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„Stability and Manufacturing Recommendations and Precautions for the Production of Amoxicillin and Amoxicillin/Clavulanate Finished Pharmaceutical Products"

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„Stability and Manufacturing Recommendations and Precautions for the Production of Amoxicillin and Amoxicillin/Clavulanate Finished Pharmaceutical Products “

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This work is dedicated to my precious blessed sons “Adam” and “Adrian”.

Abstract

Amoxicillin/clavulanate pharmaceutical products are very important antibacterial combinations. Summarizing and focusing on the most important environmental factors that affect the stability of amoxicillin/clavulanate preparations and the methods and the mechanisms to protect the product against degradative factors should be introduced, in order to provide and illustrate the ideal manufacturing conditions for amoxicillin and amoxicillin/clavulanate pharmaceutical products as an improvement for the production process.

This literature work summarizes the most important studies, articles, and books handling, treating and discussing the stability and the special manufacturing precautions of beta-lactam antibiotics generally and amoxicillin/clavulanate Products Specially.

Key words: Beta-lactam antibiotics, Amoxicillin, Potassium clavulanate, Solid state stability, Photolysis, Thermal degradation, Environmental factors, Humidity, Oxidation, Structure-activity relationships, Manufacturing and protection of amoxicillin/clavulanate.

Zusammenfassung:

Pharmazeutische Produkte aus einer Kombination von Amoxicillin/Clavulansäure sind sehr wichtige antibakteriell wirksame Präparate. Die Zusammenfassung präsentiert die wichtigsten Umweltfaktoren, die einen Einfluss auf die Stablitiät von pharmazeutischen Amoxicillin/Clavulansäure Präparaten haben können. Weiters Methoden und Mechanismen, um das Produkt vor abbauenden Faktoren zu schützen, damit die idealen Produktionsbedingungen für Amoxicillin/Clavulansäure Produkte im Rahmen des Produktionsverbesserungsprozesses eingeführt werden können. Diese Literatur Masterarbeit ist eine Zusammenfassung der wichtigsten Studien, wissenschaftlichen Posten und Bücher, die die speziellen Produktionsbedingungen und Vorsichtmaßnahmen bei der Produktion von Beta-Lactam Antibiotika und Amoxicillin/Clavulansäure Produkten beschreiben und diskutieren.

Schlagwörter: Beta-Lactam Antibiotika, Amoxicillin, Potassium Clavulanate, Festzustand Stabilität, Photolyse, Thermolyse, Oxidation, Feuchtigkeit, Umweltfaktoren, SAR, Herstellung und Schutz von Amoxicillin/Clavulanate Produkten.

Contents

1. Introduction.

Beta-lactam antibiotics are one of the most important antibiotic classes. They inhibit the synthesis of the bacterial cell wall by binding to penicillin binding protein, which is an important bacterial enzyme for the synthesis of peptidoglycan during the cross linking process of the peptide units leading to the death of the bacterial cell showing a bactericidal effect. [1]

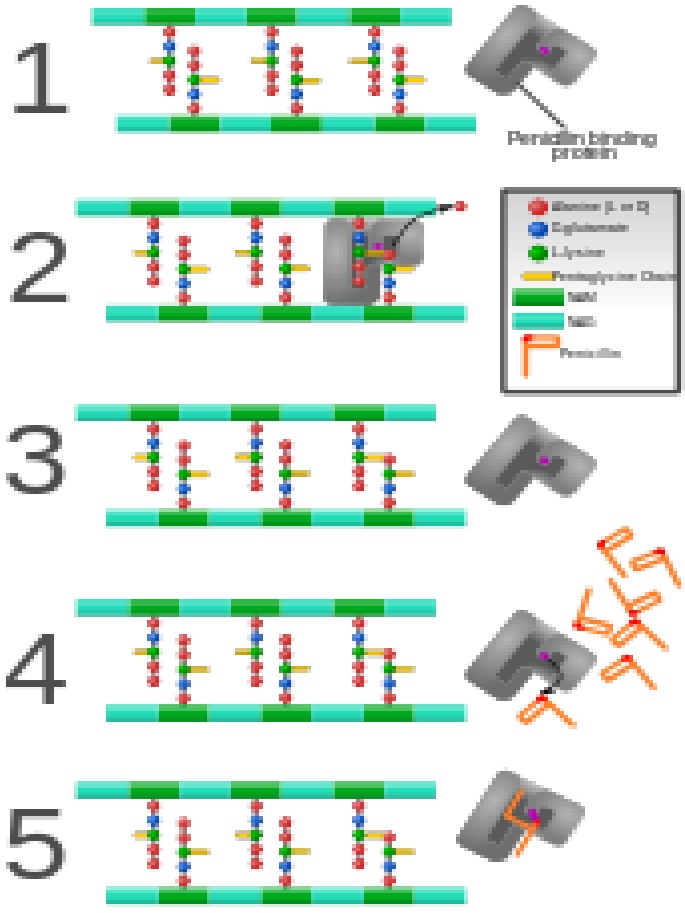

Figure 1: Beta-Lactam mechanism of action, inhibition of penicillin binding protein. [2]

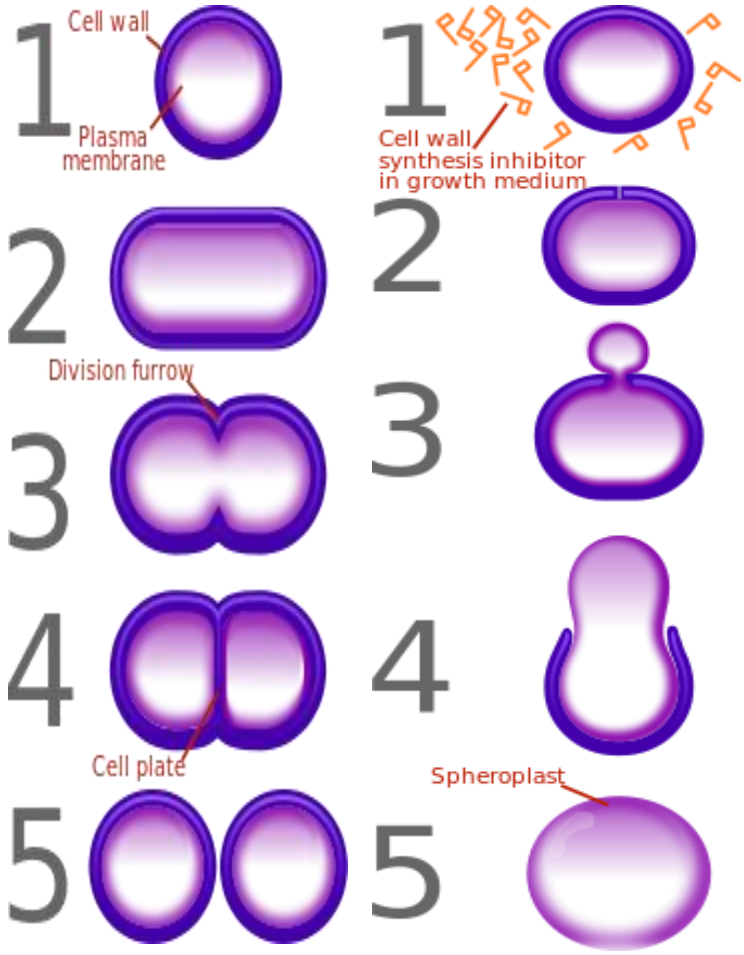

Figure 2: The role of penicillin in the inhibition of the cell wall synthesis during the bacterial cell division. [2]

