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Development of a process for Citicoline
Desarrollo de un proceso para la fabricación de *Citicolina*

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June 2018

UNIVERSITAT DE BARCELONA

Quiero agradecer a mi tutor, Dr. José María Gutierrez, por haberme hecho el seguimientio del proyecto. Además, muy especialmente a Anna Campuzano y José Lorenozo, quienes me han dado todas las facilidades y ayuda que he pedido y para acabar, todas las personas que me han apoyado durante el proyecto.

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SUMMARY

Citicoline, is an active principle that treats one of the most common neuropathies, dementia, which in its most severe condition, can develop Alzheimer's. One of the companies that produce this drug, it is Ceuber, being the product that generates more benefit. However, with the appearance of the Asian generic drug, Ceuber decided to study a process change (US Patent 3,684,652), with the aim of protecting its market share.

This study consists of two parts: first, a selection of 18 patents, and it will be selected one, the patent that cumplies better the corporative, such as: the elimination of the chromatographic column. Subsequently, basic engineering will be developed by scaling two processes in Aspen Batch process developer, the current process and the one that best meets the conditions. The last one will be simulated in two different ways, within the conditions described by the recipe, one with low productivity and the other with high, with the possibility of optimizing equipment in the favourable condition...

The results of this project show that these 18 patents, four require a more exhaustive analysis. In this analysis, it is confirmed with notoriety that patent WO2013 / 12893 obtains the best productivity. Finally, the simulation corroborates the high productivity of this process, achieving the maximum by placing two overlapping reactors, in order to reduce campaign time.

Basic engineering is not able to determine that the process meets the quality requirements. However, the information offered by the patent about quality and the high productivity obtained in ASPEN, it promotes to evaluate the process experimentally.

Keywords: Citicoline, patents, batch plant, basic engineering.

RESUMEN

La citicolina, es un principio activo que trata una de las neuropatías más comunes, la demencia, qué en su condición más severa, puede desarrollar Alzheimer. Una de las empresas que producen este fármaco es Ceuber, siendo el producto que genera más beneficio. No obstante, con la aparición del genérico proveniente de Asia, Ceuber decide estudiar un cambio de proceso (US Patent 3,684,652), con el objetivo de proteger su cuota de mercado.

Este estudio, consta de dos partes: primero, una selección de 18 patentes, en el que se elegirá el proceso que mejor se adecúe a unos requisitos corporativos definidos, como por ejemplo: la eliminación de la columna cromatográfica. Posteriormente, se desarrollará la ingeniería básica escalando dos procesos en Aspen Batch process developer, el proceso actual y el que mejor cumple las condiciones. Este último, se simulará de dos maneras distintas, dentro de las condiciones descritas por la receta, una con baja productividad y la otra con elevada, con la posibilidad de optimizar equipos en la última.

Los resultados de este proyecto muestran qué de estas 18 patentes, cuatro requieren un análisis más exhaustivo. En este análisis, se confirma con notoriedad que la patente WO2013 /12893obtiene la mejor productividad. Finalmente, la simulación corrobora la elevada productividad de este proceso, consiguiendo los máximos colocando dos reactores sobrepuestos, con el fin de reducir el tiempo de campaña.

La ingeniería básica no es capaz de determinar que el proceso cumpla los requisitos de calidad. Sin embargo, los datos que ofrece la patente en cuestión de calidad junto con la elevada productividad obtenida en ASPEN, promueven a evaluar el proceso experimentalmente.

Paraules clau: Citicolina, patentes, Batch plant, ingeniería básica.

INTRODUCTION

1.1. HISTORY

Business activity

Ceuber Internacional is a company that was founded in 1959 by Carlos Ceuber, with a view to expanding that internationally. In the 60s, the company gains relevance in the Spanish market through the commercialization of Anginiuvas, Gamusta and Saicosoma. In the decade of the 70s, Ceuber launched its flagship products, it allowed to get recognition in the European market, through the Cemicina (Citicoline) and Centro CMP Forte, a series of Nucleotides to treat peripheral neuropathies.

In the 80s, Ceuber positioned itself in the international market by acquiring companies and opening subsidiaries throughout the continent and entering in the Latin American market. Currently, Ceuber focuses its activity on medicines. However, it also has business areas in other sectors such as research, medical devices and active ingredients.

History of the medication

In 1972, Ceuber acquired the citicoline molecule from Takuda Pharmaceutics, which will be commercialized in 1974 for oral administration, once approved by the Spanish Agency for Medicines and Health Products (AEMPS), and in 1978 the injectable medication. Apart from the molecule, the laboratory also acquires the process patent (US Patent 3,666,748) to manufacture the API and reduce costs.

At the end of the 1990s, with the discovery of the new process to transform citicoline into a new more stable form (Us patent 6,057,301), Ceuber decided to change completely the way to develop the drug. Therefore, the company adopts the patent US 3,684,652 to improve the quality of the drug, because the previous method had a lot of drawbacks, such as the number of reactions, the amount of impurities, low productivity, etc.

Nowadays, after more than 30 years commercializing Cemicina, both orally and in an injectable form, Ceuber decides to optimize the process to manufacture citicoline, in order to ensure its market share because Cemicina is the drug that gives the most benefit, besides the possibility of entry into the western market APIS produced in Asian countries, specifically India and China.

Figure 1. Company Logo

1.2. DESCRIPTION OF CITICOLINE

1.2.1. Chemical description

Citicoline, also called as cytidine diphosphate-choline (CDP-Choline) or cytidine 5'-diphosphocholine is defined by the most of patents and almost studies as follows:

Citicoline is amorphous, hygroscopic powder, which has therapeutic utility, for example, as a cerebroprotectant, or a neuroprotectant. In particular, citicoline is beneficial the victims of ischemic stroke, head trauma, and, possibly, neurodegenerative disease. In addition, citicoline is used to treat unconsciousness resulting from cranial trauma, haemorrhages, cerebral thrombosis, and cerebropathies due to atherosclerosis.

(Secades J J. CDP-choline: pharmacological and clinical review. Methods Find Exp Clin Pharmacol 1995 Oct; 17 Suppl B:2-54)..

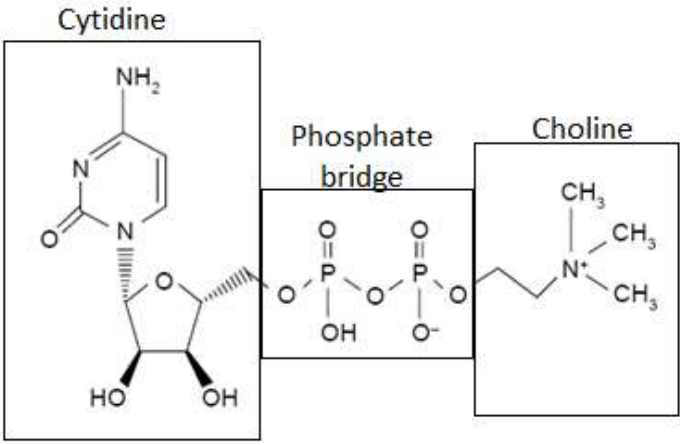

Figure 2: Chemical structure of citicoline

Citicoline is applied as its sodium salt, that is a whitish powder [figure 1] similar to talc, formed by crystals of microscopic size between 2.7 and 11 Armstrong, it is composed of two clearly identifiable parts: choline and cytidine monophosphate, joined by diphosphate bridge. One of the main characteristics of citicoline is its high hygroscopic capacity, this causes that the active principle cannot be in contact with the environment, a factor that will hinder its later storage. To prevent citicoline lost its quality as a result of humidity, in 1969 US Pat. No. 3,687,932 is applied, it is based on the transformation of citicoline to monohydrated sodium citicoline.

Figure 3: sodium citicoline

The companies that obtained the patent to Takuda, including Ceuber, to produce the citicoline homogeneously, they agreed that sodium citicoline monohydrate is stable up to a maximum value of 5-6% of water, and with a higher percentage, it was not safe the existence of this molecule in crystal form, as described above. For this reason, they reached the consensus that, with a higher percentage than the one described, the molecule is unstable. The next figure [figure la que sea]

shows the increase of stability after to apply this transformation. The continuous lines represent the Apies that are been treated, in a temperature of 17ºC and 70ºC.

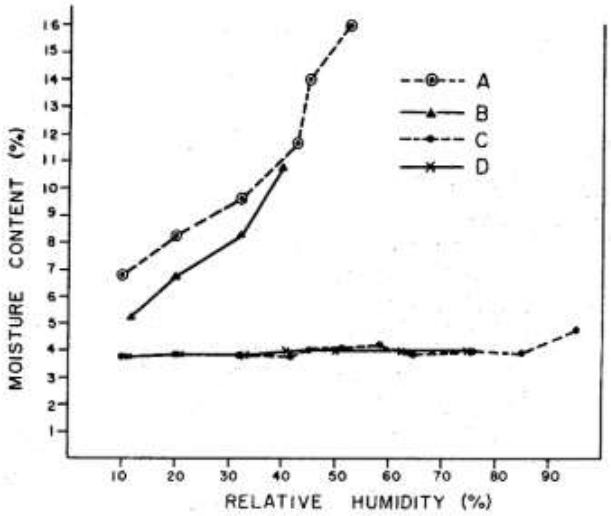

Figure 4: Humidity effect of citicoline

However, at the end of the nineties an improvement of the previous process appears, it is the patent US 6,057,301, which modifies the component until reaching the sodium citicoline tetrahydrate. This hyperhydrated form, allows to store the API in conditions more advantageous, for instance eliminating the five days to get the purified form to get this crystalize form, being its ideal percentage of water up to 12.5%. In this way, the time that elapses until the subsequent transformation of the active ingredient to Cemicina. The application of this invention, the only concern will be the hygiene of the place of storage: regardless its administration form (oral solution or intravenous route).

The active principle begins to dissolve in critical conditions to be ingested by a human, either because it contains acids or in its absence by the temperature at which citicoline is heated, 100ºC. As it can be observed in the study [24] and at the same time in the different developments that it will be described later, it implies that the maximum temperature during the reactions will be 70 ºC,

to avoid its decomposition. During the carrying out of the bibliographic search, it was verified that citicoline is distributed (for pharmaceutical use) in a range of purity between 98%, being that obtained by the process US3,687,932 at a temperature of 17º during its crystallization, up to 99.6% through genetic engineering.

Once the solid is obtained, and transformed into citicoline tetrahydrate, the drug will be elaborated, in which its pharmaceutical forms are those that will be seen below, as it is described in the SmPC (Summary of product characteristics)

Oral route:

Cemicina is a transparent oral solution of pink colour, with smell and flavour of strawberry packed in envelope. It comes in packages containing 10 and 30 sachets with 10 ml of oral solution each. Each millilitre contains 100 mg of Citicoline (as sodium salt)

The injectable route:

Cemicina 500 mg solution for injection comes in clear glass ampoules. Each 4 ml ampoule contains 500 mg of Citicoline (as the sodium salt).

In the following figures, it is shown the difference between the injectable presentation, located in the lyophilized ampoule and the oral solution, the last has its characterized pink colour.

Figure 5 & 6: Presentations of Cemicina

1.2.2. Pharmacological properties

This pharmaceutical substance is chemically equal to which is found naturally in the human organism, that has a vital role for the synthesis of phosphatidyllic acid, an agent of great relevance for neurotic growth and neuronal regeneration.

Phosphatidylcholine is a basic constituent of cells, in particular of cellular membranes. Consequently, a continuous synthesis of that, will favourite the good functioning of these membranes, and in turn, the cells. This phosphate is the precursor of glycerolcholine, a component whose purpose is to give stability and permeability to the membrane.

Subsequently, the synthesis of glycerolcholine gives rise to choline. This micronutrient is one of the few that is able to cross the blood-brain barrier, becoming an important substance for the CNS, for the production of acetylcholine. Figure 7 shows that the choline ends its synthesis in acetylcholine, one of the most important neurotransmitter (NT) in CNS (central nervous system) and it has been demonstrated that' its absence can produce Alzheimer.

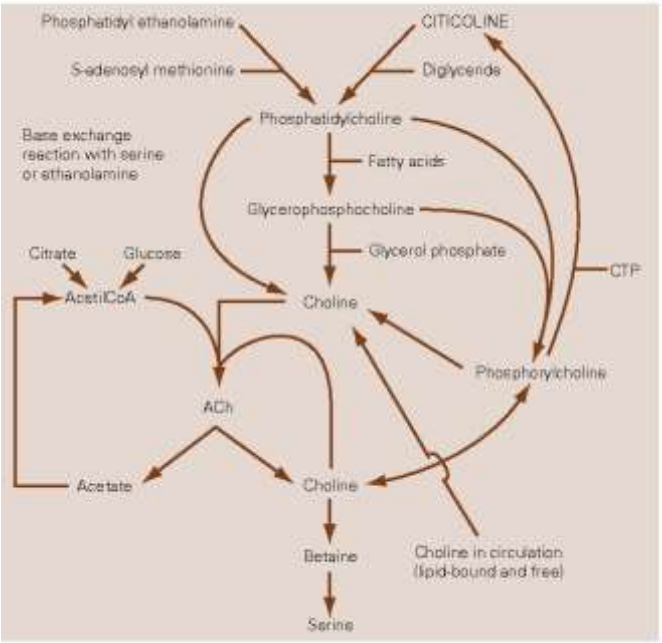

Figure 7: Synthesis of citicoline

1.2.3. Clinical use of citicoline

The Citicoline is approved by the Agency of the drug for two therapeutic areas, as indicated in the SmPC:

Treatment of neurological and cognitive disorders associated with cerebrovascular accidents (CVA). Cerebrovascular ischemia (ictus), is one of the most important causes this dementia. Mild cognitive impairment (MCI), a neurocognitive disorder recognized by the DSM-V (international reference manual of mental disorders) as a nosological entity itself., it refers to the concern of the patient, an informed person or the doctor because there has been a slight deterioration in one or more cognitive areas. MCI can be documented through a standardized cognitive assessment and that does not interfere with the capacity for independence in daily activities. [reference]

LCAs usually appears in smokers, people with dyslipidaemia (hypercholesterolemia and / or hypertriglyceridemia), in poor eating habits, high blood pressure, overweight, diabetes and sedentarism. Within this population, 1 of every 5 people can suffer MCI, and 20% will develop dementia. At times, DCL may be the beginning of the Alzheimer issue. Consequently, the size of the population sensitive to taking this drug is very high.

The effect of citicoline in the evolution of MCI and in the Quality of life (QoL) of patient was evaluated in a double blind controlled study, compared with placebo. As shown Figure 8 and Figure 9, citicoline shows the progression the MCI and improve their QoL. These results are an example by which Citicoline continues to be a drug with a great use, after almost fifty years of the beginning of the development of the molecule.

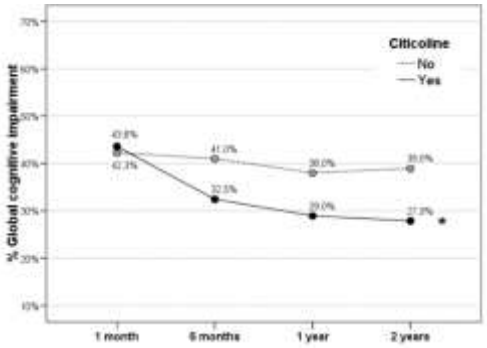

Figure 8: Progression of MCI

The other indication of Cemicina according to the Smpc is for **neurological and cognitive disorders associated with cranial traumas**. Although the most recent study, in this indication (COBRIT) not shows differences between citicoline and placebo, the study had many limitations, that difficult to stablish robust conclusions.

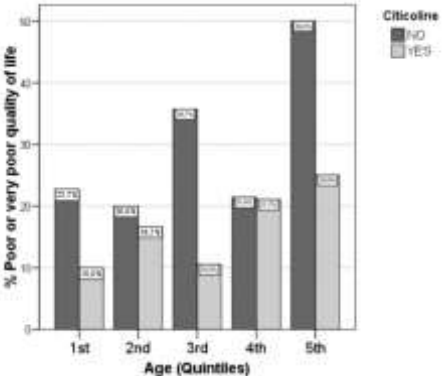

Figure 9: Progression of QoL

On the other hand, it has been demonstrated that Cemicina would be useful in different pathologies, although they are not in the table of product, also known as off-label use. This information is sensitive to the patient, for this reason it will not be referenced.

