Modeling of the Enzymatic Kinetic Synthesis of Cephalexin—Influence of Substrate Concentration and Temperature

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Abstract: During enzymatic kinetic synthesis of cephalexin, an activated phenylglycine derivative (phenylglycine amide or phenylglycine methyl ester) is coupled to the nucleus 7-aminodeacetoxycephalosporanic acid (7-ADCA). Simultaneously, hydrolysis of phenylglycine amide and hydrolysis of cephalexin take place. This results in a temporary high-product concentration that is subsequently consumed by the enzyme. To optimize productivity, it is necessary to develop models that predict the course of the reaction. Such models are known from literature but these are only applicable for a limited range of experimental conditions.

In this article a model is presented that is valid for a wide range of substrate concentrations (0–490 m*M* for phenylglycine amide and 0–300 m*M* for 7-ADCA) and temperatures (273–298 K). The model was built in a systematic way with parameters that were, for an important part, calculated from independent experiments. With the constants used in the model not only the synthesis reaction but also phenylglycine amide hydrolysis and cephalexin hydrolysis could be described accurately. In contrast to the models described in literature, only a limited number (five) of constants was required to describe the reaction at a certain temperature. For the temperature dependency of the constants, the Årrhenius equation was applied, with the constants at 293 K as references. Again, independent experiments were used, which resulted in a model with high statistic reliability for the entire temperature range. Low temperatures were found beneficial for the process because more cephalexin and less phenylglycine is formed.

The model was used to optimize the reaction conditions using criteria such as the yield on 7-ADCA or on activated phenylglycine. Depending on the weight of the criteria, either a high initial phenylglycine amide concentration (yield on 7-ADCA) or a high initial 7-ADCA concentration (yield on phenylglycine amide) is beneficial.
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INTRODUCTION

During enzymatic cephalexin synthesis an activated side-chain is coupled to the nucleus, 7-ADCA (7-aminodeacetoxycephalosporanic acid). Various activated side-chains can be used, such as phenylglycine amide (PGA), phenylglycine methyl ester (PGM) or other amides and esters. In this study, the results with PGA are reported, the results on PGM will be published later. The nucleus is produced from penicillin G using a ring expansion and a hydrolysis step (Bruggink, 1996; Bruggink et al., 1998; Verweij and DeVroom, 1993). Upon isolation, 7-ADCA can be used in the actual kinetic synthesis reaction, during which hydrolysis of the activated side-chain and hydrolysis of cephalexin also take place. This results in a temporarily high concentration of cephalexin that is subsequently hydrolyzed by the enzyme (Bruggink et al., 1998). If the reaction is allowed to continue to equilibrium, most of the cephalexin will be hydrolyzed (Schroën et al., 1999). Therefore, it is clear that for optimum productivity it is necessary to predict the precise course of the reaction, and on this basis, to stop the reaction before hydrolysis takes over.

From the literature, some models are known for bioconversions concerning antibiotics, however, these mainly deal with thermodynamic coupling (e.g., Fernandez-Lafuente et al., 1991 and 1996; Kasche, 1985) or hydrolysis of penicillin G (Spieß et al., 1999). Some attempts have been made to

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model the kinetic antibiotic synthesis process. For the kinetic synthesis of ampicillin from phenylglycine methyl ester, a model is known from literature (Kasche,1986; Kasche et al., 1984). This model was later adopted by Gonçalves and co-workers (2000) and used to describe the synthesis of another penicillin-type antibiotic, amoxicillin. Nam and co-workers (1985) developed a model for an α-acylamino-β-lactam acylhydrolase from *Xanthomonas citri* and Blinkovsky and Markaryan (1993) used a D-(−) phenylglycyl-β-lactamide amidohydrolase from *Xanthomonas sp.* Although the models could be used to describe the course of the reaction correctly, only a limited number of experiments were shown. Also, the models do not seem to be valid for a wide range of reaction conditions.

