Penicillin degradation catalysed by Zn(II) ions in methanol

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Abstract

The rates of degradation, catalysed by Zn 2+ ions, of four classical penicillins—amoxicillin, ampicillin and penicillins G and V—were followed at 20 °C in methanol by spectrophotometric assays. Kinetic schemes of the reactions of degradation catalysed by Zn 2+ ions were analogous to those given previously for the reaction catalysed by Cd 2+ ions. The methanolysis of penicillin V occurs with the formation of a single intermediate substrate–metal complex (SM), whereas the degradations of amoxicillin, ampicillin and penicillin G occur with the initial formation of two complexes with different stoichiometry, SM and S2 M, both in equilibrium. In all cases, the degradation reaction is of the first order with respect to SM, with velocity constants at 20 °C of 0.0093, 0.0288, 0.0304 and 0.0349 min⁻¹, for amoxicillin, ampicillin, penicillin V and penicillin G, respectively. The compound S 2 M degraded at a much lower rate than SM and constitutes a zero-order process. The catalytic effect of the ion Zn 2+ in the degradation of the penicillins was much weaker than that of the ion Cd 2+, owing to the lesser ionic radius of the former and the fact that in the case of the reaction catalysed by Zn 2+, the compound S 2 M occurred in a much greater amount than the SM. At the end of the degradation reaction, the corresponding penamaldic derivative of the antibiotic was produced, established by the coordination of the Zn2+ ion, forming a single complex 2:1 (derivative penamaldic–metal) in the case of amoxicillin and ampicillin; and two complexes, 1:1 and 2:1, for the other antibiotics. Finally, the molar absorption coefficients of the products of reaction at the wavelength of maximum absorption at 20 °C were calculated.

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1. Introduction

Penicillins belong to the β-lactam antibiotic group, which at present stand for a great majority of the antibiotics used in medical treatments. These antibiotics, characterised by a structure with a β-lactam ring of four members, owe their biological activity to the chemical reactivity of their ring, which has long been attributed to its inner tension [1]. The noteworthy structural tension of the β-lactam ring stems from the fact that the angles of bonding between the atoms (90°) are clearly inferior to the corresponding angles of sp³ hybridisation (109.47°) or sp² (120°); in this way, the characteristic resonance of the amide link—generally responsible for the great chemical stability of the amides—is inhibited, resulting in the instability of the structure of the β-lactam compounds [2].

This structural characteristic of the β-lactams makes them susceptible to hydrolysis and aminolysis. Penicillins in general undergo pH-dependent hydrolysis, with negligible spontaneous degradation or water-catalysed degradation. The common mechanism of hydrolysis has been described by different authors [3–6]. The α-amino penicillins such as ampicillin and amoxicillin can also undergo autoaminolysis in an aqueous solution by means of a nucleophilic attack of the amino group of the lateral chain of a molecule, on the β-lactam bond of a second molecule of antibiotic [7–9].

Transition metal ions produce an enormous increase in the velocity of hydrolysis and aminolysis of penicillins [3,10–12]. It has been proposed that catalysis occurs by means of an intermediate 1:1 complex formed between the metal ion and the antibiotic [10], and that the role of the metal ion in hydrolysis or aminolysis is to establish the tetrahedral intermediate that is formed by the addition of the nucleophilic group to the β-lactam carbonyl group [12].
Also well documented are the reactions of penicillins catalysed by alkoxide ions in aqueous solution, and certain mechanisms of the reaction have been proposed [6,13–15]. In the methanolysis of benzylpenicillin, the increase in light absorption at 280 nm has been attributed to the formation of the penicilloyl ester, which gives way to the penamaldic derivative of the antibiotic. For pH values lower than 11, the thiolate anion has been proposed to protonate rapidly, forming the corresponding thiol [15], which would be thermodynamically favoured by pH values below its pK_{a}.

The present study consists of an analysis of the absorbance data from the degradation of four /H-/?-lactam antibiotics—two penicillins (G and V) and two /H-/?-aminopenicillins (ampicillin and amoxicillin)—in methanol and in the presence of Zn^{2+}. This ion was used on the basis of its relationship with the metallo-/H-/?-lactamase enzymes, which represent the main mechanism of bacterial resistance to the lethal action of the /H-/?-lactam antibiotics. Moreover, in each case the kinetic schemes of the reaction of catalysed degradation is established, and the results obtained here are compared. Finally, we compare our results with those relative to the penicillins’ degradation in methanol in the presence of Cd^{2+} ions, described in previously published papers [16,17].