Beta-lactam antibiotics are classified into penicillins, cephalosporins, carbapenems, and monobactams. [3] Amoxicillin belongs to the penicillin class. It is a moderate spectrum antibiotic, which is effective against susceptible non beta-lactamase producing bacteria. [4]

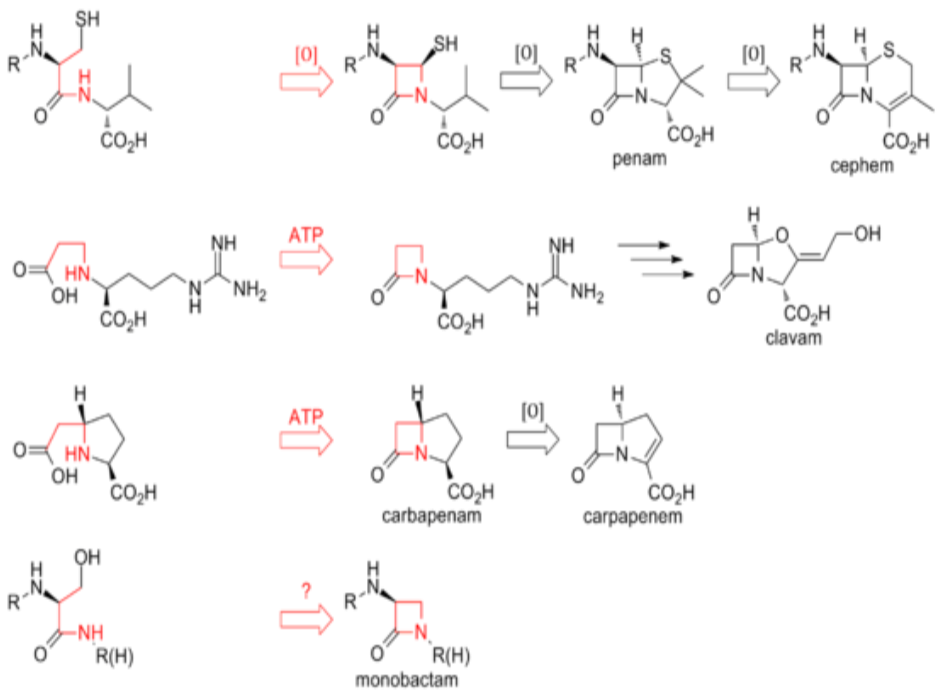

Figure 3: Different mechanisms of Beta-Lactam ring closure to produce different Beta-Lactam antibiotic classes. [2]

Beta-lactamase enzyme is a bacterial enzyme, which can destroy the beta-lactam ring of the beta-lactam antibiotics leading to inactivation of the antibacterial biological effect of the beta-lactam antibiotics. Therefore beta-lactamase producing bacteria are resistant to beta-lactam antibiotics. The mechanism of releasing and the production of beta-lactamase enzyme is considered to be the most effective bacterial resistance mechanism against beta-lactam antibiotics among other resistance mechanisms like the alteration of the bacterial penicillin binding protein, the inhibition of the entry of the beta-lactam antibiotic to the bacterial cell due to mutations of the bacterial cell wall and the transfer of the beta-lactam antibiotic outside the bacterial cell by the aid of efflux pumps. [5]

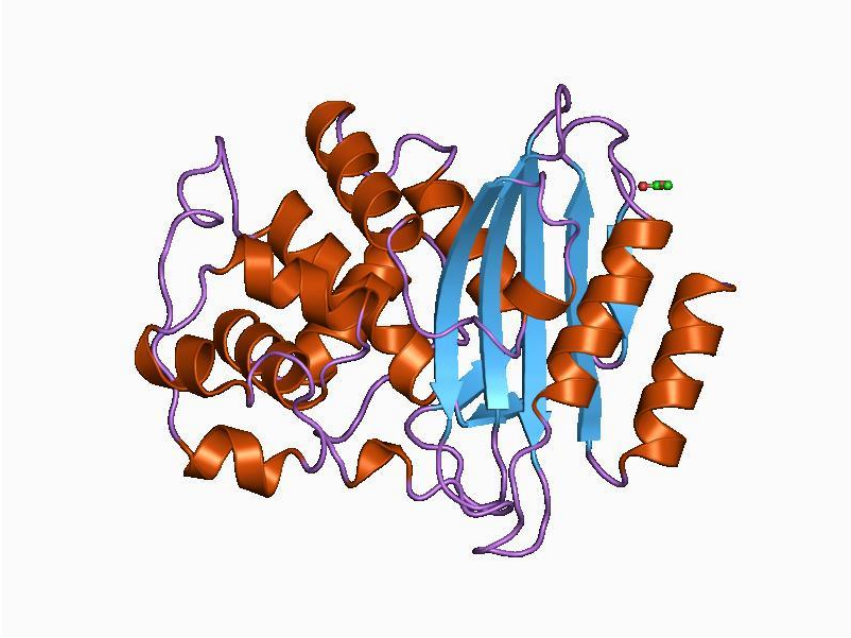

Figure 4: Structure of *Stryptomycesalbus* beta-lactamase enzyme. [2]

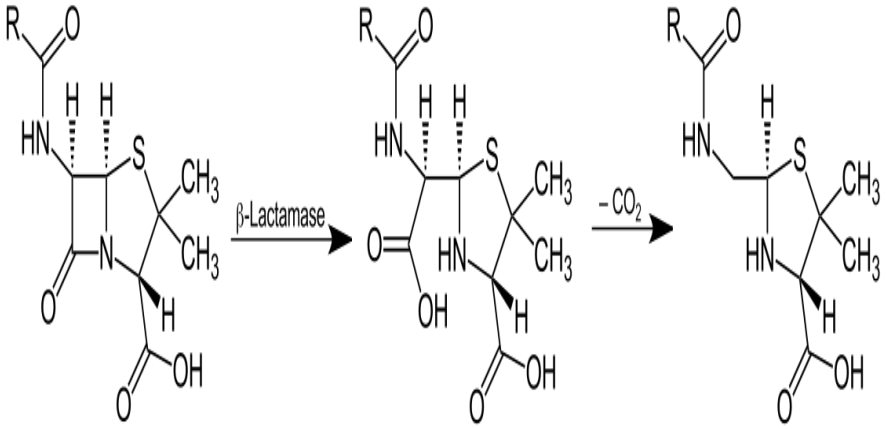

Figure 5: The action of bacterial Beta-Lactamase enzyme on the Penicillin. [2]

Clavulanic acid is a beta-lactamase enzyme inhibitor, which shows more susceptibility to the beta-lactamase enzyme than the beta-lactam antibiotic. It leads to an irreversible and time dependent inhibition of beta-lactamase enzyme, which enhances and increases the efficacy, the potency and the biological activity spectrum of amoxicillin/clavulanate antimicrobial combinations compared to amoxicillin alone. [6, 7], According to this the manufacturing of the finished pharmaceutical combinations of amoxicillin and clavulanate in different dosage forms is very important from a biologically point of view. As beta-lactam antibiotics also show high sales value about 53% of the total antibiotic market in the world by 2010 there is also an economical point of view. [5]

Manufacturing of the finished pharmaceutical products of amoxicillin/clavulanate combinations can be done in different pharmaceutical dosage forms such as film coated tablets, chewable tablets, powder for oral suspension, and powder for injection.

Pharmaceutical dosage forms can be manufactured by different manufacturing concepts like batch production, semi-batch production, semi-continuous production, and continuous production through different manufacturing steps, by which each single production step can be done according to one of these manufacturing concepts. Within batch manufacturing all materials are charged before starting the process and all are discharged together after the end of the process such as bin blending and drying processes. Within semi-batch production materials can be added during the process, but not discharged till the end of the process as in coating and wet granulation processes. Continuous and semi-continuous manufacturing are sharing the same concept of continuous charging and discharging of materials during the process. The semi-continuous step is limited to a specific time period such as the tablet compression step. [8, 9]

Understanding the different manufacturing concepts and designs is very important in order to achieve high compatibility between the process design

and the critical quality attributes of the process and the product, which include the factors affecting the product stability.