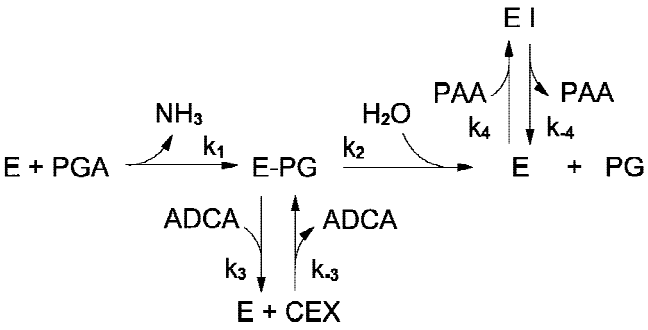
In this article, a model is presented that describes all relevant reactions that take place during cephalexin synthesis with an immobilized penicillin G acylase from *Escherichia coli*. The model was developed for reactants in solution at pH 8.0. It was built in a systematic way using the constants connected to phenylglycine amide hydrolysis at 293 K as a starting point. These constants were used as input-parameters for the calculation of the constants connected to cephalexin synthesis and hydrolysis at the same temperature. Based on the five constants necessary to describe all reactions (not only synthesis) at 293 K, the temperature dependency of all the constants was calculated using the Årrhenius equation. The thus obtained model was used to evaluate the effect of initial substrate concentration (phenylglycine amide: 0–490 m*M*, 7-ADCA: 0–300 m*M*) and temperature (273–298 K).

The model from Kasche and the model described here show considerable similarities but also one remarkable difference. Kasche (1984, 1986) describes inhibition by the nucleus while we use inhibition by phenylacetic acid, a minor component in the nucleus crystals, albeit a very strong inhibitor for the enzyme used in our study (Alkema et al., 1999). It should further be noted that the model described in this article was developed for cephalexin synthesis with phenylglycine amide, not the phenylglycine methyl ester as was the case in the work of Blinkovsky and Markaryan (1993) and Nam and co-workers (1985). The properties of the activated side-chain, and more specifically the chemical stability of this component and its solubility, determine the conditions at which the reaction should be carried out. For phenylglycine methyl ester this is a relatively low pH (6–6.5) while for the phenylglycine amide higher pH's can be used (e.g., pH 8).

THEORY

Kinetic Model

The mechanism of penicillin G acylase is described in the literature by Duggleby et al. (1995). The following simplified reaction scheme is deduced for kinetic cephalexin synthesis carried out with immobilized penicillin G acylase:



Scheme 1. Reaction scheme for cephalexin synthesis; rate equations for this scheme are given in Eqs. (1)–(5). See the text for further explanation.

In this scheme, the reactions taking place simultaneously during kinetic synthesis can be distinguished. First, the phenylglycine amide (PGA) binds to the free enzyme (E) to form an enzyme-acyl complex (E-PG: k_1). This complex can either be hydrolyzed to phenylglycine (k_2) or react with 7-ADCA to form cephalexin (CEX: k_3). Of course, the free enzyme can also bind cephalexin and give the enzyme-acyl complex (k_{-3}), which either can be hydrolyzed or react back to cephalexin. The enzyme is reversibly inhibited by phenylacetic acid (PAA: k_4 and k_{-4}) that may be present in very small amounts in 7-ADCA.

In the scheme, the reaction from phenylglycine to enzyme-phenylglycine-complex (k_{-2}) is omitted because direct synthesis of cephalexin from phenylglycine and 7-ADCA was proven to be impossible (Schroën et al., 1999). Further, the reaction from enzyme-phenylglycine complex to phenylglycine amide (k_{-1}) is not incorporated in the scheme because it was experimentally proven that this reaction hardly takes place at the reaction-pH of 8.0 (< 1%).

Using the King-Altman method (Cornish Bowden, 1995), the following reaction-rate equations were derived for Scheme 1. Because the water concentration is constant, k_2 is replaced by k_2' corresponding to k_2 multiplied by the water concentration. All concentrations are in millimoles per kilogram total.

$$\frac{d[CEX]}{dt} = \frac{k_1 \cdot k_3 \cdot [7-ADCA] \cdot [PGA] - k_2' \cdot k_{-3} \cdot [CEX]}{\Sigma} \cdot E_0 \quad (1)$$

$$\frac{d[PG]}{dt} = \frac{k_1 \cdot k_2' \cdot [PGA] + k_2' \cdot k_{-3} \cdot [CEX]}{\Sigma} \cdot E_0 \quad (2)$$

$$\frac{d[PGA]}{dt} = \frac{-k_1 \cdot k_2' \cdot [PGA] - k_1 \cdot k_3 \cdot [PGA] \cdot [7-ADCA]}{\Sigma} \cdot E_0 \quad (3)$$

$$\frac{d[7-ADCA]}{dt} = -\frac{d[CEX]}{dt} \quad (4)$$

$$\Sigma = k_1 \cdot [PGA] + k_2' + k_3 \cdot [7-ADCA] + k_{-3} \cdot [CEX] + k_4/k_{-4} \cdot [PAA] \cdot (k_2' + k_3 \cdot [ADCA]) \quad (5)$$