2. Method

2.1. Substances and reagents

Four penicillins have been tested (Fig. 1): sodium ampicillin (99% pure), sodium penicillin G (1658 IU/mg of base) and potassium penicillin V (≥98%) were supplied by Sigma. The sodium amoxicillin used was the injectable commercial brand (Clamoxyl® 1 g) from Smithkline Beecham. Methanol and ZnCl_{2} were from Merck. All reagents used were of analytical grade.

2.2. Instruments

A Perkin-Elmer lambda 16 spectrophotometer equipped with a Selecta thermostat (±0.1 °C), a computer equipped with UV-Winlab software from the Perkin-Elmer Corporation Copyright 1994–1995, and 1 cm thick spectrophotometric cells were used.

2.3. Kinetic measurements

The antibiotic degradation reaction in methanol in the presence of the Zn^{2+} ions was assessed by measuring the increased intensity of the absorption band, with a maximum near 280 nm, appearing in all cases and stabilising approximately 24 h after the reaction. The degradations of amoxicillin, ampicillin and penicillin G were measured through the increase of absorbance at 282 nm, and those of penicillin V at 285 nm. The appearance of the absorption bands in methanol, as indicated earlier, is most likely due to the formation of the corresponding penamaldic derivative of the antibiotic [15,18]. In each of our kinetic assays, two series of solutions were tested. One series had different excesses of antibiotic (1 to 500, 8 to 40, 10 to 130 and 2 to 25 times the concentration of the metal ion for amoxicillin, ampicillin, penicillin G and penicillin V, respectively) and a constant concentration of the metal ion (5 × 10^{-5} M for amoxicillin and 1 × 10^{-4} M for the other antibiotics); while in the other series, the concentration of the substrate was kept constant (5 × 10^{-5} M for amoxicillin and 1 × 10^{-4} M for the other penicillins) and different excesses of the metal ion were used (ranging from 1 to 500, 1 to 250, 1 to 40 and 1 to 20 times the substrate for amoxicillin, ampicillin, penicillin G and penicillin V, respectively). All the kinetic experiments were performed at a temperature of 20 °C. At this temperature, the degradation of the antibiotics in the presence of Zn^{2+} ions takes place very slowly, yet it allowed us to compare this reaction and that catalysed by Cd^{2+} ions [15,16], previously studied at that temperature.

The methodology followed was similar in all cases and consisted of preparing the mixture of the reagents directly in the spectrophotometric cell. Two methanolic solutions of the antibiotic and the metal ion (always as ZnCl_{2}) were prepared separately in appropriate concentrations. These solutions were kept in a thermostatic bath at the temperature of the experiment. Then, 3.4 ml of the solution of a reagent
substrate or metal ion, as were the case, was placed inside the spectrophotometer cell and once thermal equilibrium was reached, 100 μl of the other reagent was added with a micropette. The total volume was, therefore, 3.5 ml. The methanol solution of the antibiotic was used immediately after preparation for all the kinetic experiments, whereas the metal ion solution was kept for later use.

For all the substrates, the values of initial rates, \( v_{obs} \), of product appearance were calculated using the following equation:

\[
\frac{dA}{dt} = \frac{1}{\varepsilon_a} (\varepsilon_p - \varepsilon_v) \tag{1}
\]

where \( \frac{dA}{dt} \) is the slope of the linear representation of absorbance over time in the first minutes of the reaction. This value was obtained by applying the minimal square method to the linear part of the absorbance-time profiles corresponding to the kinetic experiments; \( \varepsilon_a \) is the molar absorptivity of the single product of reaction from the degradation of amoxicillin and ampicillin, and the product principally formed in the catalysed degradation reaction of the other substrates, penicillin G and penicillin V; and \( \varepsilon_v \) is the molar absorptivity of the antibiotic at the wavelength of the maximum absorbance of the reaction product. As ampicillin and penicillin G hardly present light absorbance at the mean wavelength, for these antibiotics 0 was used as the \( \varepsilon_a \) value of the Eq. (1). The \( \varepsilon_a \) values for amoxicillin and penicillin V at the respective mean wavelengths (282 and 285 nm) and at 20 °C were determined under the usual procedure, being respectively equal to 1.5 \times 10^3 l/mol cm and 1.04 \times 10^2 l/mol cm.