A lot of manufacturers in pharmaceutical industry aim to convert the whole production process from the batch concept to the continuous manufacturing concept to achieve more economic and financial gain. At the same time the product quality is enhanced, too. This will lead to the challenge of achieving a successful quality by design concept (QBD) and process analytical technology (PAT) methods, which allow and achieve rapid on-line analytical methods to support real time release tests (RTRT) concept. [10]

Therefore understanding the factors that can affect the stability, the quality, and the manufacturing of amoxicillin/clavulanate finished pharmaceutical products is essential to define the suitable precautions and protective steps for these important pharmaceutical products during batch and continuous manufacturing.

2. Pharmaceutical chemistry of beta-lactam antibiotics. [2, 11, 12, 13, 14, 15]

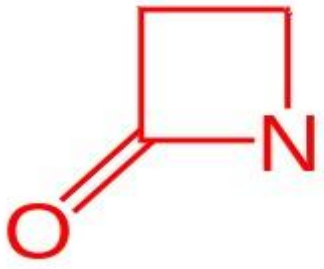

Figure 6: Structure of the Beta-Lactam ring. [2]

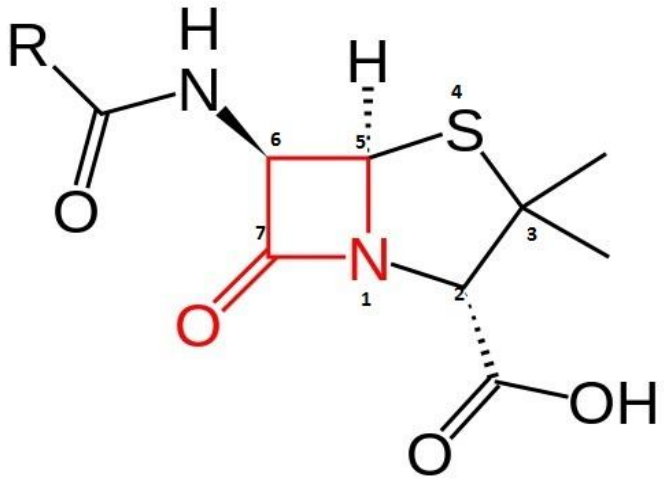

Figure 7: General structure of Penicillin class members. [2]

(USP numbering system)

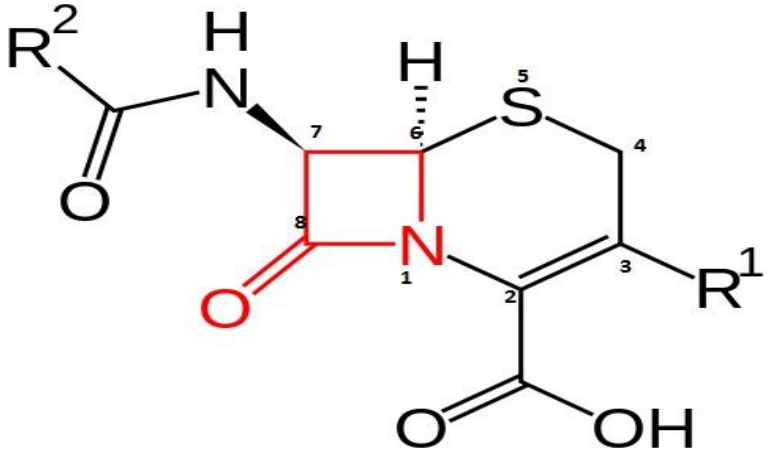

Figure 8: General Structure of Cephalosporin class members. [2]

(USP numbering system)

The activity of the beta-lactam antibiotics is related to the chemical reactivity of the four membered beta lactam ring and is directly dependent on the ring structure formed by the fusion between the beta lactam ring and the five membered thiazolidine ring (in case of penicillins) or the six membered dihydrothiazine ring (in case of cephalosporins), so that any factor or effect that can lead to the opening of this four membered ring or this ring fusion structure will abolish the activity of beta-lactam antibiotics.

The presence of sulfur on position number 4 (in case of penicillins) or on position number 5 (in case of cephalosporins), free carboxyl group on position number 2 in both penicillins and cephalosporins, two free methyl groups on position number 3 (in case of penicillins), no substitutions on position number 5 (in case of penicillins) or on position number 6 (in case of cephalosporins), carbonyl group on position number 7 (in case of penicillins) or position number 8 (in case of cephalosporins), and the presence of amide group on position number 6 of Penicillanic acid and position number 7 of Cephalosporanic acid are all essential for the biological antimicrobial activity of beta-lactam antibiotics.

Potency, efficacy, and stability against acid and enzyme degradation of beta-lactam antibiotics are greatly affected by the side chain of beta-lactam compounds. Small changes in the substitutions can show big effects on the activity of these products and also on the susceptibility to be deactivated or decomposed by beta-lactamase bacterial enzyme.

The presence of bulk groups on the side chain can protect the beta-lactam ring against bacterial enzymes. The presence of carboxamido or sulfonamido groups on the side chain will increase the activity of the beta-lactam antibiotic, while the presence of benzylamino or phenylamino groups on the side chain will decrease the activity of the beta-lactam compound.

The side chain of amoxicillin allows a wider spectrum of activity, as it increases the activity of amoxicillin against G-ve bacteria, shows higher plasma

levels, and increases the acid stability of amoxicillin, so that it can be orally administered. These effects are due to the presence of polar amide group and phenolic hydroxyl group at the para position on the side chain of amoxicillin.

Cephalosporins are considered to be less reactive than penicillins due to the presence of the six membered dihydrothiazine ring of cephalosporins, as the five membered thiazolidine ring of penicillins is considered to be more flexible that the dihydrothiazine ring, so that an unsaturation between the positions number 2 and 3, and a reactive group at position number 3 of cephalosporins are required to increase the reactivity of cephalosporins.

Structure dependent beta-lactamase inhibitors like clavulanic acid must have a beta-lactam ring, which will bind and react with the beta-lactamase enzyme by forming a stable acyl intermediate compound due to the presence of an acidic alpha Proton on position number 6. This acyl compound is stable enough to cause irreversible inhibition of the beta-lactamase enzyme, also in case of clavulanic acid, the presence of oxygen on position number 4 instead of sulfur will lead to increase the susceptibility and the reactivity of clavulanic acid against the beta-lactamase enzyme. [10, 11, 12, 13, 14, 15]