2. OBJECTIVES

The main objective of this work is to study the effectiveness changing the whole process to obtain the citicoline or its sodium salt.

To achieve this objective, a bibliographic search will be applied to obtain different patents about its manufacture. After this search, through the conditions stablished by the company, it will be selected the patent that best suits the requirements.

Afterwards, the recipes of these alternatives (the current process and the selected) will be scaled using Batch Process Developer of Aspen. Basic engineering of these alternatives will be developed and it will be simulated different option and different conditions in the selected process.

Finally, the resulting characteristics will be compared in order to decide which process give the best results for the company.

3. BIBLIOGRAPHIC RESEARCH

3.1. RESEARCH METHOD

The search patent was carried out in the succeeding portals: espace.net, patentscope and Google patent, using the following words as a key word in the search: "Citicoline Manufacture", "Sodium Citicoline Manufacture", "Sodium citicoline process" and "Citicoline process". After this research, having obtained a large number of entries; among these entries, it is important to highlight that there are entries for the clinical use of Citicoline, the process of the active principle to an intermediate form (such as that obtained by Ceuber), the transformation of the drug to its respective pharmaceutical forms, and finally the manufacturing process of Citicoline.

Firstly, it has been established to pre-select patents that show a significant change in the equipment used in order to develop citicoline, such as the elimination of chromatographic adsorption. However, Ceuber manufactures a total of 38 APIs, therefore, it is necessary to assess the number of equipment required and the low feasibility of modifying them for a single active ingredient. In the same way, it must be analysed that the final product is citicoline or the sodium salt, because it could imply a change in the process for the preparation of the drug, taking into account that the 6,057,301 patent is still valid and the company has not considered abandoning it.

Another criterion for selecting a patent, is that it demonstrates a drastic change in the method of obtaining the API, for example, the current process needs a culture for biosynthesis unlike others that are developed by chemical synthesis, how can be the WO2013 / 128393, which obtains a high productivity through the successive reactions.

Finally, for the definitive selection of the patents, the batch time, the raw materials used and the necessary equipment will be valued, but under no circumstances losing the quality of the drug. Next, it explains in detail the conditions that the new chosen process must have, in which the final result will be the one that best encompasses the different qualities.

3.2. CORPORATIVE REQUERIMENTS

To select the most suitable process for the company, it has been decided that the following requirements should be adjusted as much as possible.

First, it will be described the aspects that affects the productivity directly, these are from 1 to 5. The optimal process will meet these points, or at least, some changes that imply an improvement in manufacturing. Nevertheless, these improvements, cannot lead a worsening in the other conditions, such the quality of the drug, the appearance of a high number of secondary reactions or impurities, factors that can have an impact, such as the purification, and avoid to increase the environmental impact.

1-**Elimination of operations with high cost**. The selected patents that aim to reduce operating costs as much as possible, focus on eliminating the chromatographic column, because it is a process used only in sectors with high added value or increase the yield of the culture. For this reason, at the time of pre-selection, there will be at least one process that remove this column.

2-**Maximum adjustment to the equipment available**. One of the main characteristics when the active ingredients are produced is the type of operation, being in batch. This causes the plant equipment is used for more than one developments to manufacture other APIs, therefore, a large modification could cause problems in the other processes.

3-**Energy expenditure**. This factor is not only linked to the economic issue, it also affects to environmental issue, as will be seen in point 9. In a process, it is important to adjust the energetical usage as much as possible, adapting the selected patent to avoid loss in efficiency and environmental impact.

4-**Time of operation.** It is necessary to know the time in each batch, because it is a good indicator to approximate the time of the process. In the event that the operating time is high (valuating the slowest stage), it can have an impact on a larger amount of workforce (need for control, more exhaustive cleaning for each batch), having the same scope and the same campaign time, it causes the necessity to obtain an equipment with higher volume. In case that all the process is overlapped, it is need a large amount of control. In this way, the optimal process will need to have a shorter time or similar to the one used today.

5-Number of operations: in this case, in the same way as the operating time, an increase in the quantity could induce either a greater overall time, caused by the transport of the materials, intermediates to other equipment, the time of each procedure, etc or changing the equipment to

a high quantity or its volume. Therefore, the theoretical process will have a minimum number of operations.

It should be noted that almost patents that will be selected, they have their origin either in China or India. This will cause difficulties in the selection, because these processes are focused on cheaper raw materials, number of operations and type of operations, to have a layout as optimized as possible. On the other hand, in developed countries workforce is more expensive, between 7-9 times in the case of India and 3-5 times in relation to China. Therefore, the advantages of the processes as indicated by the patents will be assessed in a different way, taking into a consideration the batch time as the productivity of raw materials. In addition, working by campaigns is not adequate cause peaks in labour to ensure a final product homogeneous. Another difficulty when comparing patents, it will be the legislation, such as waste management, the space needed, the cost of electricity, although compared to China the difference is about 10%. As can be seen, the chosen process will encompass the best balance between the previous points.

Next, other characteristics are shown apart from the productivity issue. However, if the process cannot be adjusted to the requirements that will be seen below, it is automatically discarded, because lowering the cost of the process as a result of a loss in medicine quality, it is ethically unacceptable. In addition, medicines and active ingredients are governed by quality standards, known as Good Manufacturing Practices (GMP). GMPs are described in the European Union as follows:

Good manufacturing practice ***(GMP) describes the minimum standard that a medicines manufacturer must meet in their production processes. The European Medicines Agency (EMA) coordinates inspections to verify compliance with these standards and plays a key role in harmonising GMP activities at European Union (EU) level.***

6-Adjustment to the patent 6,057,301. As previously stated, the pharmaceutical laboratory purchased a patent to promote the storage of citicoline, which is based on hydration. Then, the final product should be either sodium citicoline or the citicoline ion and not another, because it would cause the need for a new process.

7-Quality of the active principle. The legislation in the pharmaceutical industry, whether of public origin, the EMA in Europe, and the AEMPS in Spain, or by default private legislation to self-regulate are characterized by having a high demand in the quality standards, unlike the other countries that the law is laxer. In this way, the new active principle must have at least the same purity as the previous one, not contain dangerous subproducts and in turn that these do not provoke a reaction with the future excipients of Cemicina.

8-Sustainable process. Ceuber in 2005 obtained the ISO 14001, known as *Environmental Management Systems certification*. Also, the current president has as a maximum that the company is exemplary in its environmental vision and anticipating the future. Moreover, it is expected that the legislation will be more severe in the polluting processes. Consequently, the chosen development should not create new waste streams and / or more difficult to manage, such as operations that are highly polluting.

9-Control of the demand. It is important that the new development should be capable to manage an increase in demand and not cause stock breakage, because the ability to have the drug in a short time in drugstores has a vital importance. In this way, the new process should be able to sustain an increase in demand up to 5% in the same campaign time, in the same way as the current invention.

In the next section, after describing some patents, four will be chosen (the current one used by Ceuber and three more), in order to analyses more carefully each one,

3.3. PATENT PRESELECTION

In the first search, it was founded 18 patents about the citicoline manufacturing, and in the Annex, it could be founded all patents used in this project. To avoid repetitive information, in the next paragraphs, it will be shown the preselected patents and another four, as example of discard.

3.3.1. Patent US3,687,932

This development is that Ceuber leads today, therefore, this process need to be compared with the others, to decide whether obtaining citicoline through other patents is improvable or not. This is one of the first methods to manufacture citicoline industrially, because it has its origin in the molecule development. In this way, the purpose of this patent is to obtain a stable product with a high purity, showing at the same time the great advance that this molecule represents in the field of neurology. It should be noted that other aspects are left aside: the economy of the process, waste management, etc. Moreover, the process was registered at a time when environmental legislation was almost non-existent. Therefore, it should be assumed that in almost fifty years, has appears an upgrade in citicoline manufacturing.

Despite the drawbacks mentioned above, it should be noted that this process has a total operating time similar to some contemporary processes and even in a shorter time, highlighting the relatively low reaction time, adding that, since the beginning of the application of the patent, the company has managed to improve the development in three essential aspects: cost, time and quality. However, a significantly improvement will be obtaining eliminating chromatographic adsorption and increasing the volume productivity without harming other aspects.

The adsorption in chromatography column, is based on the ionic interaction (Van der Walls forces) between the different phases, the stationary (Dowex resin) and the mobile (solution to be separated) in order to the more polar solute, KOH, will flow faster through the resin.

To carry out this operation properly, the solution containing the API must be poured into the column, being adsorbed the API and a part of the solution inside. Next, the column will be washed with water, to remove a first part of impurities, but being incapable of modifying the amount of citicoline retained in the resin. Finally, acetic acid is flowed, to separate the different products in stage, one of that, it is citicoline.

In the following images, the operation of this stage is shown in detail, in which each component (shown with colours) separates along it. The shown situation, is ideal, therefore, there will be a part of citicoline that will be lost at the time of collecting the eluent, in addition the column will retain a part of citicoline inside.

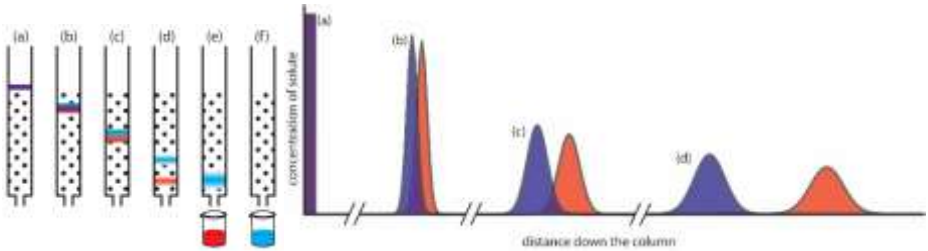

Figure 10. Ideal representation of a chromatography column

The purpose of this project is to know if news methods are capable to increase the efficiency during the citicoline manufacture, for this reason, it is necessary to simulate this process through the Aspen with some results obtained in plant, to compare with the preselected processes and finally if the acquisition of a new patent is feasible or not. Therefore, this process is **preselected.**

3.3.2. Patent WO2013/128393

The objective of this patent, as described in the summary, is to prepare sodium citicoline with high productivity through the chemical synthesis of its products (without biological reactions), in which the cultivation stage and the chromatographic adsorption are abandoned because impurities are separated by precipitation. In this case, apart from the elimination of the column, the high purity of the active principle is also highlighted, being greater than 99% (by HPLC analysis), the other point to consider is the reduced time of the chemical reaction. However, being a biochemical process, the reaction needs to be done in another space.

This patent has the advantage of being carried out very effectively, either by the reaction time, being less than 7 hours (the maximum according to the recipe), the quantity of raw materials is reduced and the detailed operations, despite not being in the current process, are common, such as frozen or precipitation reaction; In this aspect the process does not create any inconvenience.

However, a possible drawback is that the description does not mention anywhere the issue of the elimination of hazardous substances for humans and the environmental impact, factors that are found for the optimal process. In short, observing the characteristics, the patent is **preselected**.

3.3.3. Patent CN 201410247176

The purpose of this method is to obtain sodium citicoline by oxalyl chloride. This technique, in the same way as the previous procedure, seeks to reduce costs as much as possible, focusing the improvement in the following three areas: the reduction in the cost of raw materials, being these very easy to obtain; high yield in the reaction, achieving an elevate productivity and the low complexity of the operations, that avoids the use of chromatographic adsorption.

However, the complications that this method presents are: the time in the crystallization, being from 24 to 48 hours, unlike the 2 hours in the current process, the final purity of the active principle, in this case is 98.5% instead of 99%, a fact that could cause either a change in FT or non-compliance with GMP or the necessity to do a recrystallization, a factor that would increase the overall time in this process. Moreover, it does not mention the environmental impact, nor the waste management.

Once all the aspects have been observed, this process is **discarded**. Mainly for the number of hours that would be needed to achieve the purity of the current API, or another solution would be the change in the volume of the equipment, reason that would harm the efficiency during the manufacture of the other active ingredients developed by the company, because the equipment would not work at the optimum performance for what they were designed. However, this method could be interesting to manufacture in areas where the cost of labour is lower, because this procedure has been developed for domestic use, that is, in China.

3.3.4. Patent CN107488603

The following patent, presents a drastic change with respect to the other, to be manufactured by the transformation of the gene, being a process of bioengineering. This process is based on the recombination of the gene and its future transformation. This procedure marks an important difference with respect to the others, but also presents a long list of drawbacks.

The method has a high number of operations, which makes it difficult to applicate, the yield is less than 70%, away from the 77% presented by the original process. The culture used in each procedure have a single use, being a large expense in materials, a factor to avoid, and the most important, the final product is not sodium citicoline in the form of diphosphate, but monophosphate, therefore, the active principle is not the same. Finally, although the description of the process indicates that its duration is short, it is much greater than the other methods with the possibility of being pre-selected.

With the aspects defined in the previous paragraph, the patent has little viability because it does not adapt to the demands of the company for its subsequent development in the plant, therefore, the process is **discarded**.

3.3.5. Patent CN103849666B

The next method, can obtain the highest purity index in the 25 processes that has been found, obtaining up to 99.8%, as it is commented in the example. The purpose is to obtain sodium citicoline by immobilized enzymes. The main advantages of the process, according to the description are: the obtaining an active principle suitable for the pharmaceutical industry, a product of high stability, the immobilized cytidine can be used repeatedly for its synthesis, easy operations to control, a high conversion in the reaction, a low cost together with a low operating time and to conclude, environmental sustainability is described, being one of its strengths for the ability to recycle raw materials or catalysts.

In spite of the benefits that the patent refers, the use of chromatographic adsorption, breaks the corporative objectives, in the same way, the operations that should be applied, are absolutely different to the current process, an example of which are ultrafiltration and spray by ultrasound. In addition, the biological reaction requires a high reaction time, being of 26 hours and having different raw materials. Consequently, it is **discarded**.

3.3.6. Patent CN102078299A

This method consists in a variation from the active ingredient to achieve the liposomal citicoline [figure]. It is developed by reacting CDP-choline with phospholipids, this product has the characteristic of providing improvements in pharmacology and offers greater ease of storage, with respect to the non-hydrated form. Therefore, it would be applied only in case to innovate the medicine or want to change the patent US 6,057,301.

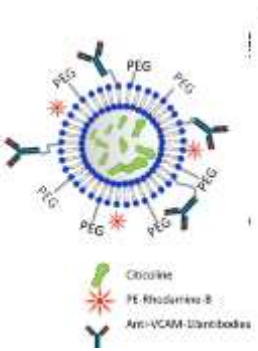

Figure 11. Liposomal ciicoline

3.3.7. Patent CN1944661A

This process is based on the selection of a new solvent and biocatalyst during biosynthesis. This development implies a greater ease in the process with a high performance in the most expensive operations, such as cultivation, which in this method can be used up to three times, with a smaller size in culture, causing lower cost, less impact on the environment and finally, higher productivity. On the other hand, the patent has a series of handicaps, such as the chromatographic stage and the need to freeze the culture for 5 days at -20 ° C, this operation can cause a difficulty in case of breaking stock.

The 104021105 patent has the same weaknesses during the citicoline manufacture, as the Japanese patent. Both processes require the chromatographic step, a high time during the cultivation and finally, the need to crystallize the final product. Despite all these drawbacks, it has **been decided to select** this process mainly for one reason, to assess the impact generated by the reuse of raw materials: in the economic incomes and the environmental impact.

3.3.8. Patent CN101538598A

This patent describes a method for obtaining pure citicoline from the improvement in the culture, an operation that causes problems due to the difficulty of recycling materials (substrates), the time required and its conversion. In this case, the process is very similar, it has a first part of culture, having the next differences: the catalysis is inside the cell, and the purification stage,

using a centrifugation, where the pure citicoline will be floating in the fluid. In addition, the patent states that no external energy needs to be applied during the reaction, reducing the energy expenditure of the process.

This patent will be **selected** because the obtaining of citicoline is very similar to the original; both in raw materials, number of stages and the method of obtaining the active principle (cultivation, reaction and finally separation) with the advantage that the chromatographic step will not be used. Finally, the products used as substrate are economical and with a high recycling capacity or with a less complex treatment, so that their environmental impact is as low as possible.