2.4. Determination of the stoichiometric ratio of the products

The stoichiometry of the reaction products of the tested penicillins was determined with the classical methods of continuous variations and molar ratio. For both, the absorbance data measured at the end of the reaction were used (approximately 24 h).

3. Results and discussion

The spectral changes produced over time in the methanol solutions of the penicillins in the presence of the Zn\(^{2+}\) ion are analogous in all cases. These changes consist of the appearance of a band with maximum absorbance near 280 nm (282 nm for amoxicillin, ampicillin and penicillin G; and at 285 nm for penicillin V). This band, which intensifies over time and reaches a constant intensity at approximately 24 h of reaction, has been associated by several authors with the corresponding penamaldic derivative of the antibiotic [15,18]. However, the intensity of the band at the end of the reaction depends, for all the antibiotics we tested, on the relationship between the antibiotic concentrations and the Zn\(^{2+}\). This can be deduced from the results by applying the classical methods used to determine the stoichiometric ratio of the reaction—that of continuous variations, and the molar ratio method—to the data of absorbance (Fig. 2). For all the antibiotics tested, the method of continuous variations gave a curve with a maximum at the molar fraction of 0.67. This indicates the formation of a compound with a stoichiometry of 2:1 (ligand-metal), \( P \). The ligand that comes to form part of this complex would be the corresponding penamaldic derivative of the antibiotic, which would be stabilised by the metal ion. Therefore, this ion, besides catalysing the degradation reaction of the antibiotic in the methanol medium, comes to form part of the final product(s) of degradation.

The product of degradation \( P \) is the only compound detected after the degradation reaction of the \( \alpha \)-aminopenicillins tested (amoxicillin and ampicillin); the concentration of a compound 1:1 is practically negligible, as the values of absorbance at the end of the reaction in the kinetic mixtures with excess or defect of metal are coherent with the formation of the compound \( P \) as the only product of reaction. This is also consistent with the result of the application of the molar ratio method, in which the formation of a single complex for a molar ratio of reagents 2:1 is observed (antibiotic-metal ion).

In no case does the representation of the method of the continuous variations present an inflexion for a molar fraction of 0.5 (Fig. 2), compatible with the simultaneous formation of a stoichiometric complex of 1:1. For penicillin G and V, however, the graph obtained by presenting the data on absorbance at infinite time using the molar ratio method (Fig. 3) suggests the formation of a 1:1 stoichiometric product in equilibrium with the compound 2:1. Moreover, the compound 1:1 has a molar absorption less than 2:1, as seen from the absorbance values obtained under a great excess of the metal ion. They are much lower than those expected assuming the formation of a single 2:1 complex, and the fact that absorbance at infinite time decreases as the excess of metal ion increases. Therefore, the compound 1:1 would only be formed in an appreciable amount when the reaction takes place under a great excess of Zn\(^{2+}\), which justifies, meanwhile, that for the molar ratios used in the method of continuous variations, there is no evidence of the compound 1:1.

Because the ion Zn\(^{2+}\) has a 3d\(^{10}\) configuration, the absorption band of the reaction products, with the maximum absorbance wavelength near 280 nm, can be attributed to a possible transference of charge between the ligand mentioned—proceeding from the degradation of the antibiotic—and the metal ion. Then, under these circumstances, the absorption toward 280 nm should bear a close relationship with the structure of this ligand rather than with that of the metal. The band of transference of charge suggested is compatible with the coexistence of two compounds with different ligand-Zn\(^{2+}\) stoichiometry that would have spectra with different intensities.

The values of the molar absorptivity of the derivatives corresponding to the \( \alpha \)-aminopenicillins studied, ampicillin and amoxicillin, in methanol and at 20 °C (\( \varepsilon_{28} \)), were calcu-
lated from the infinite time absorbance values of solutions prepared with an excess of antibiotic. The 2:1 concentration of the product coincides with the initial concentration of the metal ion. In the case of amoxicillin, the absorbance values were corrected for the undegraded amoxicillin, as this antibiotic absorbs at the mean wavelength ($\varepsilon_a = 1.5 \times 10^3 \text{ l/mol cm}$). This correction was not necessary in the case of ampicillin. The $\varepsilon_p$ values for the derivatives of amoxicillin and ampicillin are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Penamaldic derivative</th>
<th>$\varepsilon_p \times 10^{-4}$ (l/mol cm)</th>
<th>$\varepsilon_{1:1} \times 10^{-3}$ (l/mol cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>1.4</td>
<td>–</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>1.6</td>
<td>(a)</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>1.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

(a) Could not be determined because penicillin G degrades at an appreciable rate.