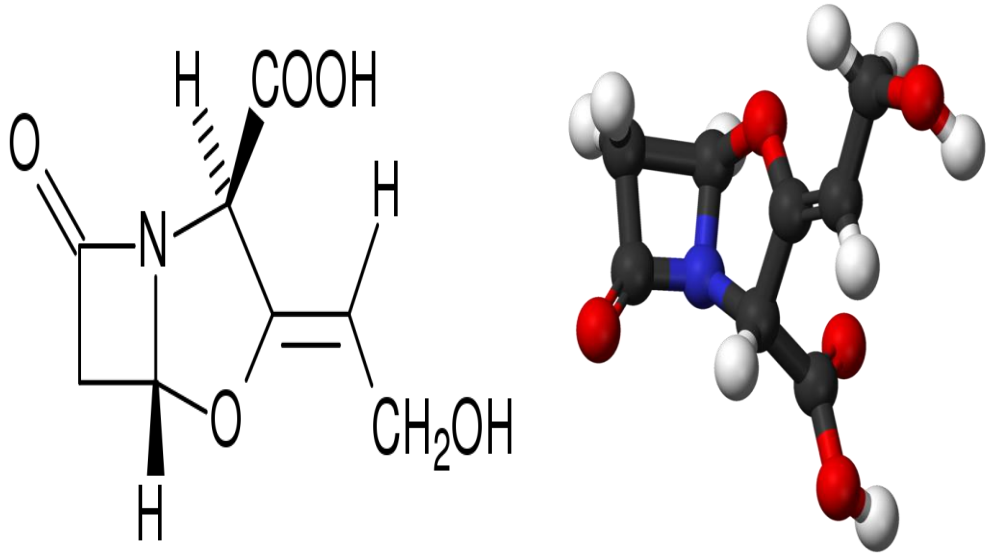

Figure 9: Structure of Clavulanic acid. [4]

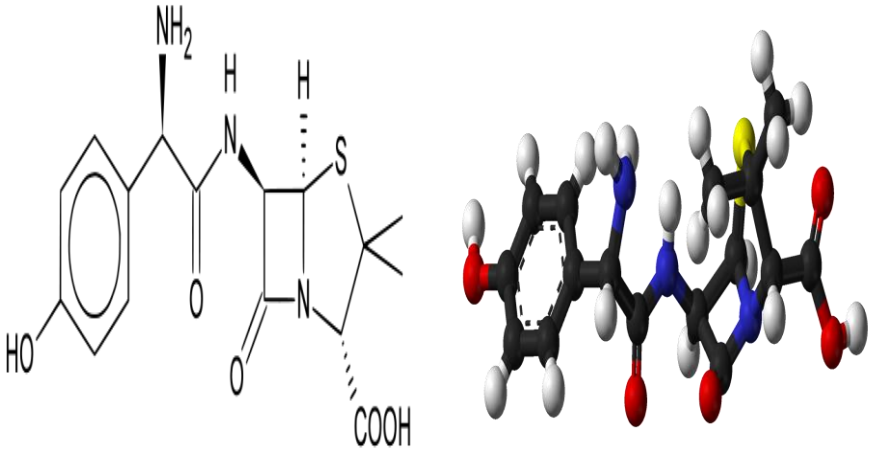

Figure 10: Structure of Amoxicillin. [4]

3. Environmental factors affecting the stability of amoxicillin/clavulanate finished dosage forms.

The antibiotic combination, amoxicillin/clavulanate combination is very effective against numerous microorganisms and can be used for the treatment of different types of infections like upper and lower respiratory tract infections and urinary tract infections.

In order to ensure high biological activity of amoxicillin/clavulanate combination, we have to ensure that the active pharmaceutical ingredients are in the active form at the infection site and in an adequate concentration to achieve the required therapeutic effect. Any degradation of the active ingredients during manufacturing or storage can lead to therapeutic failure of the antibiotic combination, as therapeutic compounds containing antibiotics tend to be sensitive to degradative enzymes produced by microorganisms and different environmental factors such as temperature and light. [16]

3.1. Effect of humidity.

Humidity is defined as the amount of water vapor in the air measured at a specific temperature that will express the absolute humidity. The relation between the absolute humidity and the maximum humidity at the same temperature defines the relative humidity. The ratio between humidity in the air mixture and the total air content is the specific humidity. [17]

Moisture can affect and change the physical and chemical stability of a lot of pharmaceutical products, as water can initiate degradative chemical reactions to the active pharmaceutical ingredients by different mechanisms such as direct hydrolysis chemical reactions or product specific reactions. [18]

Beta-lactam antibiotics are considered as sensitive carbonyl compounds, which show decomposition due to exposure to moisture and due to reaction with

water in a hydrolysis process. [19] Also amoxicillin/clavulanate combinations are known to be sensitive to moisture due to the nucleophilic attack of water as a weak nucleophile, which will lead to beta-lactam ring opening and inactivation of the combination. [12, 16] Beside the fact that clavulanic acid is a hygroscopic substance, which can absorb and hold water molecules, so that it must be stored in containers protected from air and moisture under temperature between 2-8 °C. [20, 21]

It was also reported that the amoxicillin molecule is sensitive to humidity, as high relative humidity will show significant increase of the rate of the degradation of the amoxicillin. [22] Both of amoxicillin trihydrate and potassium clavulanate show rapid degradation due to the exposure to humidity, especially clavulanate, which shows extreme sensitivity to humidity. [23]

Another study reported that clavulanic acid undergoes rapid degradation in different types of water such as tap water, acidified water and purified water obtained by reverse osmosis purification mechanism while amoxicillin show rapid degradation in acidified water. [24]

Effect of moisture on amoxicillin and clavulanic acid in solid state has been also illustrated by different studies. It was reported that moisture affects the stability of all penicillins in solid state. Amoxicillin trihydrate in solid state undergoes a first order kinetic degradation reaction started upon exposure to 25% - 90% relative humidity, while amoxicillin sodium started this degradation reaction upon exposure to relative humidity range from 50% - 90%. The rate constant of this first order kinetic degradation reaction is directly proportional to the relative humidity at constant temperature. [25, 26]

It was also reported that potassium clavulanate in solid state shows a degradation of 24.17% from the original initial concentration due to a degradative effect of humidity for 24 hours at room temperature in a neutral hydrolysis process. [27] It was also proven that humidity is the major factor influencing the degradation of potassium clavulanate in solid state, as the rate

constant of the degradation reaction of potassium clavulanate under elevated relative humidity environmental condition in a range of 50.9% to 76.5% and high temperature range of 39.85 °C to 89.85 °C was about twice as in dry air under the same high temperature conditions. [28]

Therefore protecting Amoxicillin/Clavulanate products against high moisture and relative humidity during handling, manufacturing and storage of these pharmaceutical products is very important to ensure high biological antimicrobial activity to avoid any therapeutic failure due to the degradation or the decomposition of the active pharmaceutical ingredients. , This is also important as humidity can not only cause degradation or decomposition to the moisture sensitive substances, but also can affect the drug release from tablets, the hardness of the tablets and the mechanical stress of tablets. Humidity can also prolong the disintegration time and decrease the dissolution rates due to the agglomeration of drug particles due to high moisture content. [29]

3.2. Effect of temperature.

One of the most important factors that have an effect on the stability of pharmaceuticals and that can affect the chemical and the physical properties of them is temperature. The rate of the chemical reaction, including chemical degradation reaction, greatly depends in a direct proportional relationship to the temperature according to Arrhenius equation. Temperature can also cause changes to the physical properties of the active pharmaceutical ingredients and the excipients used in the pharmaceutical formulations. These changes of physical properties can have an effect on the stability of the drug product and the formulation. They might also influence other important parameters that have an effect on the therapeutic activity of the dosage forms such as drug release, dissolution rates, and physical properties of tablets. As an example to these temperature effects it was reported that elevating the temperature from

room temperature to 55 °C during the compression process of powder mixtures containing furoic acid and microcrystalline cellulose into tablets, led to optical defects the tablets like cracks. This is related to the effect of the elevated temperature on the interaction between these two materials leading to the formation of carbon monoxide gas, which is responsible for these optical defects and cracks. [29, 30]

Beta-lactam antibiotics undergo degradation processes in solid state under the effect of temperature. This can affect the stability and can lead to therapeutic failure of the antibiotic and increase the risk of body allergic reactions to the degradation products. [31]

It was also reported that the amoxicillin molecule is sensitive to temperature. [22] Clavulanic acid is described as a thermolabile substance being very sensitive to temperature. [32, 33]