It is important to point out that the most innovative processes, those that seek to obtain a solution that covers improvements in several areas, either by cost or by their environmental impact, sometimes imply an improvement of the molecule, for example in sodium phosphate choline cell or in liposome, a component that obtains better results in clinical trials. Another aspect to evaluate is that the methods that imply an improvement in the environmental impact are carried out through genetic engineering. Consequently, if the improvement of the process is not significant, the company should do a rethink if it is viable to maintain the intermediate step to citicoline tetrahydrate and at the same time to what extent continue to develop the sodium citicoline diphosphate instead of a new form of this API.

4. PATENT SELECTION

In this section, are going to be explained the method to select the patent that offers the best solution for the company. It is important to note, that the final product is citicoline or sodium citicoline, in the main reactions a high yield is obtained and the quality of the final product is inside the required margins. Therefore, only those aspects that directly affect productivity will be studied.

To carry out this analysis, it will be done through the data offered by the four recipes. This criterion has been established because the data that comes from the plant, are significantly better than the example, because the process is optimized. Therefore, an increase in plant efficiency could be translated into each process.

4.1. ANALYTICAL METHOD

In this section, with the objective to realize a thorough analysis, the results that will show the best method, its will be obtained by the next information.

1-Productivity of raw materials: In each of the 4 recipes, the references indicated that limiting reagent is consumed between 70-90%, either in a single reaction (in the fermentation processes) or in successive necessary reactions to obtain the final product (patent WO2013). In contrast, biosynthesis reactions require a greater number of components, an example of which are substrates or amino acids, causing an increase in the substances that participate in the biological reaction

The calculus of this performance, is made by the next expression:

$$Productivity = \frac{Mass\ of\ citicoline\ obtained\ by\ the\ recipe}{Mass\ of\ raw\ materials\ used\ in\ the\ reactions} \quad \left[\frac{g}{g}\right]$$

In the tables of the different processes, it could be found that there are two yields, one taking into account the sugar and the other not, the reason for this consideration is the low environmental impact that it generates, in addition to have reduced cost.

To finish, the amount of citicoline used in the calculation is the facilitated by the example, having passed through the different stages of purification.

2-Time of operation: Pharmaceutical processes are characterized for being in discontinuous, reason that leads to use different equipment at the same time, action known as overlap. Throughout the project it has been commented the short amount of information that is detailed by the recipes, for instance the duration in every stage, only commented for the reactions and in an approximate way. Consequently, it will be considered that the time of the process is the slowest stage (usually the chemical reaction) and productivity time will be realized by the next way: It is important to point out that this productivity is evaluated with the same reaction volume, being of 1 liter in each process.

$$Productivity\ time\ (PT) = \frac{mass\ obtained\ by\ the\ recipe}{time}\ \left[\frac{g}{min}\right]$$

3-Cost of the products: Finally, it is necessary to know the approximate cost in each substance that is involved in the reaction, to assess how influence an improvement in performance in the final cost of production. This calculation will serve to guide which process needs to invest the most economic resources and not to establish the exact cost per gram. This approach has been made looking for the cost of 1Kg of each product, indicating that it will be for pharmaceutical use, in case the product is not found, it has been decided that it will have got the same cost as the most expensive product that participates in the process and results show the cost per gram in the recipe conditions. Logically, the order of compounds in high quantities is more economical and is incomparable to the product price from these results.

On the other hand, the price search has not been evaluated in substances that are recovered, because the los is zero, one of these compounds can be the catalysts, the materials present in

the different stages of purification such as resin Dowex or activated carbon. These substances are highlight in red.

The calculation will be made as follows:

$$Cost = \frac{Price\ of\ the\ material\ used\ in\ reaction}{Mass\ obtained\ of\ citicolin} \left[\frac{€}{g}\right]$$

When basic engineering is developed, the energy needed to develop the process is unknown, the only way is to approximate which stage has more energy necessities than another one. At the same time, the approximations in terms of labour cost, number of operations and type of operations, in this section it will not be evaluated, because they have already been taken into account in the pre-selection and with the results that will be obtained, it is not possible to perform correctly.

4.2. CURRENT PATENT

The process is based on a single fermentation to obtain the API, with the inconvenience to need some expensive operations and in the same time losing a lot of efficiency, according to the recipe, these losses is approximately 50%, in particular, during the purification stages.

Seen the results in the table, they present an unaffordable cost for the company, because the product value (a box of 30 envelopes, having each dose one gram) is 48 euros, a factor that demonstrates either the impossibility to carry out the manufacture of the API without the process having been optimized or the short reliability of the information.

Product	Amount by receipt (mg/ml)	Amount (g/L)	Price (€/Kg)	Price by product (€)
Cytidine 5 monophosphate	7,38	7,38	41	0,30258
Choline chloride	24	24	359	8,616
ATCC 6872	10	10	1	0,01
Monophosphate	11,6	11,6	10	0,116
Diphosphate	20	20	10	0,2
Magnesium sulphate	2,96	NA		
Sugar	10	10	0,5	0,005
Citicoline	-	1.3		
Yield (%)	0.75		Price citicoline (€/g)	30.95
Yield without sugar (%)	0.80			0
Productivity time	0.0054			

Table 1: Results of the process

One of the few positive elements of this process is its reaction time; said synthesis requires only 4 hours but obtaining a reduced amount of active principle in comparison with the products that are necessary to carry out the reaction. Regarding the purification method, it is done in two adsorption columns; it is necessary to eliminate a large quantity of impurities disposed in the solution, as a result the amount collected is only 20% respect of the obtained in the fermentation. Likewise, the operation times are high (in plant), because the process needs to be repeated up to three times in the chromatographic column, in order to recover a greater quantity of active principle.

With regard to waste management, this process is only done with organic substances (reactants) and a positive point, it is the reutilisation of the product. An example of this are some culture compounds and formic acid, which is recovered by evaporation. Moreover, the catalysts are recovered through precipitation and subsequent filtering. However, these situations are those

that appear in the plant, their recyclability will be considered non-existent, except that their reuse is specified in the recipe for elements participating in the reaction, except for the catalysts.

Finally, the overlap in each of the operations, will inspire the one that is in plant, the reason is that this modification is alien to improve the performance of operations (reactions, purifications, etc.) and is an important factor to the time to perform a scaling. To calculate the PT, it will be used the reaction time, because is the slowest stage in the reaction.

4.3. PATENT WO2013/128393

Patent WO2013/128393 is the only process in which citicoline is obtained by chemical synthesis. It is based on the same procedure as the previous ones, but with some differences that it gets optimal results: high productivity, ease of separation, high purity product, etc. These differences are the following: phosphates are not reacted as salts, but instead are found in choline chloride, using herein calcium phosphoryl choline chloride (CPCC); the use of N, N'-Dicyclohexylcarbodiimide (DCC) to condense the CMP, the morpholine to facilitate the dissociation of the salts present from the beginning of the reaction and to finish, the use of dicarboxylic acids, the use of this acid allows the impurities precipitate quickly and completely, one of these precipitates can be calcium oxalate.

This method of obtaining citicoline, is an application to the pharmaceutical industry of the synthesis described in the year 1970, can be found in the following article:
Synthesis of a Nucleotide Coenzyme, CDP-Choline KIYOMI KIKUGAWA and MOTONOBU ICHINO

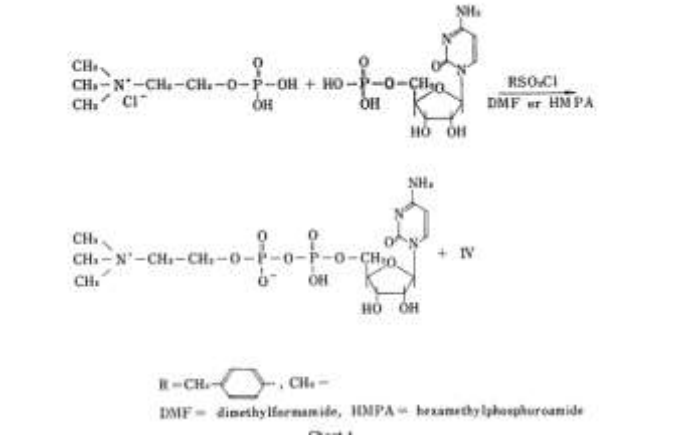

Figure 12: Chemical synthesis of citicoline

As regards the operations, the elimination of the impurities causing them to precipitate, implies suppressing the chromatographic column, one of the purposes of Ceuber. In order to improve the elimination by precipitating compounds present in the solution, a freezing is carried out together with a filtrate to increase the purity.

Producte	Amount by recipe (mg/ml)	Amount (g/L)	Price (€/Kg)	Price by product (€)
Cytidine 5 monophosphate	100,00	133,33	41,00	5,47
Morpholine	75,00	100,00	82,00	8,20
DCC	100,00	133,33	359,00	47,87
CPCC	200,00	266,67	1000,00	266,67
MSOA	160,00	213,33	1000,00	213,33
Citicoline		352,45		
yield (%)	41,63		Price citicoline (€/g)	1,54
Productivity time	1,174833333			

Table 2: Results of the process

As it has been announced in the previous section, the table 2 shows the high productivity of the process in all its areas, getting according to the recipe about 352.45 g per litter, an amount that is not capable to compare between other processes, besides abandoning as laborious stages as a 10-hour fermentation or the application of the chromatography column. However, these excellent results present significant shortcomings.

The main problems of this process are the high cost of the substances, such as € 1000 per kilo of CPCC, as opposed to choline chloride, with a value of € 359 and is the most expensive product in the other inventions. Another drawback to add is the inability to find the morpholidate salt of oxalic acid (MSOA), during the search for the price of reagents, no intermediary has been found to supply this compound, also this compound at a bibliographic level is only referenced in the description of this patent and indirectly in the article referenced above, which means that Ceuber must obtain the component, either in the pharmaceutical plant or in the plant responsible to produce fine chemicals.

On the other hand, it also presents problems in terms of the safety of the reagents or compounds that are directly involved, these products are: oxalic acid, morpholine and chlorhydric acid. However, the laboratory had previously worked with morpholine (US 3,666,748), this gives a degree of experience to the company when handling these dangerous substances. Moreover, the current process in the intravenous drug, contains traces of chlorhydric acid. As regards the other compound (oxalic acid), it is to be assumed that they will be separated in the same way as the other patents, such as distillation, condensation, etc.

Finally, the overlap of this process, it will be dominated by the slowest stage, the reactor. Having two reactions in the same recipient, it gives two possibilities to apply that patent, either use one reactor with more volume or to use two reactors with less volume. The overlap must have taken into a consideration the manufacturing of MSOA. It could be at the same time as the others, and it would be established between the faster processes, and better adjust the overlap

4.4. PATENT CN200810019854

The synthesis of the active principle is carried out through choline chloride, CMP and phosphates, with a change in the culture in which a better result is obtained than the current one.

In the following table, each of the reagents involved in obtaining citicoline is shown, without taking into account other compounds that do not intervene in the reaction, such as sodium hydroxide to adjust the pH.

Product	Amount in the example (15 L)	Amount in grams/liter	Price (€/Kg)	Price amount
Choline chloride	60mM	8	359	3,01
dihydrogen phosphate	0.2 M	23,8	56	1,33
CMP	30mM	4	41	0,16
Glucose	0.3 M	54	0,5	0,03
Magnesium Sulfate	50mM	15	n/A	N/A
KCl	2mM	n/A	n/A	N/A
Cysteine	2mM	0,24	n/A	N/A
Clay	2800g	187	359	0
Culture (40g/L)	-	40	Unknown	0
Glucose	-	2	67	0,13
Urea	-	1,5	158	0,24
Potassium dihydrogen phosphate	-	N/A	N/A	N/A
MgSO4*7 H2O	-	N/A	N/A	N/A
ZnSO4*7H2O	-	N/A	N/A	N/A
FeSO4*7H2O	-	N/A	N/A	N/A
MgCl*7H20	-	N/A	N/A	N/A
Biotin	-	4	n/A	n/A

Table 3.1: Results of the process

Yield	4,41
Yield without sugar	5,54
Price g	4.82
Productivity time	0.02

Table 3.2: Yield of the process

Table 3.2 demonstrates the need for the company to make a change in obtaining citicoline, this process multiplies the yield, with a cost per gram significantly lower than the current one, taking into a consideration that clay has its cost as the most expensive, such as the MSOA in the prior analysis. Other positive aspects of the process, is to have a single reaction, but the fermentation time is high, approximately 10 hours.

However, the procedure describes the purification method with little accuracy, because according to the recipe only a centrifugation is needed because the active principle remains on the surface of the fluid. Therefore, the purification system gives rise to doubts because all the processes described above, they require an adsorption step, either by activated carbon and / or by chromatography. In addition, it is not mentioned how to eliminate the different impurities present in the form of ions that remain in the solution, such as chlorine or metal ions, at no time refers to the addition of salts to precipitate that ions present.

It would be interesting to know the separation of the catalysts from the culture medium, because being metallic they can cause a greater impact to the medium, specifically Zinc, which is heavy metal. Another aspect related to the recycling of products, is to know the feasibility of reusing the culture in more occasions, because the patent does not mention at any time the possibility of reusing it, unlike the patent No. 6,057,301 that can be recycled up to three times.

Finally, regarding the overlap in the equipment, one solution would be to carry out the fermentation at the same time as the purification, because the necessary data are not available to coincide in each process, it is valued that this is the most optimal.

4.5. PATENT CN1944661A

The synthesis of CDP-Choline is carried out through choline chloride, CMP and phosphates with a change in the components that act on the culture, to improve its result and at the same time lower costs, because it can be reused.

In the following table, each of the reagents involved in obtaining citicoline is shown, without taking into account other compounds that do not intervene in the reaction, such as sodium hydroxide to adjust the pH.

Product	Amount in the example (800 kg of water)	Amount in grams/litter	Price (€/Kg)
Phospho choline	3 Kg	3,75	359
CMP	1 Kg	1,25	41
Glucose	10 Kg	12,5	N/A
Potassium Hydroxide	2 Kg	2,5	N/A
Culture	30/3 Kg	12,5	N/A

Table 4: Results of the process

The main reason why this process has been chosen, has been the emphasis on the recycling of materials (point mentioned in the advantages of the patent), specifically the culture, which it is multiplied per 0.33 in the tables, to be used up to three times. In addition to presenting these benefits, it gets a better performance than the current one, in the same way as the other processes.

The table does not show results regarding productivity time, cost per gram and yield, this is due to the fact that the final amount obtained from sodium citicoline is not available in the recipe.

The main inconveniences of the process, in addition to not offering any data to the amount of citicoline produced, and the scant description of each of the operations, it is the high number of operations. In the recipe, a total of fourteen operations are shown, which include columns of active carbon and chromatographic absorption, requiring a change in the resin, being the 711 (Cl-). With

the experience transmitted in the plant, it can be reasoned that such a quantity of operations or an optimisation will be necessary. or a considerable amount of the final product will be lost and by company policy, it is desired to eliminate this type of operations.

Referring to overlap, the process is difficult to be apply, having 14 operations, and the only stage that is discussed is 6 hours. The process, even in the most favourable circumstance, that the reaction time is the slowest stage and a large number of equipment is available to perform this operation, will continue to have a lower productivity time for two reasons: the number of moles per litter is inferior to the process CN200810019854 having as reference the same yield, consequently the amount of API will be lower; and this process needs a culture of 24 four

4.6. PROCESS COMPARISON

Once the data have been obtained, the patent that shows the most advantages to be applied in the plant will be selected. It is important to note that each of the processes has an improvement over the currently used, either substantially increasing the performance, productivity time and cost per gram. Therefore, these data corroborate the need to make a change in the process.

The WO2013 has been **selected** for the following reasons:

First, the 2013 patent obtains a longer productivity time than the other processes. As a result, the company could reduce campaign time or use smaller equipment, such as reactors of 1.6 and / or 2 cubic meters, reactors that are currently used to manufacture other APIs, or use the equipment for the manufacture of paracetamol, with volumes between 10 to 15 cubic meters for the reaction.