Also in Table 1 are the molar absorptivity values in methanol and at 20°C of the degradation products from penicillins G and V. These coefficients were also evaluated from the absorbance data of the kinetic mixtures measured in infinite time. For the calculation of the $\varepsilon_p$ absorbance values at 24 h corresponded to antibiotic solutions with $\text{Zn}^{2+}$ prepared with enough excess antibiotics to allow the absorbance value to reach the maximum value. This procedure could not be applied to determine the $\varepsilon_p$ value of the 2:1 compound of penicillin G because it degrades directly to an appreciable degree, and total absorbance in infinite time in solutions with an excess of antibiotic can be considered as the sum of absorbance of the compound 2:1 and of the degradation compound of non-coordinated penicillin. In such a case, the molar absorptivity of the compound 2:1, $\varepsilon_p$, was calculated from the ordinate at the beginning of the curve representing the infinite time absorbance values with respect to the concentration of solution with excess antibiotic, and of the initial concentration of the metal ion in the solutions. In these solutions, the concentration of P coincides with that of the metal ion ($1 \times 10^{-4}$ mol/dm$^3$).
The value of the ordinate in the origin is associated with absorbance due to the P product. The value of the molar absorptivity of the final product 1:1 was assessed from absorbance values of solutions with a great excess of Zn$^{2+}$, under which only this one compound is formed.

In the kinetic study of the reaction, for each of the antibiotics two series of methanolic solution, with antibiotic and metal ion, were studied. In one series, the amount of antibiotic was constant while the amount of metal ion varied, and vice versa in the other series. From the absorbance data in the first minutes of the reaction, and using the values of $\varepsilon_p$ and $\varepsilon_a$, rate was calculated for each using the Eq. (1). The slope of the absorbance-time representations of the first minutes of the reaction were drawn, for which a linear relation between the two variables was seen. The slope was obtained by adjusting the data using the minimum square method. The $\varepsilon_p$ values used to calculate initial rates are given in Table 1.

For the four penicillins tested, the initial rate of degradation, $v_{\text{obs}}$, increased with increased concentrations of the metal ion, whereas the amount of antibiotic was constant (e.g. amoxicillin, Fig. 4). Moreover, rate reached a maximum value for a given concentration of metal, after which the rate became independent of the Zn$^{2+}$ concentration. When testing with fixed concentrations of the metal ion and variable antibiotic concentrations, amoxicillin, ampicillin and penicillin G showed an initial decrease in reaction rate when the concentration of substrate increased, and eventually saturation took place. These findings suggest that the degradation reaction catalysed by Zn(II) ions of the three above penicillins take place through the formation of two metal complexes formed by the coordination of the Zn$^{2+}$ ion to one or two molecules of intact penicillin—respectively, SM and S$_2$M. These compounds decompose at different rates to give identical reaction products. The kinetic scheme shown in Fig. 5 might be used to summarize this. In the mentioned scheme, S represents the intact antibiotic; SM and S$_2$M are two complexes formed between the antibiotic and Zn$^{2+}$ with stoichiometric ratios of 1:1 and 2:1, respectively; $K_1$ is the constant of formation of the compound SM; $K_e$ that of the formation of S$_2$M from SM; $k$ is the rate constant of the first order with respect to SM; and $k_0$ is the rate of the zero-order rate constant process by which the intermediate S$_2$M is degraded. The scheme proposed (Fig. 5) for the reactions catalysed by Zn$^{2+}$ in methanol coincides with that put forth in previous works [16–18] for the degradation reactions in methanol of amoxicillin, ampicillin and penicillin G catalysed by Cd$^{2+}$ ions.