A study had been carried out to illustrate the effect of temperature on beta-lactam antibiotics by studying the stability and the rate of the hydrolysis of different beta-lactam antibiotics at temperatures of 25 °C, 50 °C, and 60 °C. It was found that the hydrolysis rate constantly increased from 2.5 to 3.9 folds for each 10°C increase of the temperature. The recorded half-life of the beta-lactam antibiotics is greatly affected by temperature such as the half-life of ampicillin of initial concentration of 5 mg L^{-1}, which is decreased from 27 days under the conditions of 25 °C and PH 7 to 1.2 day at 50 °C and PH 7 to 1.1 day at 60 °C and PH 7, proving that the hydrolysis and the half-life of beta-lactam antibiotics greatly depend on temperature. [34] It was also reported that a small rise in the temperature can increase the degradation rate of the main structure of the penicillins (6-amino penicillanic acid) by three folds. [35]

Thermal degradation of amoxicillin trihydrate in solid state was reported. The thermal degradation reaction was a first order kinetic reaction at a temperature of 37 °C and 50 °C, while the thermal degradation reaction was a zero order kinetic degradation reaction at a temperature more than 50 °C, which means

that, when the temperature was more than 50 °C, there was no increase of the thermal degradation rate constant of amoxicillin trihydrate in solid state upon increasing the temperature. It was also reported that this thermal degradation reaction is accompanied with production of gas [25, 26], which can lead to optical defects and cracks during compression process [29, 30]. Heating of amoxicillin trihydrate at temperatures range from 30 °C to 250 °C led to dehydration and mass loss. The first mass loss was detected at a temperature of 30 °C and complete dehydration was detected at a temperature more than 250 °C. After the complete dehydration the anhydrous form of amoxicillin will show a degradation process due to oxidation by air, so that it is important to control the temperature during processing and storage of amoxicillin trihydrate to avoid any mass loss, dehydration and decomposition of amoxicillin trihydrate. [36]

Increasing the temperature will lead to increase the hydrolysis rate of amoxicillin trihydrate in aqueous solutions at different pH values from 1 to 10. The mechanism of the hydrolysis of amoxicillin trihydrate differs according to the different pH values. Acid catalyzed hydrolysis and water mediated opening of the beta-lactam ring was reported at acidic pH, while at pH from 5 to 7 the hydrolysis was mainly an intramolecular interaction due to the attack of the amino group on the side chain, at pH more than 8 the hydrolysis is mediated by hydroxide ion. [37]

The thermal degradation of amoxicillin is one of the used methods to eliminate the antibiotic traces from the environment and the food products such as cow milks. Increasing the temperature led to the decrease of the half-life of the amoxicillin traces in milk, as the recorded half-lives of the initial concentration of 5000 µg/Kg were 323 minutes at 70 °C, 129 minutes at 80 °C, 81 minutes at 90 °C and 50 minutes at 100 °C. This degradation of amoxicillin is greatly affected by the elevated temperatures, however it is not limited to the temperature effect alone, but also the high water content of milk play an important role in this degradation reaction. [38]

Amoxicillin show a thermal degradation of 13.12% due to the exposure to 50 °C for 3 hours compared to the assay of non-treated amoxicillin sample of the same concentration at room temperature, while potassium clavulanate show a thermal degradation of 17.80% under the same conditions. [39] In another study it was also reported that potassium clavulanate in solid state showed a thermal degradation of 19.96% in a tablet dosage form due to the exposure to a temperature of 60 °C for 12 hours. [27]

Temperature has a great effect on the stability of clavulanic acid, as it was reported within a study on the stability of clavulanic acid under different conditions including different temperatures that the stability of clavulanic acid strongly decreased, when the temperature increased to be more than 25 °C at the same pH values. [40]

The effect of temperature on the physical interaction between amoxicillin trihydrate and potassium clavulanate in solid state was studied, and it was reported that this physical interaction is affected and stimulated by elevated temperatures (50 °C for 30 minutes). The mechanism of this interaction was investigated and illustrated, and it was due to the interaction between the hydrate molecules of amoxicillin trihydrate and the amino or carbonyl group of potassium clavulanate through the formation of hydrogen bonds. This will change the crystal form of the mixture and cause changes to the physical and chemical properties of the mixture. [41, 42] Also this type of physical interactions can change the biological activity and the potency of the antibiotic combination depending on the formulation and the molar ratio between the two active components and which component will domain and control the solid mixture after the physical interaction. Increasing the molar ratio of clavulanate to amoxicillin will lead to decrease the potency, as clavulanate will dominate the new crystal form of the solid solution. [43] Also Increasing the ratio of amoxicillin to clavulanate in aqueous solution will lead to the inhibition of the activity and the role of clavulanate as beta-lactamase enzyme inhibitor due to

the opening of the beta-lactam ring of clavulanate caused by the nucleophilic attack of the free amino groip at the side chain of amoxicillin to the beta-lactam ring of clavulanate. [44] So that the selection of an appropriate molar ratio between amoxicillin and clavulanate in solid state and aqueous solution and controlling the temperature are very important factors to maintain the stability and the biological activity of amoxicillin/clavulanate combination.

3.3. Effect of light.

The prolonged exposure to light can initiate or stimulate chemical reactions, which can change the chemical properties of the drug and lead to the degradation of the active pharmaceutical ingredients. [45]

Photolysis or photo-degradation is one of the frequent degradative mechanisms of the pharmaceutical products, by which drug molecules can absorb energy from a light source till they reach the activation energy, at which the molecules start the chemical decomposition reaction either by photolysis or photo-oxidation of the covalent bonds of the molecule in a reaction, by which the formation of free radicals is involved. [19, 30]

The wavelength of the light plays an important role in the photo-degradation reaction. In general, when the wavelength of the light is shorter the effect of the light on the pharmaceutical products, will increase. [30, 46] The ability of the compound to absorb light is also an important factor that affects the photo-degradation reaction, as molecules that can show absorption of light at a wavelength shorter than 280 nm will show photo-degradation under sunlight. [19]

Photo-degradation can occur in different mechanisms such as direct drug photo-degradation, by which the drug molecules absorb the light and produce energy, which can be emitted as fluorescence or can initiate chemical degradation reaction. There is also the indirect photo-degradation mechanism,

by which another material or an excipient absorbs light and form reactive radicals that can cause photo-degradation or photo-oxidation to the active pharmaceutical ingredients. Photo-degradation can also happen through self-sensitizing photo-degradation mechanism, by which drug molecules itself can form the reactive radicals and compounds due to light absorbance that lead to photo-degradation of the drug molecules. [47] It was also reported that photo-stability of solids is influenced by the presence of humidity, as the photo-degradation is greatly increased and affected by water absorption in solid state. [48]

It was reported that amoxicillin undergoes self-sensitizing photo-degradation due to the formation of reactive oxygen species like singlet oxygen, which are generated by the photolysis of the phenolic hydroxyl group at the side chain of amoxicillin. The photo-degradation of amoxicillin in water will follow directly self-sensitizing and indirect photo-degradation mechanisms [49], while clavulanic acid shows a degradation of 40% from the initial concentration due to the exposure to the classic lamp light for 4 hours. The supposed mechanism is direct photo-degradation, as clavulanic acid absorbs light leading to energy production and temperature elevation followed by thermal degradation. [33]