Secondly, the possibility of manufacturing citicoline from this process offers to abandon operations of high cost and in which a high amount of citicoline is lost, such as the chromatographic column. In addition to eliminating these operations, a product with high purity is achieved, according to the 99% recipe analysed by HPLC (high performance liquid chromatography). However, with regard to equipment, this patent requires a greater number and different, such as frozen to separate substances. In spite of this, the balance is favourable to apply it because the different operations are common in the pharmaceutical industry, and in the case of the others, another column of chromatography (in this case chlorine) or microfiltration must be applied.

Another relevant aspect for having selected WO2013 is the description of the patent, that is, this recipe contains some examples and a more concise explanation. Inside the patent, the use of each reagent (along with its quantities) and the operations applied to obtain the final product are explained in detail. However, the CN1944661A patent specifies the reactants at a qualitative level, but not the quantities required, and for the patent CN200810019854 the purification of the product is not precisely detailed. Therefore, more accurate data implies more reliable results in its subsequent simulation using the ASPEN Batch process developer.

It is important to point out that the objective is to perform the basic engineering to obtain approximate data that confirm a change in the manufacturing method, therefore, it will not be possible to quantify some aspects of the process, such as the recycling of substances, future recirculation, etc. On the other hand, apart from the environmental issue, the morpholidate salt of Oxalic Acid had been identified as an inconvenience. Despite not being in the recipe, this reaction will need to be taken into account throughout the process, an alternative could be to place it in series during the fastest stage.

5. PROCESS SIMULATION

Once the selection has been made, the two processes will be simulated in order to assess and compare the impact of the new manufacturing method for Ceuber. Given the short information provided by the recipe, it is necessary get numbers that best approximate the reality. Consequently, the data from plant will be applied, unless it is clearly indicated in the recipes from patents.

In the following table is shown the different operations. The common stages, they will have the same operating time, an example of this is “transfer”.

Operation	Ceuber process	New process	Time (min)
Transfer	■	■	10
Mix	■		15
Filter	■	■	15
Crystallisation		■	125
Charge	■	■	5
Dry	■	■	40
Carbon Column (overall)	■	■	200
Concentrate		■	40
Column chromatography	■		300
Fermentation	■		231
Chemical synthesis		■	300-420
Cool		■	25
Precipitation reaction		■	120-240

Table 5: Changes applied in equipment

Another operation that appears in both process, is “filter”. One Characteristics of the filter, is the amount of liquid that the solid retains. Thus, the moisture will retain the 5% of the liquid, in every simulation.

In the next paragraphs, it will be simulated until the citicoline obtention, not arriving until its sodium salt, because patent US 6,057,301 is capable to be applied in both forms.

It will be focus mainly in the stages that gets the most influence in batch, the reaction for its transformation and being the slowest stage, and the purifications, because of the losses in these stages are significant. On the other hand, other equipment will be studied in case of being applied the process in plant.

5.1. CURRENT PROCESS

The simulation of the 3,684,652 process is intended to be as similar as possible to reality, for this reason it will be studied under the same conditions developed in plantt. However, it is necessary to know the simplifications and / or approximations used in Aspen:

The actual process obtains a yield of 50% in the different stages of purification, this is due to the solution obtained from the fermenter, it passes up to three times through the chromatography column, this leads to have a longer operation time. Therefore, in Aspen it will be shown as a single process, but the operating time and performance will be real.

The volume of occupation, will be the maximum that can support the fermenter, being of 95%. The reason for applying this critical condition is to study if the new process with an occupation of 90% gets the same scope.

The initial reaction has all the components in solid phase; One of these components are the cells. However, the cells appear as a solid acetone, to simplify calculations, and dextrose as sugar.

The different stages of purification, aim to show the losses in citicoline not in other compounds. Consequently, the final purity of the API can be different from reality.

Finally, to compare each one of the simulations, it will be done by the number of Batches made in 3 weeks, that is, the duration of each campaign.

5.1.1. Process simulation

In order to facilitate the compression of the process and look the equipment, the layout obtained by the simulation is attached. The image does not represent the original plant layout. However, in basic engineering, it does not affect the results that are required.

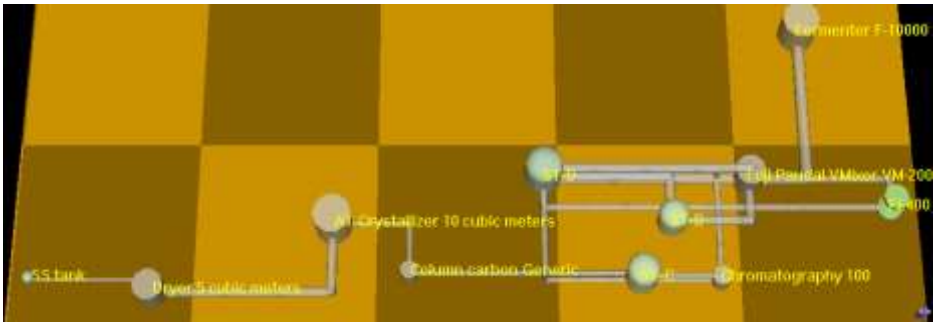

Figure 13: Process Layout

In the next pages it is add the Aspen recipe, it could be seen, the differences between the example from patent and the next.

1. Charge Fermenter F-10000 with 4749,93 liter of Cultivo.

2 Mix the contents of unit Fermenter F-10000. The mixing time is 30 min. Continuously add 4749,93 liter of WATER.

3. Ferment in unit Fermenter F-10000. The yield of CDP-CHOLINE in the Liquid phase is 0,028133605 (General Range: +/- 4 Percent.), of CHOLINE CHLORIDE in the Solid phase is 0,10774572, of DEXTROSE in the Solid phase is 0,055668622, of ACETONE in the Solid phase is 0,598587334 (General Range: +/- 4 Percent.), of MAGNESIUM-SULFATE in the Solid phase is 0,01652101, of POTASSIUM DIHYDROGEN PHOSPHATE in the Solid phase is 0,075422004, of DIPOTASSIUM-PHOSPHATE in the Solid phase is 0,10774572 and of CYTIDINE-5' MONOPHOSPHATE in the Solid phase is 0,010175985 (General Range: +/- 4 Percent.). The fermentation time is 231 min. The final temperature is 30 C. The heat of reaction is 15000 kJ/kg of component CDP-CHOLINE in Liquid transformed. Use 100 C Hot Water on the Entire Jacket.

4. Transfer contents of unit Fermenter F-10000 to ST-D. This is the key step output. The transfer time is 10 min.

5. Filter the batch from unit ST-D in filter FI-400. The transfer time of the slurry is 30 min. Transfer 50% of the batch to the filter. The mother liquor, named Solución, is sent to Fuji Paudal VMixer VM-2000. Transfer the mother liquor via Tubing. The cake contains 91% of CHOLINE CHLORIDE in the solid phase, 96% of POTASSIUM DIHYDROGEN PHOSPHATE in the solid phase, 100% of MAGNESIUM-SULFATE in the solid phase, 99% of DIPOTASSIUM-PHOSPHATE in the solid phase, 89% of DEXTROSE in the solid phase, 75% of CYTIDINE-5' MONOPHOSPHATE in the solid phase and 99% of ACETONE in the solid phase. The moisture content in the final cake is 5%.

6. Mix the contents of unit Fuji Paudal VMixer VM-2000. The mixing time is 15 min. Maintain the temperature of the batch at 25 C. The agitator is Spinbar. The rotation speed of the agitator is 200 Rev/min. Continuously add 952,42 liter of Hydroxide solution.

7. Transfer contents of unit Fuji Paudal VMixer VM-2000 to ST-B. This is the key step output. The transfer time is 10 min.

8. Clean unit FI-400. Cleaning time is 20 min.

9. Filter the batch from unit ST-D in filter FI-400. The transfer time of the slurry is 30 min. Transfer 100% of the batch to the filter. The mother liquor, named Solución almacenada, is sent to Fuji Paudal VMixer VM-2000. Transfer the mother liquor via Tubing. The filter separates 100% of all solids. The moisture content in the final cake is 5%.

10. Mix the contents of unit Fuji Paudal VMixer VM-2000. The mixing time is 15 min. Maintain the temperature of the batch at 25 C. Continuously add 952,42 liter of Hydroxide solution.

11. Transfer contents of unit Fuji Paudal VMixer VM-2000 to ST-D. This is the key step output. The transfer time is 10 min.

12. Transfer contents of unit ST-B to ST-D. This is the key step output. The transfer time is 10 min.3. Separation (chromatography)

13. Load the column Chromatography 100. Use 100% of the material in ST-D. The transfer time is 60 min. The resin retains 92% of CDP-CHOLINE.

14. Wash the column Chromatography 100. Use 3809,7 liter of WATER. The transfer time is 30 min. The column retains 30 liter of the solution. The solution removes 11,429% of the column

resin contents. Spent Wash Stream: The spent wash is sent to ST-D. Transfer the spent wash via Tubing.

15. Elute the column Chromatography 100 with 6666,96 liter of FORMIC-ACID. The elution time is 68 min. Collect 6190,75 liter at 476,21 liter into the elution. The cut is sent to ST-C. The solvent removes 87% of CDP-CHOLINE. Unspecified components are sent to waste.

16. Load the column Column carbon Generic. Use 100% of the material in ST-C. The transfer time is 50 min. The resin retains 98% of CDP-CHOLINE.

17. Elute the column Column carbon Generic with 285,72 liter of WATER. The elution time is 49 min. Collect 238,11 kg at 28,572 kg into the elution. The cut is sent to AT Crystallizer 10 cubic meters. The solvent removes 86% of CDP-CHOLINE.

18. Crystallize the batch in unit AT Crystallizer 10 cubic meters. The following components are separated in the crystal phase: 91% of CDP-CHOLINE. The crystallization time is 117 min. Continuously add 2857,27 liter of WATER.

19. Transfer contents of unit AT Crystallizer 10 cubic meters to Dryer 5 cubic meters. Transfer 100% of vessel contents. The transfer time is 10 min.

20. Dry the batch in unit Dryer 5 cubic meters. The drying time is 2 h. The drying temperature is 50 C. The moisture content in the final cake is 5%.

21. Transfer contents of unit Dryer 5 cubic meters to SS tank. This is the key step output. The transfer time is 10 min.

5.1.2. Results discussion

The results obtained from ASPEN, demonstrates the two main inconvenience in that process, the fermenter and the purification. The biological reactor, is the step that requires the most time during the manufacture, therefore is the controlling stage. It implies, that an increase of production, pass through a change in the volume of the reactor, being more than 10.000 litters. In addition, the yield of the reaction is higher than the recipe, because it is also optimized, obtaining 133 kg of crude citicoline, and the CMP it's consumed the 77%. If the operation gets the same yield as the recipe, it will get 119.7 Kg as crude citicoline, in the same volume, the 95% of the fermenter.

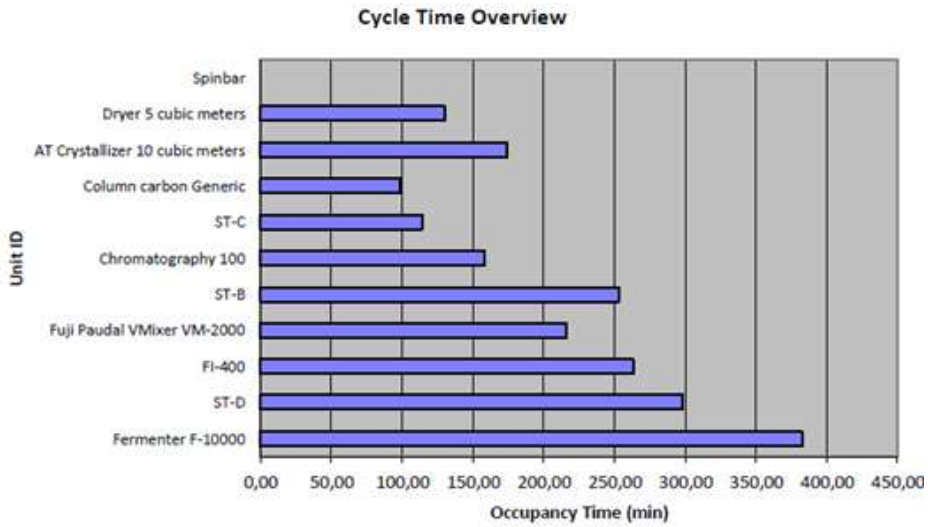

Figure 14. Size utilization

In the campaign results, the data obtained are in 72 batch, obtaining 69kg/Batch and the time necessary to get this scope is 19.9 days. This process it will be repeated until 6 times per year, to obtain almost 30.000 Kg

This Figure 14 is the occupation in each batch, and the reactor, is the equipment that has the highest percentage of volume, excepted both columns, because during the process, the average components that flows through that, is higher than its capacity. Moreover, in the average cycle time, the reactor is the equipment that consumes the most of time, it implies its bottleneck condition [Figure 15]

The ressults obtained in ASPEN demonstrate that a improvement in the process should pass through either reducing the reaction time or mprove the volum productivity and get beter results in the purification stages.

In this plant, the number of API that requires a bioreactor of this volume is minimum, moreover it is the most manufacture process in this plant, before the paracetamol (it use a chemical reactor). Therefore, to increase this volume has low viability, and the existence of the generic drug, implies that Cemicina will not increase its demand considerably. Then, the corporative necessity is get better productivity to reduce costs.

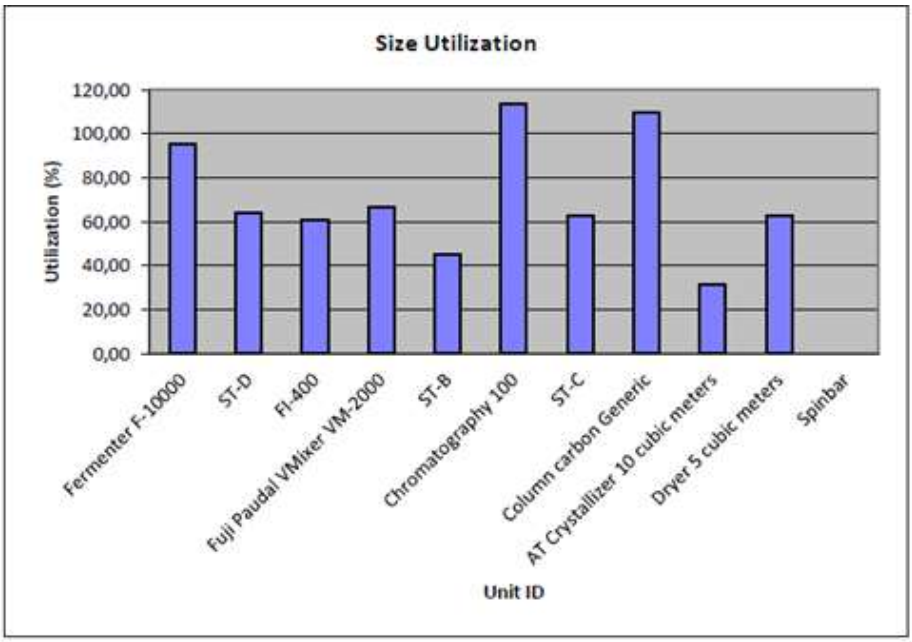

Figure 15. Cycle time.

In the chromatography column, despite the citicoline lost in this stage, at the moment is not possible to get better yield, because as it is said before, it has been optimized, increasing the yield up to 50% in this stage. The only alternative is either get another cycle, but it could provoke that the slowest stage will become the purification or obtain another patent to purification the citicoline, such as the KR101311571B1.

About the results obtained in the campaign, the low quantity achieved in each batch requires an optimisation in the process, concretely in the overlap. For this reason, it is used some tanks to avoid that an inexpensive stage, it can produce a higher time per batch. Next images, show the importance of that, because the time necessary to get 10 batches, is much higher without overlap.