If indeed amoxicillin, ampicillin and penicillin G degrade in the presence of Zn$^{2+}$ as proposed before, the constants implied vary greatly depending on the antibiotic considered. Thus, for ampicillin and amoxicillin, the complex S$_2$M formed by the intact antibiotic has a formation constant much greater than that of the compound SM, because when the reaction is carried out with a slight excess of antibiotic (twice that of the metal ion), rate holds a minimum and constant value, comparable to the degradation rate constant of the complex S$_2$M. Therefore, in the case of these antibiotics, the most stable complex from the thermodynamic

$$S + M \underset{K_1}{\overset{K_e}{\rightleftharpoons}} SM \quad S + S \underset{K_0}{\overset{k}{\rightleftharpoons}} S_2M \quad \text{Products}$$

Fig. 5. Kinetic scheme of reaction for the decomposition in methanol of amoxicillin, ampicillin and penicillin G ($T = 20^\circ$C).
stand point, $S_2M$, is also the one with the greatest thermal stability. For the other penicillin that fits the above scheme, penicillin G, the constant of formation of the intermediate compound is lower than in the other two antibiotics. This is demonstrated by the fact that as the excess antibiotic increases, initial rate decreases progressively, until arriving at a minimal constant value (Fig. 6). This slow decrease of the initial velocity can be associated, according to the above scheme, with the formation of a greater amount of the compound $S_2M$ from $SM$ when excess substrate is increased. It is precisely this displacement of balance that serves as the base for the present study of the simultaneous determination of $k$ and $K_e$ in the degradation of penicillin G, as we will see later on. It also constitutes the base of the calculation of these constants in the reactions in methanol catalysed by Cd$^{2+}$ ions [16,17,19] of amoxicillin, ampicillin and penicillin G.

In order to determine the initial velocity of penicillin V, kinetic experiments were also carried out under with excess metal while keeping the amount of antibiotic constant; and in others the substrate concentration was constant while metal varied. The initial rate of degradation, $v_{obs}$, was found to increase when excess Zn$^{2+}$ increased in the first group of assays. The value of $v_{obs}$ likewise increased when the concentration of penicillin and that of the metal ion were fixed. In both cases the initial rate was constant and had a maximum value for a given specific concentration of excess reagent. Thus, in representing the values of initial velocity with respect to the reagent in excess, whether substrate or metal ion, graphs analogous to that of Fig. 6 were obtained. These results suggest that the catalysed degradation reaction of penicillin V takes place through the formation of a single intermediate complex of stoichiometry 1:1. Hence, for the decomposition of penicillin V, we can propose the kinetic scheme shown in Fig. 7. In this scheme, $SM$ is the intermediate complex formed between the substrate and the metal ion, $K_1$ and $k$ are the constant of formation and the first-order rate constant with respect to $SM$, respectively. The value of the first-order rate constant with respect to $SM$, $k$, has been determined in all cases. For the catalysed degradation of amoxicillin, ampicillin and penicillin V this constant was obtained from the absorbance data gathered in the presence of a sufficiently great excess of the metal ion for which it is assumed that all the antibiotic is forming the $SM$ complex, and therefore the reaction takes place according to a pseudo-first-order process. Accordingly, the constant of velocity, $k$, was obtained from the fit of the absorbance data from the full course of the reaction to the integral first-order equation, giving in all cases a coefficient of determination very close to the unit. The values of the velocity constants are shown in Table 2.

The value of the constant $k$ corresponding to the degradation of penicillin G was determined from the kinetic data through assays with a variable excess of antibiotic and a constant quantity of metal ion. The initial reaction rate in each experiment was calculated, as for the other antibiotics, from Eq. (1). The values of velocity, $v_{obs}$, were represented with respect to the initial concentration of substrate under Eq. (2), which was deduced in a previous study [16].

$S + M \rightleftharpoons SM \rightarrow \text{Products}$

Fig. 7. Kinetic scheme of reaction for the decomposition in methanol of penicillin V ($T = 20^\circ$C).
Table 2

Values of the first-order rate constant, \( k \), and the equilibrium constant, \( K_e \), for antibiotic degradation reactions catalysed by Zn\(^{2+}\) ions and Cd\(^{2+}\) (\( T = 20 \degree C \))

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Catalysed by Zn(^{2+})</th>
<th>Catalysed by Cd(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k \times 10^2 ) (min(^{-1}))</td>
<td>( K_e \times 10^{-2} ) (l/mol)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.93</td>
<td>1.70 [19]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2.88</td>
<td>3.87 [16]</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>3.04</td>
<td>6.10 [17]</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>3.49</td>
<td>1.95</td>
</tr>
</tbody>
</table>

\[
\frac{1}{v_{obs}} = \frac{1}{k [M]_T} + \frac{K_e}{k [M]_T [S]_T}
\]

(2)

where \([M]_T\) represents the total concentration of metal and \([S]_T\), that of the substrate.