Photo-degradation of amoxicillin is a common method, which is applied to remove the antibiotic residuals from the environment in a Photo-reaction catalyzed by different catalysts like Fe^{+3}/H_2O_2 (photo-fenton reaction), TiO_2, or ZnO. These catalysts can form reactive radicals and singlet oxygens, which can cause decomposition of the amoxicillin. Photo-degradation of amoxicillin without the aid of catalysts was also reported, but it was much lower than the photo-degradation in the presence of catalysts after the exposure to UV lamp of wavelength 350 nm – 400 nm. Another factor that can affect the photo-degradation of amoxicillin other than catalysts is the light wavelength, as the absorbance spectrum of amoxicillin found to be between 200 nm – 290 nm which means that, if a light source can provide light covering the wavelength

between 200 nm – 290 nm would be used, this increase the rate of the photo-degradation reaction of amoxicillin. The effect of the presence of catalysts and the light wavelength on the photo-degradation of amoxicillin in aqueous solution was reported, as the photo-degradation of amoxicillin in aqueous solution due to the exposure to UV lamp of a wavelength of 240 nm – 400 nm for 30 minutes was 14% without the presence of any catalysts and was 99% in the presence of TiO_2 as a catalyst under the same conditions. [50, 51, 52, 53, 54, 55]

The effect of light on potassium clavulanate in solid state was also reported, as potassium clavulanate showed a photolytic degradation of 16.64% in a tablet formulation due to the exposure to UV light of 254 nm wavelength for 24 hours. [27] It was also reported that amoxicillin and potassium clavulanate showed a photo-degradation of 8.59% and 10.21% respectively due to the exposure to UV light source of 254 nm wavelength for 3 hours [39], so that exposure to light, the exposure duration and the wavelength of the light have a big effect on the stability of the pharmaceutical preparations containing amoxicillin and clavulanic acid as active pharmaceutical ingredients.

3.4. Effect of oxidation.

Oxidation is a chemical reaction, by which a substance loses electrons and become oxidized. [56] An oxidizing agent is a substance that can cause other substances to lose electrons due to the ability to gain electrons like oxygen, ozone, oxides, peroxides, and other oxidizing agents. [57] Oxidation can also be defined as the process of oxygen addition or hydrogen removal. [19]

One of the well-known mechanisms that can cause chemical degradation to pharmaceuticals is oxidation, in which pharmaceuticals are exposed to environmental oxygen during handling, manufacturing and storage processes. This degradation mechanism greatly depends on the presence of an oxidizing agent such as oxygen and reactive oxygen species and the chemical structure of

the pharmaceutical product. The rate of the oxidation of an active pharmaceutical ingredient also greatly depend on the presence of oxygen, such as photo-degradation reactions following photo-oxidation mechanisms directly depend on the concentration of oxygen, which can be excited by light to produce reactive singlet oxygens, which can cause photo-oxidation to the pharmaceutical products. [30]

Changes at the penicillin five membered thiazolidine ring like penicillin sulfoxides can greatly decrease the biological activity of penicillins. [58] Aerobic oxidation of beta-lactam members to the corresponding sulfoxides under aerobic oxygen at room temperature was reported. This reaction was in the presence of cobalt (III), acetylacetonate, and isobutyrlaldhyde, as these materials served as catalysts and as preventative agents to prevent the recovery of the oxidized substances. [59]

Oxidation of beta-lactam antibiotics by the aid of O_3 and $OH^{\bullet}$ to the corresponding sulfoxides is a method to abolish the biological activity of beta-lactam antibiotics.It was also reported that (R)-sulfoxides of beta-lactam members showed some potency, while (S)-sulfoxides were completely inactive. [60] Also the oxidation of beta-lactam antibiotics to (R)-sulfoxides and (S)-sulfoxides in water using ferrate (VI) oxidation method decreased the potency of the oxidized compound to be lower than 5% of the potency of the parent beta-lactam antibiotic. [61]

Phenols, amines, and carboxyl groups are all functional groups that are sensitive to oxidation and can be oxidized. [19] All these chemical groups can be found in the chemical structure of amoxicillin. [10]

The effect of oxidation on potassium clavulanate and amoxicillin was also illustrated, as potassium clavulanate showed an oxidative degradation of 23.15% as an active pharmaceutical ingredient due to the treatment with 0.3% V/V H_2O_2 at room temperature for 10 minutes [27], while amoxicillin showed an

oxidative degradation of 5.01% upon exposure to 0.1% V/V H_2O_2 at room temperature immediately. [39]

Summing up all those previous studies prove that amoxicillin/clavulanate pharmaceutical products are affected and degraded by several environmental factors such as humidity, temperature, light, aerobic oxygen and oxidizing agents, however the effect of light and aerobic oxygen on solid state amoxicillin/clavulanate pharmaceutical preparations during handling, manufacturing and storage processes without the presence of any other catalysts or factors and under the normal production conditions need to be more deeply and clearly studied and examined, not only the effect of each factor a lone, but also the effect of these both factors (light and aerobic oxygen) together, as the risk of degradation of amoxicillin and clavulanate as active pharmaceutical ingredients according to the summarized previous studies increase in the presence of light and aerobic oxygen, especially if these two factors are not been taken in consideration during the manufacturing process.

4. Protection of amoxicillin/clavulanate pharmaceutical products during handling, processing and storage against degradative environmental factors.

According to the previously summarized results, studies and books, it is very clear that special precautions and steps during handling, manufacturing and storage steps are required in order to protect the pharmaceutical preparations containing amoxicillin and clavulanate materials as active pharmaceutical ingredients against the environmental factors that can cause degradation to this antimicrobial combination such as humidity, temperature, light and oxidation process.

4.1. Protection against humidity

Within a successful validation study of amoxicillin/clavulanate powder for injection manufacturing process it was reported that environmental factors such as humidity and temperature during the processing of amoxicillin/clavulanate pharmaceutical products are considered as critical process parameters. The actual applied values of temperature and relative humidity during the filling process within this validation study to produce stable finished product were 19.21 °C – 22.44 °C for temperature and 18.23% - 27.50% for relative humidity. [62]

It was also recommended that the filling process of the moisture sensitive amoxicillin sodium active pharmaceutical ingredient as powder for injection should be done under controlled environmental conditions of relative humidity lower than 25% and temperature not more than 27 °C. [63]

The dry granulation method is preferred instead of the wet granulation method to produce formulations of amoxicillin/clavulanate powder mixtures to be compressed into tablets, as amoxicillin/clavulanate combination show high sensitivity to humidity and temperature. The processing of amoxicillin/clavulanate preparations and mixtures should be done under controlled environmental conditions of 15 °C – 25 °C for temperature and 20% – 25% for relative humidity. While handling and processing of clavulanate alone before mixing with any other materials (active pharmaceutical ingredients or excipients) is recommended to be under very restricted humidity control not more than 5% for relative humidity. Also continuous measurement and tracking of the equilibrium relative humidity (ERH) of the preparations containing amoxicillin/clavulanate active ingredients during the different manufacturing steps for the half finished products such as mixtures, tablet cores, and coated tablets is required and recommended. It is also recommended that the equilibrium relative humidity of the coated tablet just before packaging to be lower than 5%. These all recommendations are necessary to avoid degradation and discoloration of clavulanic acid, as the processing of amoxicillin/clavulanate mixed powder preparations at solid state under the environmental conditions of 25 °C for temperature and 30% for relative humidity showed discoloration and degradation of clavulanate. [64]

Excipients play an important role in the protection of moisture sensitive active pharmaceutical products. As the use of excipients with low content of active water (free water) is recommended to prevent the degradation of the moisture sensitive active pharmaceutical ingredient by the free water of excipients. Also the use of hygroscopic excipients such as colloidal silicon compounds, tri-magnesium citrate, microcrystalline cellulose, and hypromellose that can bind moisture and water molecules and show a high capacity of water binding, which will prevent the degradation of the moisture sensitive active pharmaceutical ingredient by decreasing the amount of the available moisture

to interact with the moisture sensitive materials. [65] Therefore clavulanic acid is commercially available as 1:1 mixture with other drying protective agents such as Syloid (silicon dioxide) and Avicel PH 112 (microcrystalline cellulose) to protect clavulanic acid against moisture and to enhance the safety properties during the handling of clavulanic acid. Clavulanic acid mixture with Avicel PH 112 show better characters than the clavulanic acid mixture with Syloid. [23]