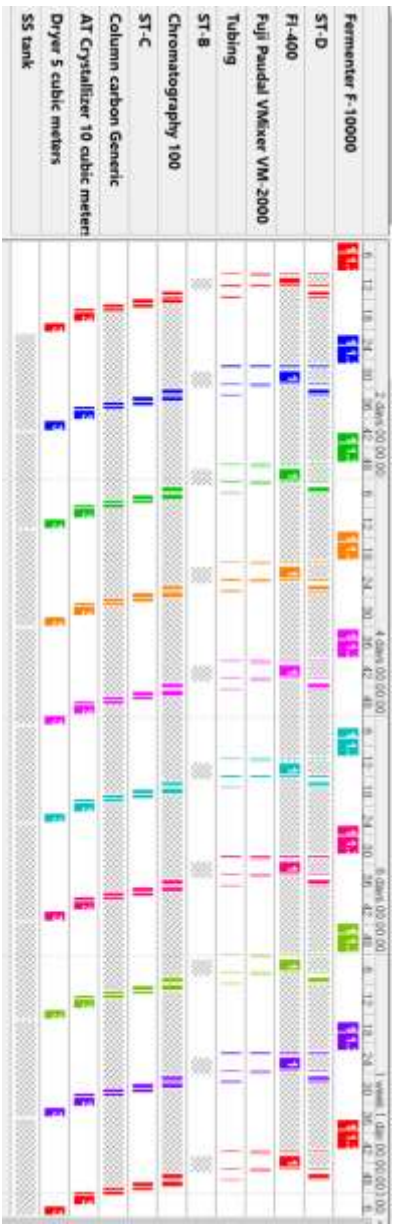

Figure 17 . Campaign without overlapping

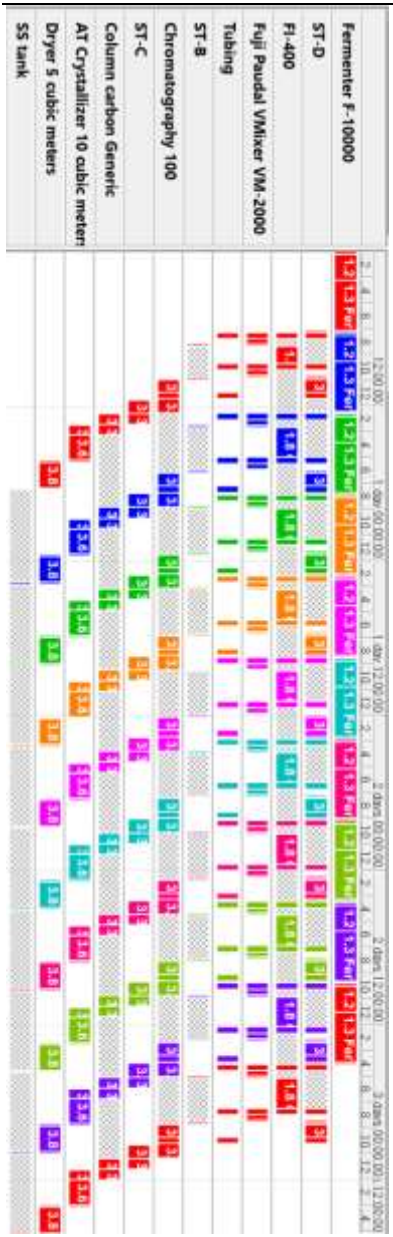

Figure16. Campaign overlapping

5.2. PROCESS WO2013/128393

In order to obtain coherent information to make future adjustments, the results should be the most similar with reality, then an escalade will be carried out to get it. The scale consists of three parts, the recipe (text offered by the patent), after that, the process is developed in a pilot plant to adjust, and finally develop it in plant. In the annex is possible find each of the recipes.

The simulation of the process 128393 in Aspen, it will be carried out in two situations, an unfavourable condition compared with a more favourable (modifying equipment, yield reaction and yield in purifications), with the aim to quantify the improvements and study if in both conditions is obtained the scope. However, the reaction times will be the same, because it is indicated in the patent recipe.

5.2.1. Unfavourable situation

The process will be developed in the most disadvantageous conditions possible, always within the margins offered by the patent, or extrapolated from the patents previously exposed (only the performance of the reaction). These conditions are the following:

Performance of the reaction: In both syntheses, the limiting reagent will have a conversion of only 75% CMP. However, the yield obtained in the recipe, it is unreliable because the CMP represents in weight approximately the 75%, then if is add 100g of CMP, it is not possible get 2625g of citicoline.

Equipment of the process: The equipment will not undergo any modification, except in the reactor, this will affect the equipment to work in lower capacities than the designed. However, this process is carried out by chemical synthesis, so it will use a reactor available during the Cemicina campaigns. This reactor has a volume of 1600 liters, but it will be used in two possibilities, the 90% of volume, using 900 liters, to compare with the favourable situation and evaluating the effect of the losses in reaction yield and purification, because the favourable situation use a reactor of 1 cubic meter in the second reaction.

Stages of purification: One of the characteristics that the citicoline production is so expensive; it is its low efficiency in the purification stages. The following simulation will show a productivity that is far from the one described in the example.

5.2.1.1. Process simulation

In the Annex of the project, it could be founded the process in the different stages of the scalation, the recipe (laboratory), pilot plant and the generic plant. To simulate the described condition, it has been modified the capacity of adsorption in the carbon column, and the crystallization (the precipitation reaction) the amount of citicoline no precipitated, is higher.

The elaboration of this simulation it has been done at the same time that the real condition, for this reason, it will be studied in two ways the process in ASPEN, to get results with the same quantity of raw materials in each situation (the unfavourable and the real one) and finally comparing the influence of the variations in yield (reactions and precipitation) and finally, using the 90% of the reactor, as is has been stipulated.

To get the results, it has been scaling every stage and finally, to replace the equipment from the current process. During the replacement, it has been appearing some problems about the volume, because the amount of liquid, is considerably minor than the current, hence, some operations are not developed in the design condition, such as the crystallizer.

5.2.1.2. Results discussion

The application of this method in Aspen, demonstrates the high capacity of this invention, even though, having the worst situation described in the patent. In this condition, using a 26.91 day, the campaign objective is obtained in 64 batch (79 kg/batch). It shows that is not possible obtain the scope, due to reactor time.

Figure 17 indicates the low occupation in each stage, having a reduce operation volume, but increasing considerably the obtention of crude citicoline, if it is compared with the current one. However, the utilization of only one reactor, difficult the optimization of the process, because its reactor requires elevate time of operation and the reactant volume should be moved to different stages, such as the cooler, staying the reactor empty and losing time to manufacture citicoline. The differences between figure 18 and figure 19 is adding the time when reactor is empty, it shows the inconvence in use a single reactor.

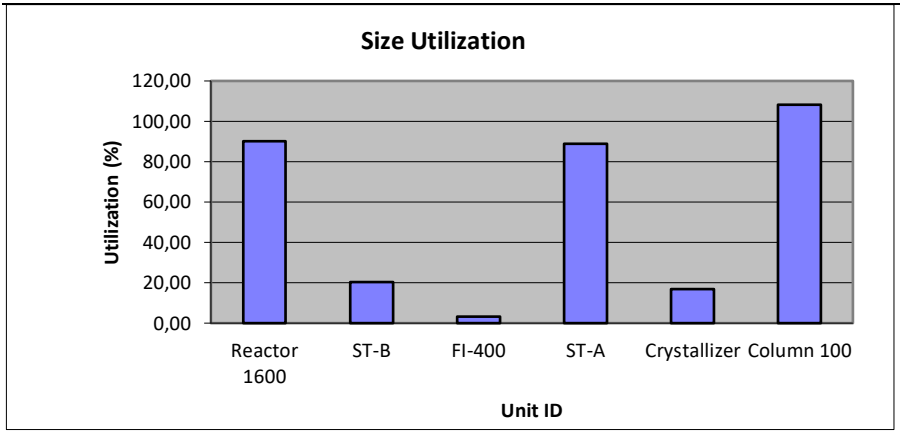

Figure 17. Size utilization.

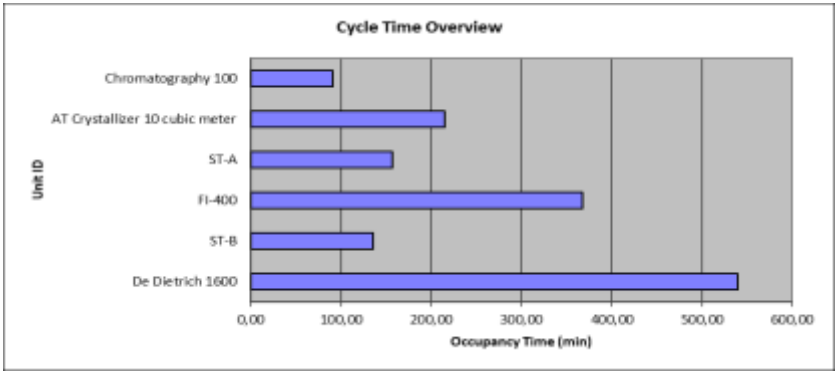

Figure 18. Cycle time overwiew.

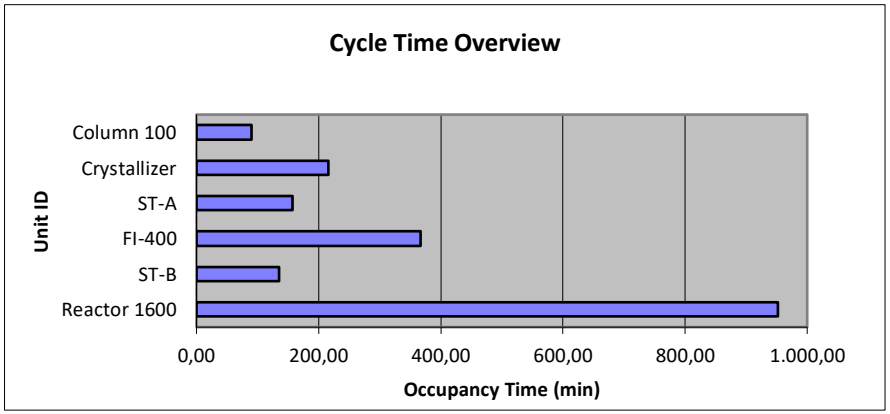

Figure 19: Cycle time overwiew evaluating when reactor is empty

Purifications stages, gets better productivity removing chromatography column. In the first process, the losses caused by the different stages of purification represents the 47%, getting as a product 133.62Kg of crude citicoline in front of 69.72Kg as purified product. In this case, the losses represent 9.56%.

Both increases in productivity, shows the problems can be solved through changing the process to manufacture this API. However, a reduction of time in the imitating stage, it will offer to Ceuber gets the same scope with less time, given that the citicoline obtention is developed in two reaction, a possibility is install other reactor. Finally, the column needs this large number of hours [Figure 21] because is directly related with the reactor, because it is in use but no working, as it shown in Figure 20.

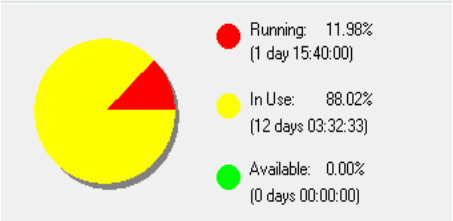

Figure 20. Time used by column.

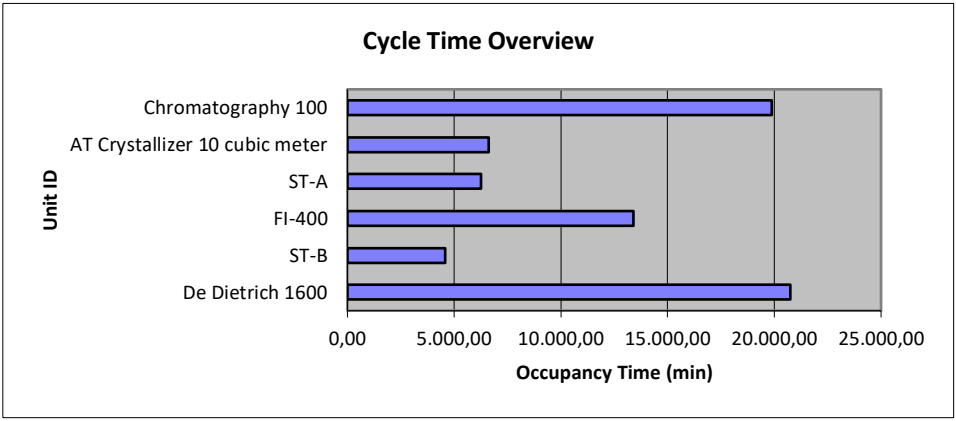

Figure 21. Cycle time Overwiew

On the other hand, applying the changes to get better yield in purification and reaction it will increase substantially the productivity, but is not the most significative point. In case to apply these improvements, the citicoline manufactured is 112.Kg per Batch, in this situation, the process is capable to obtain the scope in the time that is determined by campaign. Next paragraphs make

evident that another reactor should be install to get better productivity and improve the overlapping, next figure confirms its problems.

Figure 22. Shedule process.

5.2.2. Real situation

In this case, the process will be carried out with an optimization of the equipment, in order to obtain a better productivity time, the reactions will be a yield of 85%, unlike 75%. Finally, in the different purification stages (carbon column and precipitation reaction), the losses in the active principle will be lower.

Another consideration to realize the simulation, it is the no preoccupation to use a large number of equipment, for instance the tanks. The reason to manage this quantity of tanks, it is for do no create bottleneck in operations that the cost is not elevated. Moreover, the tanks used, there are available in plant (the tanks with high volume) and simulating news, with less volume, for example the “Tank Set”.

In the following table, it shows some changes applied in the process:

Equipment	Worst situation	Real situation
Reactor	De Dietrich 1600 liters	De Dietrich 1000 liters
		DIN Reactor 800 liters
Filter	Fi-400 (4000 liters)	Belt Filter (100 liters)
		Filter P100 (100 liters)
Crystallizer	AT crystallizer 10000 liters	AT crystallizer 2000 liters

Table 6: Changues applied

5.2.2.1. Process simulation

The method to realize this simulation, it is using the previous one and changing the different equipment in order to get better results but using the same amount of raw materials, to observe the influence in productivity time and in the same time, how affect that in campaign time. The prior results, demonstrates that the reactor is the operation that needs more time, being two reactions in the same recipe, taking into account that the prior has a reaction volume of 1.6 cubic meter, it will be applied two reactors (one of 1000 liters and another one of 800 liters), these changes implies a reduction of more than three hours in each batch.

However, after these changes, it appears problems in tanks and filter, becoming these equipment that needs almost time, to face with this problem, it has been applied some tanks and clean two times the filter per batch.

Finally, every equipment that is used in this simulation, are predefined in ASPEN (with the exception of the first reactor), it could imply that exists equipment that suits better this improvement and some equipment are reused, such as the carbon column. This ASPEN recipe, it could be founded in the annex with all details.

NITROGEN	28,01	0,0189	0,0227
OXYGEN	32,00	0,0057	0,0069
Morpholidiate cytidi	428,17	1,16	1,06
HCl-methanol	685,00	0,0297	
METHANOL	32,04	0,4124	
ISOPROPYL-ALC	60,09	0,1095	
MORPHOLINE	87,12	0,0363	
WATER	18,02	3,03	
Cytidine monophos	323,20	3,76e-004	3,06e-004
1,3-dicyclohexylcar	206,33	0,0010	9,33e-004
CITICOLINE	488,33	74,11	74,11
Calcium phosphory	257,66	0,4726	0,2332

Table 7. Citicoline obtained

5.2.2.2. Results discussion

Table 7 shows the number of products in the last stage in that simulation, the changes applied in the reaction yield and the different stages of purification (the adsorption column and the precipitation), it allows for getting an increase of 19kg in each batch, using the same quantity of raw materials and the same reaction volume, respect the simulation described above. Obtaining 80Kg in each batch, the number of batches it will decrease, and finally, the campaign time.

To get the same scope as the first simulation (current process) it is necessary to apply 68 batches, in conditions of overlapping, then the campaign time will decrease considerably, being 22721 min, or 15.778 days. As it is said in the simulation, putting a second reactor increase considerably the productivity reducing the batch time. Now, the slowest stage continues being the reaction, but reducing its time that occurs in 5 hours and 40 minutes (charge the recipient, the reaction and transfer). In the requirements, it has been pointed out that the reaction volume is the 90% to get the scope in a less severe conditions to manufacture, because the fermenter it has been designate to be operate with a capacity of 90% not the 95% as it has been simulated, that is its maximum capacity. Figure 23 shows the different capacity in each operation and Figure 24 the time applied in all campaign in each recipient.