Fig. 8 offers the kinetic data represented according to Eq. (2). The curve has been adjusted by means of the minimum square method. The value of the coefficient of linear determination (\( r^2 \)) was 0.995. The first-order constant, \( k \), was calculated from the ordinate in origin (2.86 \( \times 10^5 \) dm\(^3\) min/mol) and its value is shown in Table 2. Once known, along with the value of \( k \), and the slope of the curve (5.59 \( \times 10^1 \) min), we calculate the constant of association (\( K_e \)), whose value is also shown in Table 2.

In Table 2, together with the values of \( k \) and \( K_e \) determined in the present study, we give their values established by previous studies [16,17,19] for the degradation reaction catalysed by Cd\(^{2+}\) (II) at the same temperature (20 \degree C). Degradation in methanol of the penicillins in the presence of Cd\(^{2+}\) ions is also apparent that the constant of velocity with respect to the intermediate compound SM, which is formed by the coordination of the metal ion to the intact antibiotic, is greater in all cases for Cd\(^{2+}\). This can be explained by the greater radius of the latter ion (0.97 \( \hat{\text{A}} \)) as opposed to 0.74 \( \hat{\text{A}} \) of Zn\(^{2+}\); indeed, the relationship between the constants is nearly the same as that existing between the ionic radius of the two ions, coinciding exactly in the case of ampicillin. Moreover, in the reactions catalysed by Cd\(^{2+}\) ions, the relationship between the concentration of intermediate compound with stoichiometry 1:1, SM, and of the 2:1 compound, S\(_2\)M, is greater than for the reaction catalysed by Zn\(^{2+}\) ions, as reflected in the values of the constants of association between the two compounds (Table 2). This is another reason by which Cd\(^{2+}\) catalyses the degradation of the penicillins at a greater velocity than Zn\(^{2+}\), and the compound SM is degraded at a much greater rate than the intermediate complex S\(_2\)M. Therefore, the values of the constants of formation of S\(_2\)M, \( K_e \), for the degradation reactions in methanol of the penicillins studied in the presence of Zn\(^{2+}\) are greater than the corresponding constants when the reactions are catalysed by Cd\(^{2+}\) ions. The values of these constants for the degradation reactions of ampicillin and amoxicillin in the presence of Zn\(^{2+}\) could not be determined precisely because their high value does not allow the application of the principles of calculation considered in the reactions catalysed by the Cd\(^{2+}\) ions.

Fig. 8. Plot of \( 1/v_{obs} \) vs. penicillin G concentration, \([S]_0\), at 20 \degree C.
4. Conclusions

The degradation reaction catalysed by Zn(II) ions of amoxicillin, ampicillin and penicillin G take place through the formation of two metal complexes of intact penicillin, namely SM and $S_2M$. Both complexes decompose at different rates to give identical reaction products. For ampicillin and amoxicillin, the complex $S_2M$ has a formation constant much greater than that of the compound SM. Moreover, $S_2M$ has a remarkable thermal stability. For the penicillin G, the formation constant for the intermediate $S_2M$ is lower than those found for the other two antibiotics. The reaction of penicillin V takes place through the formation of a single intermediate complex, SM.

Degradation in methanol of the penicillins in the presence of Cd(II) takes place at a greater reaction rate than when in the presence of Zn$^{2+}$ ions. In all cases, the constant of velocity corresponding to the intermediate SM, is greater for the reactions catalysed by Cd$^{2+}$. The relationship between the constants is nearly the same as that existing between the ionic radii of the two ions. Moreover, in the reactions catalysed by Cd$^{2+}$ ions, the relationship between the concentration of intermediate SM, and that of $S_2M$, is greater than for the reaction catalysed by Zn$^{2+}$ ions. This is another reason by which Cd$^{2+}$ catalyses the degradation of the penicillins at a greater velocity than Zn$^{2+}$.

References