One of the reported methods to protect moisture sensitive drugs against humidity is co-crystallization as co-crystallization of moisture sensitive active pharmaceutical ingredient with other substances in the formulation can decrease the unwanted solid physical changes of the active pharmaceutical ingredient caused by moisture effect. [66]

Film coating process is considered one of the applied mechanisms to protect tablet solid dosage forms against degradative environmental factors like humidity. The deposition of a polymer film on the tablet by using a suitable polymer (film former) such as hydroxypropylmethylcellulose (HPMC), polyvinyl alcohol, poly (methacrylate – methylmethacrylate), or Shellac can provide high protection against moisture, light and temperature. It was also reported that Shellac, polyvinyl alcohol, and poly (methacrylate – methylmethacrylate) showed better protection and lower water uptake than the most widely used polymer HPMC. [67, 68, 69] Also the use of a mixture between the two film formers HPMC and polyvinyl alcohol enhanced the protection provided by the applied film coat against the humidity compared to HPMC alone.[70]

Hygroscopicity of the used film can affect the efficiency of the applied film as an effective barrier against moisture, as it was reported that the use of coating film using polymer with lower hygroscopicity like (methacrylic acid – ethyl acrylate co-polymer) will show higher protection against humidity compared to other polymers that show some higher hygroscopicity such as polyvinyl alcohol based film or hypromellose, Avicel and stearate based film. [71]

Non-aqueous solvents based film coating is preferred over the aqueous based film coating solvents for the pharmaceutical products that can undergo moisture or thermal degradation, as due to the non-aqueous nature of these solvents the temperature required for the drying process after the coating step for most widely used organic solvents is lower that the drying temperature in case of aqueous based film coating. However environmental protection and safety against explosion must be taken in consideration in case of that non-aqueous based solvent for film coating is decided. [67]

A method was introduced to protect clavulanic acid against degradation by humidity during the manufacturing and storage of amoxicillin/clavulanate chewable tablets by the aid of the tablet dry coating mechanism, which is also known as jacketed tablets mechanism, by which clavulanate is applied as a core of the tablet surrounded by a layer of amoxicillin, hydrophobic lubricant (stearic acid), and other excipients in a bilayer tablet form or even in triple layer tablet form, in which the middle layer is composed of the protective hydrophobic layer. This formulation showed a remarkable stability enhancement for clavulanate compared to the amoxicillin/clavulanate chewable tablet formulations already present and available in the market. As the concentration of clavulanic acid in case of the normal marketed preparations is decreased to be about 35% from the initial concentration due to the exposure of the tablets to 75% relative humidity for 10 hours, while the clavulanic acid concentration in chewable tablets formulated as triple layer tablets was 80% - 75% of the same initial concentration as the marketed chewable tablets under the same conditions of 75% relative humidity for 10 hours and it took about 20 hours under 75% relative humidity to reach a concentration of 35% clavulanic acid from the initial concentration in the modified formulation. This stability enhancing effect is related to the formulation of clavulanic acid as a core of a multiple layer tablet and the centralization of the clavulanic acid core and not due to the presence of the hydrophobic stearate substance. This is known

because, the stability of amoxicillin/clavulanate chewable tablet as one single layer tablet with the same components did not provide the required and the desired protection against moisture. [23]

Micro-encapsulation of clavulanic acid and silicon dioxide 1:1 mixture as a raw material using acetone as an organic solvent and Eudragit E as a coating polymer during the spray drying micro-encapsulation technique increased the stability of clavulanic acid against moisture under the environmental conditions of 40 °C for temperature and 75% for relative humidity compared to the reference marketed product. This stabilization technique against moisture showed a delay to the dissolution profiles. The use of a proper plasticizer during the micro-encapsulation process can enhance the efficiency of the process and produce more stable clavulanate with better dissolution profiles. [72]

Protection of solid oral dosage forms and powders for reconstitution against moisture can be achieved by the proper selection of the primary and secondary packaging materials. Plastic bottles made from high density or linear poly-ethylene and glass bottles are good barriers against moisture, also applying a foil laminate inner sealing membrane will increase the protection against moisture. [29, 73, 74, 75] It was also reported that cold form aluminum strips will provide a very sufficient protection against degradation by humidity for moisture sensitive pharmaceuticals, as the concentrations of a tested moisture sensitive substance (PGE – 7762928) formulated as tablets and packaged in different packaging materials after the exposure to the environmental conditions of 40 °C for temperature and 75% for relative humidity for 6 months compared to the same starting initial concentration were 84% when PVC blisters are used, 91% in case of cyclic olefic blisters, in blisters made from aclar the concentration was 97%, high density poly-ethylene bottle with foil inner sealing membrane achieved a concentration 99%, while in cold form aluminum strips the concentration of the tested moisture sensitive substance

after these stressful conditions was 100% from the initial concentration showing full protection against degradation under these tested environmental conditions. [75]

4.2. Protection against temperature.

It is recommended that handling and processing of pharmaceutical products containing amoxicillin and clavulanate as active pharmaceutical ingredients under temperature that do not exceed 25 °C [62, 64], and that the storage of clavulanic acid as a raw material should be under temperature between 2 °C – 8 °C and must also be protected from light and moisture. [76] It is also generally typically reported that an increase of 10 °C in the temperature can lead to a 2 – 5 fold increase in the degradation rate of pharmaceuticals. [77]

The crystal type, the degree of crystallinity, and the storage conditions have a big effect on the thermal stability of beta-lactam antibiotics in solid state condition. Increasing the degree of crystallinity of beta-lactam antibiotics will enhance the thermal stability of the product, as the trihydrate crystal forms such as amoxicillin trihydrate are more thermostable than the corresponding anhydrous amorphous forms. [78]

Increasing the pH of amoxicillin trihydrate powder to be between 4.39 – 4.97 will increase the length of amoxicillin trihydrate crystals and will improve the thermal stability, the purity, and the quality of amoxicillin trihydrate crystals. This increase in the powder pH is directly related to the crystallization pH during the synthesis of amoxicillin trihydrate. [79]

The application of non-aqueous solvent based film coating process will help in protecting thermolabile compounds against the elevated temperature during the coating and the drying processes, as the temperature and time required to evaporate and dry the solvent is lower than the temperature and time required

in case of aqueous based film coating. Safety and environmental issues must be taken in consideration in this case. [67]

Also the use of vacuum dryers and freeze dryers allow a drying process at lower temperatures because of the decreased pressure, so that these dryers are recommended for thermo-liable drugs. [80, 81]

Protecting amoxicillin/clavulanate products against light during handling, processing and storage will help in protecting amoxicillin/clavulanate products against thermal degradation, as it was reported that the elevated temperature due to energy gained because of absorbance of light is the recommended mechanism for the degradation of clavulanic acid in light. [33]

4.3. Protection against Light.

ICH (international conference of harmonization) guidelines recommend carrying out photo-stability studies for the active pharmaceutical ingredients produced. These photo-stability studies are forced degradation studies applied to the substances in solid state, solutions, or suspensions to evaluate the degree of the photosensitivity of the substance and the degradation mechanisms. These ICH recommended Photo-stability studies also include confirmatory studies beside the forced degradation studies to provide information and precautions required for handling, processing, packaging, labeling and storage of the photosensitive drugs. [82] Generally as previously reported protection against light must be provided during the handling and storage of clavulanic acid containing pharmaceutical products. [76]

Photo-degradation of solid pharmaceuticals due to ultraviolet or visible light irradiations depend mainly on the penetration depth and the wavelength of these irradiations, as it was reported that only the outer external layers of photosensitive tablets showed photo-degradation [48], so that formulating the amoxicillin/clavulanate tablets as multiple layers tablets, by which clavulanate

is the core part [23] will help in protecting clavulanic acid against photo-degradation.