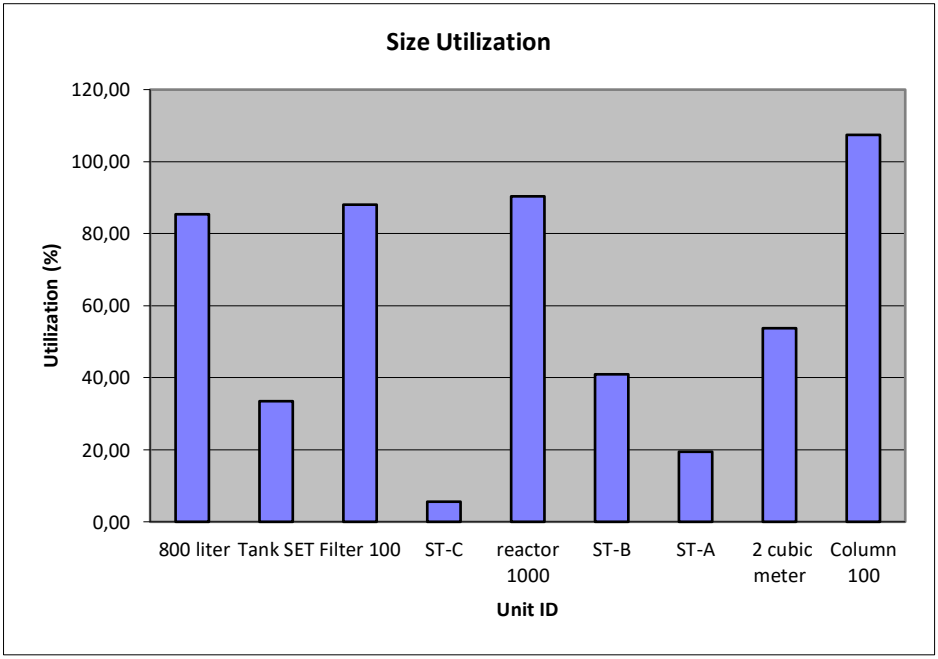

Figure 23. Size utilization

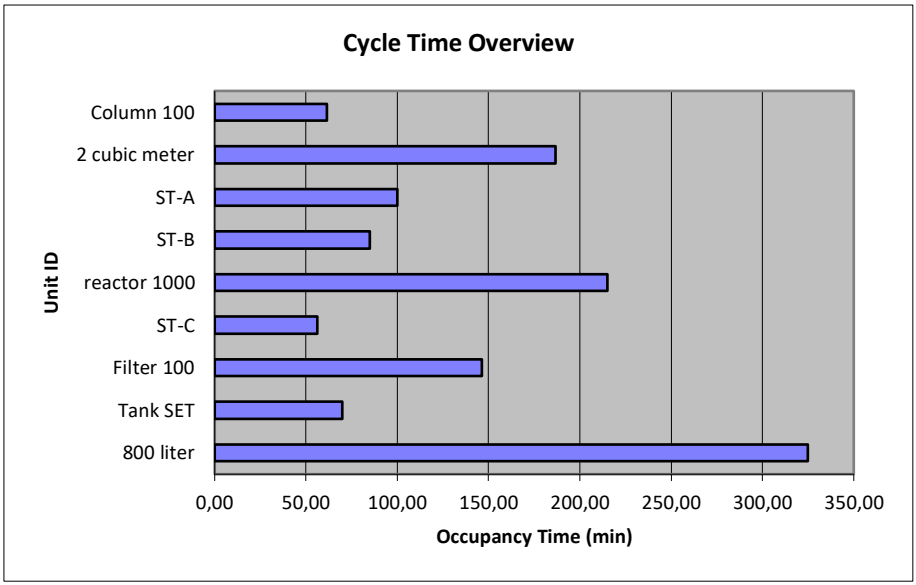

Figure 24. Cycle time overwiew.

This renovation offers to the company two ways to carry out, either reducing the campaign time or use equipment with less volume:

The ability to manage better the campaign time, it allows the company to ensure its production time in case of an important fail. No having results about the necessities of cleanliness in this new process, it could imply a longer time to do this operation, and it affects the process time. Finally, a reduce number of cycles, it provokes a longer life in the equipment.

On the other hand, the reduction in the equipment volume, implies a reduction in the energetical necessities in plant, reducing costs and reducing environmental impact per batch.

5.3. PROCESS COMPARATION

Process	Kg per Batch	Number of Batch	Campaign time	Total Kg	Volume capacity (reactor)
Current	69	72	19,9 days	4968	95
Unfavourable condílion	102	49	20.63days	4998	90
favorable condílion	74.11	68	15.778 days	5032	90

Table 8: Summary of results

* unfavourable condition (yield 75% in reactions) is not developed because no gets the scope.

In order to compare which process has the best results, and finally, make a decision about the viability to Ceuber to change the process, it will be done through the next results: Productivity time and productivity time volume, as the prior section, but using productivity time column because the three reactors have got different volume. For this reason, it has not been into a consideration the influence of the other recipient volumes, because the stage that modifies these results, are the reactors. It is important to point out that the reactor volume for the "real" will be the biggest one, with 1000 liters.

The productivity time volume (PTV) is evaluated as follows:

$$PTV = \frac{Mass\ of\ pure\ citicoline}{Reactor\ volum * canpaign\ time}\ \left[\frac{Kg}{L * day}\right]$$

Then, the results obtained in each process, are the next.

Process	PT (Kg/days)	PTV (Kg/liter*day)
Current	249.65	$2.49*10^{-2}$
Unfavourable condition	242.26	0.15
Favourable condition	318.92	0.318

Table 9: Results obtained

The results, demonstrates the high viability to study this process experimentally and analyse its future development in plant. Obtaining an increase of 1.5 in times PT and 13 times PVT, it indicates that the corporative objective is obtained, because exists a process that gets greats results with low volume in the reactor and eliminating the chromatography column.

The two different simulations, regardless the modifications in the different reactions, indicates that there are two ways to apply this process: Either through overlapping the two different reactors, or using only one:

The management of one reactor, it provides Ceuber some positive aspects. The space of one reactor, its less than two, such as its security distance between others, and in a plant, the saving space is important. Increasing this reactor volume, it also provokes that the cycles per campaign is less than the other option. The control of one reactor, it could be easier than two and in the same time, it avoids to change less the products between different equipment and this possibility, that obtains the requirements to be applied, even though in bad conditions about yield, it gives the possibility to reuse its chemical reactor.

The most important of this point is the possibility to develop the process with the equipment available in plant, as a condition of the requirements. This application has some alternatives, but everyone has an inconvenience, such as the low occupancy in the different stages, being a waste in energy, or the other situation is applicate the paracetamol reactors, but these will create a great problem to storage the API.

On the other hand, the PTV in the situation that the rector is overlapped, it is much higher than the another one, even though using the same productivity in each stage. Other reason to discard this option, is the chemical reaction, because it consists in two parts, having in the middle a separation that implies freeze the solution, then, the solution must be moved from the reactor. Moreover, the option to use two reactors, it adequate better in the current process, because the reaction needs more or less the same time, easing the application in plant.

It concludes that, with the exception of the low space available in plant, taking into account that Ceuber will not move the fermenter, because it is used in other APIs; the best solution is apply two reactors, as it has been simulated in ASPEN, using a 90% of occupation. Despite having been simulated this option, the company could apply modifications in the reactors volume to

obtain the same scope in higher time and less volume, ever depending in the available necessities of the company, such as space, workforce, etc and affecting as little as possible, in the manufacture of other APIs. The application of this process in this condition, ensure its obligation in case of adverse event, such as an equipment fail and an unexpected demand.

However, despite having a large number of benefits aspects, it should be taking into a consideration the next points:

Firstly, a change in a pharmaceutical process it ought to be notice to EMA, and finally needs to get the approbation of this agency. The no obtention of this permission because of no approves the GMP, prevent the commercialisation of Cemicina. Then, if it is decided to change the process, it should be ensuring that this process comply and the no possibility to reduce the quality of this API.

The aspects that are related with products are the next: there are not suppliers that offer the morpholidiate salt of oxalic acid, provoking the necessity to manufacture this product. Despite offering other options in the description of this patent, the utilization of other component, it could affect in the precipitate reaction, getting worst results. Therefore, in pilot plant, it should be studied the different possibilities in order to get the best results and in case of manufacturing this salt, to analyse which option is the best for the company: either manufacture this compound in its fine chemical plant, to increase the batch time or adding a new stage during the overlap. Other consideration about these compounds, are this elevate hazard to human, then, it should be studied the possibility of traces, such as HCL, oxalates, to avoid its dangerous effects.

The reduction of volume in the different equipment, provoke the reduction in energy and its environmental impact. However, this new process, it should be analysed with detainment to not lose the ISO 14001.

To conclude, the results obtained in ASPEN studying the basic engineering, that are capable to demonstrate the high viability of the process. Although, this process should be evaluated experimentally in order to evaluate its development, because of these numbers are not able to ensure its future application in plant.

6. CONCLUSIONS

-The bibliographic search demonstrates the advances made in the production of citicoline; corroborating the need to analyse the possibility to obtain a new process.

-Throughout the project, it is confirmed that the WO2013128393 patent is the one that is adapted to improve the corporate requirements, eliminating high cost operations.

-In the simulation of the two processes using Aspen Batch process developer, results are obtained exceeding expectations, increasing PTV up to 20 times.

-The maximum productivity, under the conditions established by the company, is obtained through the application of the patent WO2013128393 overlapping two reactors.

-It is not possible to say categorically that the application of this process is the best for the company. However, the need to be studied more thoroughly is demonstrated in order to obtain data that does not reach basic engineering.

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ACRONYMS

(API) Active Pharmaceutical Ingredients

(SmPC) Summary of product characteristics

(GMP) Good Manufacturing Practice

(HPLC) High performance liquid chromatography

(ISO). International Organization for Standardization

(MSOA).Morpholidiate salt of oxalic acid

(PT) Productivity Time

(PTV). Productivity time volume

(CMP). Cytidine Monoophosphate

(DCC). N,N'-Dicyclohexylcarbodiimide

APPENDICES

APPENDIX 1: STUDIED PATENTS

Patent CN104031105A

Method for preparing citicoline sodium

Abstract

A method of preparing the citicoline sodium, oxalic acid was added to the aqueous solution of calcium phosphate, choline chloride, and the precipitate was removed after the water, in a solid phosgene, cytidine is reacted with an acid, and then purified by recrystallization cell sodium phosphate choline.

Patent CN1944661A

Process for preparing citicoline sodium

Abstract

The present invention is process of preparing citicoline sodium. The preparation process includes the biotransformation of material including 5'-cytidylate, phosphorylcholine, potassium hydroxide and glucose with culture as the biocatalyst; extraction and separation with active carbon as the adsorbing carrier, Cl- type ion exchange resin as the separating carrier and re-compounded water-alcohol mixture as the analyzing reagent; and product purification with alcohol solvent as the crystallizing solvent. Compared with available technology, the present invention has the advantages of high product yield and high product purity.

Patent CN 105732752A

Citicoline and synthetic method thereof

Abstract

The invention discloses citicoline and a synthetic method thereof.The synthetic method includes subjecting cheap and easily available cytidine, serving as a raw material, and dichlorophosphoryl morpholine to condensation at a 5' position highly selectively so as to obtain 5'-phosphorylmorpholinylcytidine; subjecting the 5'-phosphorylmorpholinylcytidine and phosphocholine chloride calcium to condensation in solvents so as to obtain the citicoline.Total yield is up to 58% only by the two steps.The synthetic method is cheap in and easily available to raw materials, expensive cytidine monophosphate is unused, operation steps are simplified, and reaction scale can be expanded to synthesize 500 g of citicoline.The synthetic method has a potential application prospect by providing a novel citicoline synthetic route.

Patent CN 103849666 A

Method for catalytically producing citicoline sodium with immobilized enzyme

Abstract

The invention provides a method for catalytically producing citicoline sodium with an immobilized enzyme. The method comprises the following steps: by utilizing engineered escherichia coli of a molecularly cloned cytidine phosphotransferase gene for fermentation, preparing cytidine phosphotransferase, and preparing cytidine phosphotransferase liquid through purification; immobilizing cytidine phosphotransferase; by using phosphorylcholine and cytidine disodium triphosphate as raw materials, catalytically generating citicoline with immobilized cytidine phosphotransferase. The method is simple in production process, short in production cycle and low in production cost and can be widely applied to industrial production of citicoline.

Patent CN 102586383

Process for preparing cytidine diphosphate choline

Abstract

The invention relates to a process for preparing cytidine diphosphate choline. According to the method, denovo synthesis of pyrimidine is utilized as a basic reaction and choline materials are subjected to a reaction at the reaction temperature ranging from 20 DEG C to 38 DEG C for 10 to 72 hours with pH controlled at 5 to 7.5 in the presence of culture solution of microorganism A or further in the presence of culture solution of microorganism B or treatment so as to accumulatively produce cytidine diphosphate choline. Simple starting materials of carbon and nitrogen sources are utilized, in culture solution containing the choline materials, precursor of the cytidine diphosphate choline, namely cytidine monophosphate is synthesized by means of metabolic process, and cytidine diphosphate choline is cumulatively produced in the reaction solution. According to the method, expensive cytidine monophosphate or the precursor materials are avoided so that the preparing cost of the cytidine diphosphate choline is greatly reduced.

Patent CN 102199643 A

Preparation method of citicoline

Abstracte

The invention discloses a preparation method of citicoline. The method comprises the following steps: the culture of a single gene engineering bacterium or the treatment material of the culture is used as enzyme source to catalyze the reaction of a substrate comprising ammonium chloride, orotic acid and phosphocholine and ensure that citicoline is generated and accumulated in the reaction solution, and citicoline is extracted from the reaction solution. The invention has the following beneficial effects: (1) the single microorganism is utilized to catalyze the reaction, reaction conditions are easy to control; (2) the cost of the substrate is lower so that the production cost of citicoline is lower; and (3) the reaction is fast and the conversion rate is higher. The method can be widely used in the preparation of citicoline.

Patent CN 101265453 A

Screening and application of beer microzyme containing CMP kinase and CDP kinase

Abstract

The invention relates to a method for screening Saccharomyces cerevisiae with high-activity CMP kinase and CDP kinase, and the use of the Saccharomyces cerevisiae in the production of cytidine triphosphate, and belongs to the technology field of biological engineering. The Saccharomyces cerevisiae strains is preserved in the China General Microbiological Culture Collection Center (CGMCC) with accession number of CGMCC NO.2448. The Saccharomyces cerevisiae strains with high-activity CMP kinase and CDP kinase are screened by means of nitrosoguanidine (NTG) mutagenesis. In the production of the cytidine triphosphate, the Saccharomyces cerevisiae strains are used to be prepared into immobilized cells of various grain diameters with carrageenan as carriers by different controlled stirring speeds realized through embedding. Compared with manual cutting, the screening method of the invention has the advantages of small cell leakage, regular grains with high strength, low labor intensity, simple grain-producing procedure, no complicated equipment and instrument, and is suitable for industrialized production. Furthermore, bean oil used in the invention is safe and nontoxic solvent, suitable for various requirements and easy to recycle.

Patent CN201410247176

Method for preparing citicoline sodium by utilizing oxalyl chloride

Abstract

The invention discloses a method for preparing citicoline sodium by utilizing oxalyl chloride. The method is characterized by comprising the following steps of: by taking choline chloride calcium phosphate (P-choline) as a raw material, dissolving in organic amine not containing reactive hydrogen, adding oxalyl chloride for reacting for 0.5-2 hours after adding benzene for removing water in an azeotropic manner, and then, adding cytidylic acid (5'-CMP) for reacting.

Patent CN107488603A

Overexpressed phosphorylcholine cytidine transferase saccharomyces cerevisia genetically engineered bacteria as well as construction method and application thereof.

Abstract

The invention relates to overexpressed phosphorylcholine cytidine transferase saccharomyces cerevisia genetically engineered bacteria as well as a construction method and application thereof. The construction method comprises the steps of cloning saccharomyces cerevisia sourced gene cct capable of encoding phosphorylcholine cytidine transferase to a pYES2.0-Kanmx carrier so as to construct recombinant plasmid pYES2.0-Kanmx-cct, and transferring the recombinant plasmid pYES2.0-Kanmx-cct into S.cerevisiae HG, so as to obtain the saccharomyces cerevisia genetically engineered bacteria. The yield of a solid matter of the strain to fermentable sugar is 50%-56%, the strain can be used for manufacturing a citicoline product from 5'-cytidine monophosphate and phosphorylcholine through bioconversion, and after the strain reacts for 7 hours, the molar conversion ratio reaches up to 73%. The yield of the strain in a fermentation system for producing and culturing the strain is high. When applied to the production of citicoline, the strain has the advantages of low cost and manufacturing energy consumption, short reaction period and the like.

