and its standardization. The scale-up process afforded enough products to continue the technological and clinical development of a new neuroprotective API.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Mondelo Rodriguez A	González León D	Verdecia Reyez Y	Forte Mesa R	Nuñez Figueredo Y	Ochoa Rodríguez E
Concepts or ideas	x	x	x	x	x	x
Design	x	x	x			x
Definition of intellectual content	x	x	x		x	x
Literature search	x					x
Experimental studies	x	x	x	x		x
Data acquisition	x			x		
Data analysis	x	x				x
Statistical analysis	x	x				
Manuscript preparation	x					
Manuscript editing	x	x	x		x	x
Manuscript review	x	x	x	x	x	x

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