Photo-stabilization of solid pharmaceuticals can be achieved by the use of solid particles with irregular surface, which can show more reflections to the exposed light. Larger particle size and larger tablet size will decrease the total surface area exposed to light and decrease the effect of light. Crystalline forms of particles are more photo-stable and thermostable than the corresponding amorphous forms. The use of ultraviolet absorbers, colorants and opacifiers such as Eusdex 9020 for external use, Curcumin E100 and Riboflavin E101 for oral dosage forms as excipients can increase the protection of photosensitive substances against photo-degradation. Iron oxides as opacifier showed better photo-protection than the widely used titanium dioxide especially for substances sensitive to light of wavelength between 400 nm – 420 nm. By applying film coating to the tablets supported with components that can reflect light away or absorb the light by itself such as TiO_2 preventing the photo-degradation of the active pharmaceutical ingredients increasing the protection against photo-degradation. The degree of Photo-protection provided by the film coating depends on the thickness of the coat, the wavelength of the light, the particle size of the used protective excipients, and the photosensitivity of the drug. [48, 83]

It was also reported that soft gelatin capsules do not provide sufficient photo-protection to the photosensitive drugs. Tablets and hard gelatin capsules showed more photo-protection compared to soft gelatin capsules. Increasing the thickness of soft gelatin capsules and incorporating of photon absorbers to the capsule shell will increase the photo-protection provided by soft gelatin capsules. [48]

Protection of the drug from light during manufacturing and packaging can also be provided by the use of light sources with wavelength longer than 500 nm such as sodium vapor lamps and LED lamps. It was also reported that

clavulanic acid is stable under LED lamps and sodium vapor lamps, and LED light sources have the advantage of more convenient production environment and more normal lighting conditions. [33, 84, 85]

Primary and secondary packaging materials play an important role in protecting photosensitive drugs against photo-degradation by the use of packaging materials, which can show resistance to the ultraviolet and visible light irradiations such as brown glass bottles. Plastic packaging materials can achieve photo-stabilization only by the use of pigments, coloring agents, and substances that can absorb ultraviolet irradiations, as neat plastic alone can show absorption of ultraviolet and visible light irradiations of wavelength shorter than 280 nm. Aluminum foil packaging materials like aluminum strips, tubes and bags show 100% protection against photo-degradation. The use of cartons as secondary packaging materials will achieve very high photo-protection, as cartons serve as a strong barrier against ultraviolet and visible light irradiations. [29, 86]

4.4. Protection against oxidation.

Generally active pharmaceutical ingredients should be studies, if they are susceptible to oxidation by aerobic oxygen (Auto-oxidation) or not. This can be tested by investigating the stability of the active pharmaceutical ingredient under the environmental condition of 40% oxygen compared to the stability of the same substance maintained under inert atmosphere at room temperature. [19]

Protection of pharmaceuticals that can be degraded by oxidation like penicillin [87] can be achieved by different techniques and mechanisms during handling, processing and storage of these products. Protection from light and avoidance of high temperatures are recommended to protect these oxidation sensitive pharmaceutical products against oxidative degradation, as light will lead to photo-oxidation of these active pharmaceutical ingredients and elevated

temperatures will accelerate the rate of the oxidation degradation reactions. [19, 30, 87]

Incorporation and the use of antioxidants as components of the formulation will provide protection against oxidative degradation. Antioxidants work by different mechanisms and they may be: A) water soluble antioxidants such as sulfurous acid salts, ascorbic acid, and thiol derivatives, which can be oxidized instead of the active pharmaceutical ingredients due to the high standard oxidation potential of these antioxidants, B) water-insoluble antioxidants such as alpha-tocopherol and propyl gallate, which act as free radicals scavengers and prevent the interaction between the free radicals and the active pharmaceutical ingredients, and C) metal-sequestering antioxidants, which can prevent the process of free radicals formation. [19, 30, 87]

The use of solutions with pH lower than 3.5 (acidic in nature) will lead to decrease the redox potential of the solution and increase the stability of the products against oxidation [87].However it was also reported that beta-lactam antibiotics and clavulanic acid are exposed to acid catalyzed hydrolysis, especially if the pH was lower than 3, so that this mechanism is not the preferred mechanism to protect amoxicillin/clavulanate products and solutions against oxidative degradation. [40, 34]

The replacement of air (aerobic oxygen) from the formulations and from the primary packaging materials with inert gases such as nitrogen or carbon dioxide and maintaining the product under inert atmosphere will protect the active pharmaceutical ingredients against oxidative degradation. [63, 87, 88] This mechanism is recommended for the filling of amoxicillin/clavulanate powder preparations. [63]

Packaging materials play an important role in the protection of the products against degradation by oxidation such as the use of amber colored glass bottles supported with inner sealing membrane or amber colored vials with rubber stoppers. Also the use of good sealed aluminum-aluminum strips or the use of

packaging materials supported with antioxidant and drying materials and parts like closure systems supported with antioxidant and silica gel. All these techniques and mechanisms can provide protection against oxidative degradation of pharmaceutical products. [19, 30, 87]

5. Conclusion.

Amoxicillin trihydrate and potassium clavulanate combination is one of the most important antibiotic combinations, which have great antimicrobial biological activity against different types of infections such as respiratory tract infections and urinary tract infections. This antibacterial combination also shows very high economic value.

Structure activity relationships of beta-lactam antibiotics show that minor and small changes in the chemical structure of beta-lactam antibiotics will lead to major and great changes in the biological activity and the stability of these pharmaceutical products.

Stability of amoxicillin and clavulanic acid can be affected by different environmental factors during handling, processing and storage of these pharmaceutical products such as humidity, temperature, light and aerobic oxygen. These factors can cause degradation to amoxicillin by different mechanisms, which can lead to therapeutic failure.

Precautions and protective mechanisms against each of these environmental factors should be applied during the different manufacturing steps and the storage of amoxicillin/clavulanate pharmaceutical products in order to prevent the expected therapeutic failure and instability due to the degradation or even to increase the shelf life and to enhance the stability of these pharmaceutical products, and also to develop and improve the production process.

Understanding these degradative factors and the protective methods is very important especially in case of continuous manufacturing is required, by which

several continuous production steps are applied such as continuous feeding, continuous mixing, continuous compression, continuous coating and drying. Taking all these factors in consideration will be very important to design the right and an efficient process analytical technology methods to provide online analysis and detection of all the possible degradative pathways, critical quality attributes, critical process parameters and critical material attributes to achieve a successful real time release concept to achieve the economic gain expected from the continuous manufacturing.

Production sites should take all these degradative environmental factors in consideration and study the stability of the product under the actual applied environmental conditions during the production in order to define the possible enhancements that can be applied to the process or to make sure that the applied environmental conditions achieve the most stable product and any other modifications will not deliver better stability or quality.

Further long term and short term studies are required to illustrate the effect of different light sources, light wavelength, light exposure time, and aerobic oxygen (the effect of each of these factors alone and also the effect of light and oxygen together) on the different amoxicillin/clavulanate preparations especially in solid state under the normal manufacturing conditions without the presence of any other catalysts or affecting factors during the different manufacturing steps and conditions and during the different storage conditions.

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