Patent CN101906126B

Method for separating purified cytidine diphosphate choline by hydrophobic chromatography

Abstract

The invention discloses a method for separating purified cytidine diphosphate choline by hydrophobic chromatography. The method comprises the following steps of: (1), performing enzyme inactivation, centrifugation and ultra-filtration of a cytidine diphosphate choline conversion liquid; (2), after adjusting the pH of the pre-processed cytidine diphosphate choline conversion liquid to between 2.0 and 14.0, adding an inorganic salt to prepare column-loading liquid which contains 0.01 to 5mol/L of inorganic salt and 0.1 to 40g/L of cytidine diphosphate choline with the cytidine diphosphate choline conversion liquid, absorbing the column-loading liquid with a

hydrophobic chromatography resin and eluting with pure water of which the pH value is 2.0 to 14.0; and (3), nano-filtering, desalinating and concentrating the eluate, and obtaining the cytidine diphosphate choline through crystallization. In the method, the separation process is simple; through regeneration, the resin after elution can be repeatedly used; the separation cost is low; the product is easily crystallized; and the CDP-choline product with high purity and high yield can be obtained.

US patent 3.684.652

Method for preparing cytidine diphosphate choline

Abstract

Cytidine diphosphate choline is prepared by cutlturing an aqueous reaction liquor containing (a) at least one of the class consisting of choline or phosphoryl choline; (b) at least one of the class consisting of cytidine, cytindine 5''monophosphate, cytidine-5''-diphosphate or cytidine-5''-triphosphate; (c) enzyme systems of bacteria, cultures or molds; (d) phosphate ion and (e) at least one of the class consisting of magnesium ion and manganese ion and recovering cytidine diphosphate choline from said culturing liquor

KR101311571B1

Method for purification of cytidinediphosphoric choline

Abstract

The present invention, pH is 0.5 or greater than 5.0 of cytidine is by phosphoric acid choline solution in contact with H-type strong acid cation exchange resin, the cytidine water or ion concentration of 0.1 with phosphoric acid choline adsorbed onto the resin containing the nucleic acid related substances by elution with an aqueous solution of below mol / ℓ, the cytidine and cytidine characterized in that the purified phosphoric acid choline is an object of the invention to provide a method for purifying phosphoric acid choline.

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Patent JPS6125358

Purification of cytidine-5'-diphosphate choline

Abstract

PURPOSE: To purify the titled compound economically on an industrial scale, by contacting an aqueous solution of crude cytidine-5'-diphosphate choline with a strongly acidic ion exchange resin and a weakly basis ion exchange resin, thereby specifically adsorbing and removing the impurity components from the solution.

CONSTITUTION: An aqueous solution of a chemically synthesized crude cytidine-5'-diphosphate choline (abbreviated as CDP-choline) is made to contact with a strongly acidic ion exchange resin (e.g. a resin produced by sulfonating a crosslinked polystyrene) and a weakly basic ion exchange resin, preferably by using a column, and usually, the treated aqueous solution is concentrated. The CDP-choline can be crystallized by purifying the concentrated solution with an alcohol. The CDP-choline solution used as the raw material has a CDP-choline concentration of preferably 3W10wt%.

Patent WO2013128393A1

A process for preparing pure citicoline (cdp-choline)

Abstract

Disclosed herein is a process for preparing highly pure Citicoline (CDP-Choline) or sodium salt of Citicoline with the aid of dicarboxylic acid or its salts. The process of the present invention results in Citicoline with a purity of more than 99% measured by HPLC.

Patent CN102010454B

Citicoline sodium compound and new method thereof

Abstract

The present invention provides a citicoline sodium compound and new method thereof, wherein purification is performed via active carbon adsorption, acidification reaction and preparing

chromatographic column, finally a citicoline sodium compound with high purity is obtained; the shortage of low raw material purity in the prior production is recovered, simultaneously the quality of preparation product is improved, and side effects are reduced. Compared with the prior art, the method has the advantages of simple and easy technology, low cost, high yield and high product purity; the method is suitable for industrial production; and the citicoline sodium prepared by the refining method of the present invention is suitable for being used in preparation of neural activation agent pharmaceuticals.

US Patent 6.057.301

Hyperhydrated citicoline, process and use

Abstract

A hyperhydrated form of citicoline and its formulations, which exhibits desirable characteristics, including crystal formation, moisture resistance, improved storage stability, and formulation versatility, is disclosed. Methods of preparing the citicoline hyperhydrate and its use in the manufacture of stable pharmaceutical dosage forms are also described.

US Patent 3.687.932

Crystalline cytidine-5{40 -diphosphate choline monohydrate and production thereof

Abstract

Crystalline cytidine-5''-diphosphate choline monohydrate is produced by a. adding a hydrophilic organic solvent to an aqueous solution system of cytidine-5''-diphosphate choline in the ph range of from about 2 to about 4, or b. keeping cytidine-5''-diphosphate choline standing at a relative humidity of not less than about 32 percent and at a temperature in the range of from about 17*C to about 70*C for not shorter than about 5 days.

US patent 3666748

Method for production of cytidine (or deoxycytidine)-5{40 -diphosphate choline and intermediates therefor

Abstract

Cytidine-5''-diphosphate choline and deoxycytidine-5''-diphosphate choline are produced by reacting cytidine (or deoxycytidine)-5''monophosphate with a choline phosphoramidate. Choline phosphoramidates of the formula WHEREIN R1 and R2 represent hydrogen, a hydrocarbon residue having at most seven carbon atoms, or R1 and R2 taken together represent a five- or six-membered heterocyclic ring are prepared by reacting phosphorylcholine with an amine.

APPENDIX 2: ASPEN RESULTS

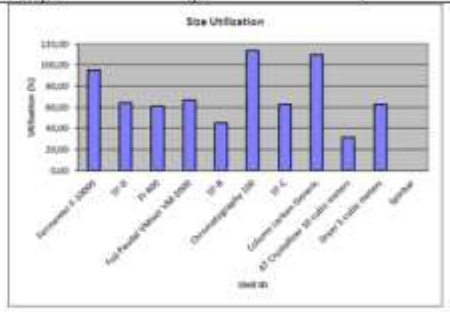
Step Executive Summary (Batch)

Process (Version):	Process Fermentation(1.0)	**Key Input Intermediate:**	POTASSIUM-HYDROXIDE
Step (Version):	Piloto (Receta - Copy - Copy)	**Key Output Intermediate:**	CDP-CHOLINE (as crude citicoline)
Simulation Date:	15/06/2018 11:42	**Plan Quantity:**	133,62 kg

Batch Size (kg)	Potential Batch Size (kg)	Cycle Time (min)	Batch Time (min)	Production Rate (kg/min)	# of Batches	Campaign Time (min)
133,62	117,82	408,55	1.199,03	0,33	1	1.199,03

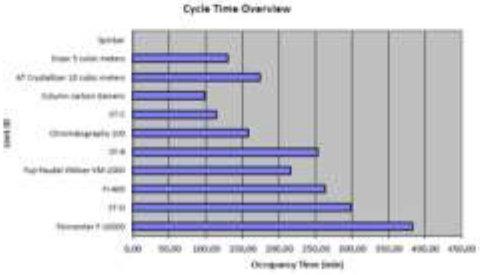
Input Material

Component	Per Batch Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)
CHOLINE CHLORIDE	647,89	647,94	647,89	647,94
CYTIDINE-5' MONOPHOSPHATE	199,23	199,24	199,23	199,24
ACETONE	2.699,55	2.699,74	2.699,55	2.699,74
FORMIC-ACID	8.091,29	8.666,96	8.091,29	8.666,96
POTASSIUM-HYDROXIDE	1.232,30	664,87	1.232,30	664,87
MAGNESIUM-SULFATE	79,91	79,91	79,91	79,91
DEXTROSE	269,96	269,97	269,96	269,97
DIPOTASSIUM-PHOSPHATE	539,91	539,95	539,91	539,95
WATER	12.862,53	12.942,59	12.862,53	12.942,59
NITROGEN	107,65	94.028,33	107,65	94.028,33
OXYGEN	32,69	24.994,90	32,69	24.994,90
POTASSIUM DIHYDROGEN PHOSPHATE	313,15	313,17	313,15	313,17
Total Input Material	27.076,07	144.047,58	27.076,07	144.047,58



Output Material

Component	Per Batch Amount (kg)	Campaign Amount (kg)
CHOLINE CHLORIDE	511,75	511,75
CYTIDINE-5' MONOPHOSPHATE	46,33	46,33
ACETONE	2.843,05	2.843,05
FORMIC-ACID	7.339,58	7.339,58
POTASSIUM-HYDROXIDE	1.232,30	1.232,30
MAGNESIUM-SULFATE	78,47	78,47
DEXTROSE	264,40	264,40
DIPOTASSIUM-PHOSPHATE	511,75	511,75
WATER	12.805,64	12.805,64
NITROGEN	107,65	107,65
OXYGEN	32,69	32,69
POTASSIUM DIHYDROGEN PHOSPHATE	358,22	358,22
CDP-CHOLINE	121,49	121,49
Total Output Material	26.255,33	26.255,33



Requirements (Campaign)

Requirement	Steps
72 batches of CDP-CHOLINE via step Piloto (Receta - Copy - Copy)	
	72 batches Piloto (Receta - Copy - Copy)

Step Summary

Step Name (Version)	Average Batch Size (kg)	Potential Batch Size (kg)	Average Cycle Time (min)	Average Batch Time (min)	Average Production Rate (kg/min)	Number of Batches	Campaign Time (min)	Campaign Intermediate Production (kg)
Piloto (Receta - Copy - Copy)	133,62	117,82	408,70	15.510,21	0,33	72	90.216,80	9.620,88

Equipment Capacity

Unit ID	Class	Capacity	Max Capacity	Size Utilized	Unit of Measure	Size Utilization (% Max)	Occupancy Time (min)	Time Utilization (%)
Fermenter F-10000	Fermenter	10.000,00	10.000,00	9.524,56	liter	95,25	27.551,87	94,44
ST-D	Tank	15.000,00	15.000,00	13.321,45	liter	88,81	21.483,55	73,64
FI-400	Filter - Pot	4.000,00	4.000,00	2.434,97	liter	60,87	18.955,56	64,97
Fuji Paudal VMixer VM-2000	Mixer	5.000,00	5.000,00	5.238,74	liter	104,77	15.529,30	53,23
ST-B	Tank	7.500,00	7.500,00	5.238,74	liter	69,85	18.235,56	62,51
Chromatography 100	Column - Chromatography	1.250,00	1.000,00	1.149,68	liter	114,97	29.173,65	100,00
ST-C	Tank	10.000,00	10.000,00	6.294,87	liter	62,95	8.222,60	28,19
Column carbon Generic	Column - Chromatography	1.000,00	900,00	1.002,04	liter	111,34	29.114,81	99,80
AT Crystallizer 10 cubic meters	Crystallizer	10.000,00	10.000,00	3.143,79	liter	31,44	12.685,83	43,48
Dryer 5 cubic meters	Dryer	5.000,00	5.000,00	9.077,71	liter	181,55	29.147,77	99,91
Spinbar	Agitator - Propellor					#N/A	0,00	0,00

Patent WO2013/128393 (No optimized)

Escalade: 1st step: Laboratory process

Recipe:

1. 1st part (R1 +C1 +F1)

1.1.Charge Erlenmeyer Flask, 1000 ml with 137,5 g of CMP MET MORPH DCC. Charge Erlenmeyer Flask, 1000 ml with 375 ml of METHANOL. Dissolve 0,1% of all solids.

1.2.React in unit Erlenmeyer Flask, 1000 ml via R1. Reaction occurs over 5 h. The final temperature of the batch is 55 C.

1.3.Cool unit Erlenmeyer Flask, 1000 ml to -30 C. The cooling time is 35 min.

1.4.Filter the batch from unit Erlenmeyer Flask, 1000 ml in filter FILTER FLASK, 1000 ml. The transfer time of the slurry is 25 min. Transfer 100% of the batch to the filter. The mother liquor, named Solución 1, is sent to Round Bottom Flask, 1000 ml. The filter separates 99% of all solids. The moisture content in the final cake is 5%.2.

2nd part (R2+C2+F2)

2.1.Transfer contents of unit Round Bottom Flask, 1000 ml to Erlenmeyer Flask, 1000 ml. This is the key step output. The transfer time is 10 min.

2.2.Charge Erlenmeyer Flask, 1000 ml with 200 g of Calcium phosphorylcholine chloride. Dissolve 0,01% of all solids.

2.3.React in unit Erlenmeyer Flask, 1000 ml via R2. Reaction occurs over 3 h. The final temperature of the batch is 50 C. Continuously add 10 ml of HCl-methanol.

2.4.Cool unit Erlenmeyer Flask, 1000 ml to -30 C. The cooling time is 25 min. Continuously add 100 ml of ISOPROPYL-ALCOHOL.

2.5.Filter the batch from unit Erlenmeyer Flask, 1000 ml in filter FILTER FLASK, 1000 ml. The transfer time of the slurry is 30 min. Transfer 100% of the batch to the filter. The mother liquor, named Crudo de Citicolina, is sent to Round Bottom Flask, 1000 ml. The filter separates 99,7% of all solids. The moisture content in the final cake is 5%.

2.6. Transfer contents of unit Round Bottom Flask, 1000 ml to Crystallizing Dish, 1000 ml. The transfer time is 10 min.3.

Purification

3.1. Crystallize the batch in unit Crystallizing Dish, 1000 ml. The following components are separated in the crystal phase: 99,2% of CITICOLINE, 0,1% of Calcium phosphorylcholine chloride and 3% of Morpholidiate cytidine 5-monophosphate. The crystallization time is 125 min. Continuously add 69,15 g of Morpholidiate cytidine 5-monophosphate.

3.2. Load the column Columna cromatográfica. Use 100% of the material in Crystallizing Dish, 1000 ml. The transfer time is 20 min. The resin retains 98% of CITICOLINE. Displaced Liquid Stream: The displaced liquid belongs to category: Organic Waste.

3.3. Wash the column cromatográfica. Use 10 liter of WATER. The transfer time is 10 min. The column retains 2 liter of the solution. The solution removes 89.934% of the column resin contents. Spent Wash Stream: The spent wash is sent to Round Bottom Flask.

3.4. Filter the batch from unit Round Bottom Flask, 1000 ml in filter Filter Flask, 125 ml. The transfer time of the slurry is 10 min. The moisture content in the final cake is 5%.

3.5. Concentrate the batch in unit Filter Flask, 125 ml. The operation time is 40 min. The overhead is sent to Round Bottom Flask, 250 ml. Separation is: 99% of CITICOLINE goes to Bottoms. Unspecified materials go to Overhead.

Escalade: 2st step: Pilot plant

Recipe:

1. 1st part (R1 +C1 +F1)

1.1. Charge Reactor De Dietrich DIN Reactor AE 63 with 13057,11 g of CMP MET MORPH DCC. Charge Reactor De Dietrich DIN Reactor AE 63 with 35610,31 ml of METHANOL. Dissolve 0,1% of all solids.

1.2. React in unit Reactor De Dietrich DIN Reactor AE 63 via R1. Reaction occurs over 5 h. The final temperature of the batch is 55 C.

1.3. Transfer contents of unit Reactor De Dietrich DIN Reactor AE 63 to SS tank 429.

1.4. Cool unit SS tank 429 to -30 C. The cooling time is 35 min.

1.5.Filter the batch from unit SS tank 429 in filter Bag Filter 100. The transfer time of the slurry is 25 min. Transfer 100% of the batch to the filter. The mother liquor, named Solución 1, is sent to SS tank 429. The filter separates 99% of all solids. The moisture content in the final cake is 5%.2.

2nd part (R2+C2+F2)

2.1.Transfer contents of unit SS tank 429 to Reactor De Dietrich DIN Reactor AE 63. This is the key step output. The transfer time is 10 min.

2.2.Charge Reactor De Dietrich DIN Reactor AE 63 with 18992,16 g of Calcium phosphorylcholine chloride. Dissolve 0,01% of all solids.

2.3.React in unit Reactor De Dietrich DIN Reactor AE 63 via R2. Reaction occurs over 3 h. The final temperature of the batch is 50 C. Continuously add 949,61 ml of HCl-methanol.

2.4.Transfer contents of unit Reactor De Dietrich DIN Reactor AE 63 to SS tank 429.

2.5.Cool unit SS tank 429 to -30 C. The cooling time is 25 min. Continuously add 9496,09 ml of ISOPROPYL-ALCOHOL.

2.6.Filter the batch from unit SS tank 429 in filter Bag Filter 100. The transfer time of the slurry is 30 min. Transfer 100% of the batch to the filter. The mother liquor, named Crudo de Citicolinba, is sent to SS tank 429. The filter separates 99,7% of all solids. The moisture content in the final cake is 5%.

2.7.Transfer contents of unit SS tank 429 to Crystallizer 630. The transfer time is 10 min.3.

Purification

3.1.Crystallize the batch in unit Crystallizer 630. The following components are separated in the crystal phase: 99,2% of CITICOLINE, 0,1% of Calcium phosphorylcholine chloride and 3% of Morpholidiate cytidine 5-monophosphate. The crystallization time is 125 min. Continuously add 6566,54 g of Morpholidiate cytidine 5-monophosphate.

3.2.Wash the column column carbon. Use 100% of the material in Crystallizer 630. The transfer time is 10 min. The column retains 300 ml of the solution. The solution removes 47,481% of the column resin contents.

3.3. Wash the column carbon. Use 1000 liter of WATER. The transfer time is 10 min. The column retains 2 liter of the solution. The solution removes 89.934% of the column resin contents. Spent Wash Stream: The spent wash is sent to SS tank.

3.4. Filter the batch from unit SS tank 429 in filter Bag Filter 100. The transfer time of the slurry is 10 min. The moisture content in the final cake is 3%.

3.5. Concentrate the batch in unit Bag Filter 100. The operation time is 40 min. The overhead is sent to Round Bottom Flask, 250 ml. Separation is: 99% of CITICOLINE goes to Bottoms. Unspecified materials go to Overhead.

Escalade: Last step: Generic plant

Recipe:

1. 1st part (R1 +C1 +F1)

1.1. Charge De Dietrich 1600 with 298.33 kg of CMP MET MORPH DCC. Charge De Dietrich 1600 with 813.64 liter of METHANOL. Dissolve 0.1% of all solids.

1.2. React in unit De Dietrich 1600 via R1. Reaction occurs over 5 h. The final temperature of the batch is 55 C.

1.3. Transfer contents of unit De Dietrich 1600 to ST-B. The transfer time is 10 min.

1.4. Cool unit ST-B to -30 C. The cooling time is 35 min.

1.5. Filter the batch from unit ST-B in filter FI-400. The transfer time of the slurry is 25 min. Transfer 100% of the batch to the filter. The mother liquor, named Solución 1, is sent to ST-A. The filter separates 99% of all solids. The moisture content in the final cake is 5%.2.

2nd part (R2+C2+F2)

2.1. Transfer contents of unit ST-A to De Dietrich 1600. This is the key step output. The transfer time is 10 min.

2.2. Charge De Dietrich 1600 with 433.94 kg of Calcium phosphorylcholine chloride. Dissolve 0.01% of all solids.

2.3. React in unit De Dietrich 1600 via R2. Reaction occurs over 3 h. The final temperature of the batch is 50 C. Continuously add 21.697 liter of HCl-methanol.

2.4. Transfer contents of unit De Dietrich 1600 to ST-B. The transfer time is 10 min.

2.5. Cool unit ST-B to -30 C. The cooling time is 25 min. Continuously add 216.97 liter of ISOPROPYL-ALCOHOL.

2.6. Filter the batch from unit ST-B in filter FI-400. The transfer time of the slurry is 30 min. Transfer 100% of the batch to the filter. The mother liquor, named Crudo de Citicolinba, is sent to ST-A. The filter separates 99,7% of all solids. The moisture content in the final cake is 5%.

2.7. Clean unit FI-400. Cleaning time is 20 min.

2.8. Transfer contents of unit ST-A to AT Crystallizer 10 cubic meter. The transfer time is 10 min.3.

Purificacation

3.1. Crystallize the batch in unit AT Crystallizer 10 cubic meter. The following components are separated in the crystal phase: 99.2% of CITICOLINE, 0.1% of Calcium phosphorylcholine chloride and 3% of Morpholidiate cytidine 5-monophosphate. The crystallization time is 125 min. Continuously add 150.04 kg of Morpholidiate cytidine 5-monophosphate.

3.2. Load the column Chromatography 100. Use 100% of the material in AT Crystallizer 10 cubic meter. The resin retains 90% of CITICOLINE.

3.3. Wash the column Chromatography 100. Use 4318.37 liter of WATER. The transfer time is 10 min. The column retains 30 liter of the solution. The solution removes 89.934% of the column resin contents. Spent Wash Stream: The spent wash is sent to ST-A.

3.4. Filter the batch from unit ST-A in filter FI-400. The transfer time of the slurry is 10 min. The moisture content in the final cake is 5%.

3.5. Concentrate the batch in unit FI-400. The operation time is 40 min. The overhead is sent to ST-A. Separation is: 95% of CITICOLINE goes to Bottoms. Unspecified materials go to Overhead.

Step Executive Summary (no optimized)

Process (Version):	Process1 (1.0)	**Key Input Intermediate:**	CITICOLINE
Step (Version):	Step1 (1.0 - Copy - Copy)	**Key Output Intermediate:**	METHANOL
Simulation Date:	15/08/2018 12:18	**Plan Quantity:**	639,00 kg

Batch Size (kg)	Potential Batch Size (kg)	Cycle Time (min)	Batch Time (min)	Production Rate (kg/min)	# of Batches	Campaign Time (min)	Campaign Intermediate Production (kg)
639,00	590,46	600,00	952,70	1,07	1	952,70	639,00

Input Material

Component	Per Batch	Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)
Morpholidiate cytidine 5-monophosphate		150,04	150,97	150,04	150,97
HCl-methanol		21,56	21,70	21,56	21,70
METHANOL		642,46	813,64	642,46	813,64
ISOPROPYL-ALCOHOL		169,63	216,97	169,63	216,97
MORPHOLINE		81,56	81,62	81,56	81,62
WATER		4.291,66	4.318,37	4.291,66	4.318,37
NITROGEN		44,74	39.078,48	44,74	39.078,48
OXYGEN		13,59	10.387,96	13,59	10.387,96
Cytidine monophosphate		108,48	108,49	108,48	108,49
1,3-dicyclohexylcarbodiimide		108,48	108,49	108,48	108,49
Calcium phosphorylcholine chloride		433,94	436,64	433,94	436,64
Total Input Material		6.065,95	55.723,34	6.065,95	55.723,34

Output Material

Component	Per Batch	Amount (kg)	Campaign Amount (kg)
Morpholidiate cytidine 5-monophosphate		177,39	177,39
HCl-methanol		21,56	21,56
METHANOL		642,46	642,46
ISOPROPYL-ALCOHOL		169,63	169,63
MORPHOLINE		59,45	59,45
WATER		4.291,66	4.291,66
NITROGEN		44,74	44,74
OXYGEN		13,59	13,59
Cytidine monophosphate		27,10	27,10
1,3-dicyclohexylcarbodiimide		56,60	56,60
CITICOLINE		91,61	91,61
Calcium phosphorylcholine chloride		385,60	385,60
Total Output Material		5.961,50	5.961,50

Plan executive

Summary

Step Name (Vendor)	Average Batch Size (kg)	Potential Batch Size (kg)	Average Cycle Time (min)	Average Batch Time (min)	Average Production Rate (kg/mh)	Number of Batches	Campaign Time (min)
Intact (1.8 - Copy - Copy)	645,77	968,48	680,04	903,14	1,06	64	58.755,22

Input Material

Component	Campaign	Amount (kg)	Campaign Amount (liter)
Monofruktidate cytidine 5-monophosphate		8.602,58	8.862,33
KCl (wäßrig)		1.380,02	1.388,81
METHANOL		41.117,59	52.272,89
ISOPROPYL-ALCOHOL		10.858,58	12.886,08
MORPHOLINE		6.007,15	6.210,88
WATER		274.666,04	276.373,68
NITROGEN		1.215,51	1.081.318,65
OXYGEN		668,08	282.178,33
Cytidine monophosphate		8.842,95	8.843,44
1,3-dicyclohexylcarbodiimide		3.842,95	3.843,44
Calcium phosphanylcholine chloride		17.772,18	17.845,05
Total Input Material		380.071,21	1.744.134,19

Output Material

Component	Campaign	Amount (kg)
Monofruktidate cytidine 5-monophosphate		11.377,02
KCl (wäßrig)		1.380,02
METHANOL		41.117,59
ISOPROPYL-ALCOHOL		10.856,58
MORPHOLINE		3.809,98
WATER		274.666,04
NITROGEN		1.215,51
OXYGEN		359,08
Cytidine monophosphate		1.742,49
1,3-dicyclohexylcarbodiimide		3.822,08
CYTOSINE		3.805,98
Calcium phosphanylcholine chloride		14.602,84
Total Output Material		380.678,22

Unscheduled Batches

Patent WO2013/128393 (optimized)

1. 1st part (R1 +C1 +F1)

1.1. Charge Reactor 800 liter with 193,28 kg of CMP MET MORPH DCC. Charge Reactor 800 liter with 527,14 liter of METHANOL. Dissolve 0,1% of all solids.

1.2. React in unit Reactor 800 liter via R1. Reaction occurs over 5 h. The final temperature of the batch is 55 C.

1.3. Transfer contents of unit Reactor 800 liter to Tank SET. The transfer time is 10 min.

1.4. Cool unit Tank SET to -30 C. The cooling time is 35 min.

1.5. Filter the batch from unit Tank SET in filter Belt Filter 100. The transfer time of the slurry is 25 min. Transfer 100% of the batch to the filter. The mother liquor, named Solución 1, is sent to ST-C. The filter separates 99% of all solids. The moisture content in the final cake is 5%.

1.6. Clean unit Belt Filter 100. Cleaning time is 20 min.2.

2nd part (R2+C2+F2)

2.1. Transfer contents of unit ST-C to De dietrich reactor 1000. This is the key step output. The transfer time is 10 min.

2.2. Charge De dietrich reactor 1000 with 281,14 kg of Calcium phosphorylcholine chloride. Dissolve 0,01% of all solids.

2.3. React in unit De dietrich reactor 1000 via R2. Reaction occurs over 3 h. The final temperature of the batch is 50 C. Continuously add 30 liter of HCl-methanol.

2.4. Transfer contents of unit De dietrich reactor 1000 to ST-B. The transfer time is 10 min.

2.5. Cool unit ST-B to -30 C. The cooling time is 25 min. Continuously add 140,731 liter of ISOPROPYL-ALCOHOL.

2.6. Filter the batch from unit ST-B in filter Filter P100. The transfer time of the slurry is 30 min. Transfer 100% of the batch to the filter. The mother liquor, named Crudo de Citicolinba, is sent to ST-A. The filter separates 99,7% of all solids. The moisture content in the final cake is 5%.

2.7. Clean unit Filter P100. Cleaning time is 20 min.

2.8.Transfer contents of unit ST-A to AT Crystallizer 2 cubic meter. The transfer time is 10 min.3.

Purification

3.1.Crystallize the batch in unit AT Crystallizer 2 cubic meter. The following components are separated in the crystal phase: 99,2% of CITICOLINE, 0,1% of Calcium phosphorylcholine chloride and 1% of Morpholidiate cytidine 5-monophosphate. The crystallization time is 125 min. Continuously add 97,212 kg of Morpholidiate cytidine 5-monophosphate.

3.2.Load the column Chromatography 100. Use 100% of the material in AT Crystallizer 2 cubic meter. The resin retains 90% of CITICOLINE.

3.3.Wash the column Chromatography 100. Use 4000 liter of WATER. The transfer time is 10 min. The column retains 30 liter of the solution. The solution removes 98% of the column resin contents. Spent Wash Stream: The spent wash is sent to ST-B.

3.4.Filter the batch from unit ST-B in filter Filter P100. The transfer time of the slurry is 10 min. The moisture content in the final cake is 5%.

3.5.Concentrate the batch in unit Filter P100. The operation time is 40 min. The overhead is sent to ST-A. Separation is: 95% of CITICOLINE goes to Bottoms. Unspecified materials go to Overhead.

Step Executive Summary

Process (Version): Process1 (1.0)
Step (Version): Step1 (1.0 - Copy - Copy)
Simulation Date: 15/06/2018 12:29

Input Material

Component	Per Batch	Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)
Morpholidiate cytidine 5-monophosphate		97,21	87,82	97,21	87,82
HCl-methanol		29,81	30,00	29,81	30,00
METHANOL		416,23	527,14	416,23	527,14
ISOPROPYL-ALCOHOL		110,03	140,73	110,03	140,73
MORPHOLINE		52,71	52,88	52,71	52,88
WATER		3.975,26	4.000,00	3.975,26	4.000,00
NITROGEN		40,47	35.344,46	40,47	35.344,46
OXYGEN		12,29	9.395,37	12,29	9.395,37
Cytidine monophosphate		70,28	70,29	70,28	70,29
1,3-dicyclohexylcarbodiimide		70,28	70,29	70,28	70,29
Calcium phosphorylcholine chloride		281,14	282,89	281,14	282,89
Total Input Material		5.155,72	50.011,87	5.155,72	50.011,87

Output Material

Component	Per Batch	Amount (kg)	Campaign Amount (kg)
Morpholidiate cytidine 5-monophosphate		109,35	109,35
HCl-methanol		29,81	29,81
METHANOL		416,23	416,23
ISOPROPYL-ALCOHOL		110,03	110,03
MORPHOLINE		36,63	36,63
WATER		3.975,26	3.975,26
NITROGEN		40,47	40,47
OXYGEN		12,29	12,29
Cytidine monophosphate		10,60	10,60
1,3-dicyclohexylcarbodiimide		32,18	32,18
CITICOLINE		76,33	76,33
Calcium phosphorylcholine chloride		240,87	240,87
Total Output Material		5.090,05	5.090,05

Shedule in 5 Batches

Requirements Campaign (WO2013 optimizated)

Requirement	Steps
68 batches of Calcium phosphorylcholine chloride via step	Step1 (1.0 - Copy - Copy) *68 batches Step1 (1.0 - Copy - Copy)*

Step Summary

Step Name (Version)	Average Cycle Time (min)	Average Batch Time (min)	Average Production Rate (kg/min)	Number of Batches	Campaign Time (min)
Step1 (1.0 - Copy - Copy)	325,03	946,22	0,00	68	22.721,28

Input Material

Component	Campaign	Amount (kg)	Campaign Amount (liter)
Morpholidiate cytidine 5-monophosphate		6.610,42	6.651,56
HCl-methanol		2.027,38	2.040,00
METHANOL		28.303,98	35.845,52
ISOPROPYL-ALCOHOL		7.481,91	9.569,71
MORPHOLINE		3.584,47	3.595,93
WATER		270.317,43	272.000,00
NITROGEN		886,22	774.063,23
OXYGEN		269,13	205.763,90
Cytidine monophosphate		4.779,29	4.779,62
1,3-dicyclohexylcarbodiimide		4.779,29	4.779,62
Calcium phosphorylcholine chloride		19.117,52	19.236,52
Total Input Material		348.157,02	1.338.325,62

Output Material

Component	Campaign	Amount (kg)
Morpholidiate cytidine 5-monophosphate		7.435,95
HCl-methanol		2.027,38
METHANOL		28.303,98
ISOPROPYL-ALCOHOL		7.481,91
MORPHOLINE		2.490,53
WATER		270.317,43
NITROGEN		886,22
OXYGEN		269,13
Cytidine monophosphate		720,96
1,3-dicyclohexylcarbodiimide		2.188,53
CITICOLINE		5.190,23
Calcium phosphorylcholine chloride		16.378,92
Total Output Material		343.691,15