E_0 denotes the initial enzyme concentration (g enzyme per g total). Two mass-balance equations, one for the nucleus

and one for the side-chain, are valid. In case no product is present at time zero, as is the case in our experiments, these equations are as follows:

$$[7-\mathrm{ADCA}_0] = [7-\mathrm{ADCA}] + [\mathrm{CEX}] \quad (6)$$

$$[\mathrm{PGA}_0] = [\mathrm{PGA}] + [\mathrm{PG}] + [\mathrm{CEX}] \quad (7)$$

In which the subscript 0 denotes the concentration at time zero. For hydrolysis of phenylglycine amide ([CEX], [7-ADCA] and [PAA] equal zero), the reaction-rate equations (2) and (3) reduce to:

$$-\frac{\mathrm{d}[\mathrm{PGA}]}{\mathrm{d}t} = \frac{\mathrm{d}[\mathrm{PG}]}{\mathrm{d}t} = \frac{k_2' \cdot [\mathrm{PGA}]}{[\mathrm{PGA}] + \frac{k_2'}{k_1}} \cdot \mathrm{E}_0 \quad (8)$$

Temperature

For the temperature dependency of all reaction-rate constants, the Årrhenius equation is used (e.g., Van'tRiet and Tramper, 1991).

$$k_{n,T} = k_{n,T_{ref}} \cdot e^{\frac{-\Delta H_n^*}{R} \cdot \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \quad (9)$$

In this equation $k_{n,T}$ is the reaction-rate constant at temperature T, while $k_{n,Tref}$ is the reaction-rate constant at reference temperature (T_{ref}) 293 K and ΔH_n^* is the activation-enthalpy change (further called reaction enthalpy).

MATERIALS AND METHODS

Chemicals

The water used throughout was double-distilled. Cephalexin, phenylglycine amide, and samples of 7-ADCA with various amounts of phenylacetic acid, were in-house chemicals of DSM Research (Geleen, The Netherlands) and DSM Anti-Infectives (Delft, The Netherlands). Sodium hydroxide (99+%) and hydrochloric acid originated from Merck (Darmstadt, Germany).

Enzyme

Assemblase® is immobilized penicillin G acylase from *E. coli*, an in-house enzyme of DSM Anti-Infectives (Delft, The Netherlands). The enzyme was isolated as described in Kaasgaard et al., (1992). Immobilization was carried out as described in DeVroom (1997). The amoxicillin synthesizing activity of Assemblase was 3800 units/kilogram dry weight (one unit is the amount of enzyme that produces 1 gram of amoxicillin · $3H_2O$ from 6.5% 6-amino-penicillic acid and 6.5% hydroxyphenylglycine methyl ester at 293 K). Before use in the experiments, the immobilized enzyme was washed with a 0.2*M* sodium-phosphate buffer (with the same pH as applied in the actual experiment) and filtered through a ZapCap CR (0.45 μm, nylon filter, Schleicher & Schuell, FP 030/2, Dassel, Germany). The volume of buffer that was used for washing was approximately 3 times the volume of the enzyme. Under continuous suction the enzyme was removed from the ZapCap, most water between the enzyme particles was thus removed and reproducible amounts of enzyme could be weighed in.

Hydrolysis of Phenylglycine Amide

Measurement of the initial hydrolysis rate of phenylglycine amide took place in a temperature- and pH-controlled (automatic titrater by Titrino 719s, Metrohm, Herisau, Switzerland) reaction vessel. Prior to addition of the enzyme, the phenylglycine amide-containing solution was brought to the desired pH and temperature. Initial reaction rates were measured at pH 8.0, for different initial concentrations (0–430 m*M*) and at two temperatures, 277 and 293 K. The enzyme concentration was typically 0.2% (w/w). The reaction was monitored for 45 minutes. Every 3 minutes samples were taken and analyzed by HPLC.

Cephalexin Synthesis and Hydrolysis Reactions

The same experimental set-up was used as for the phenylglycine amide experiments. The pH was 8.0 for all experiments. Prior to addition of the enzyme, the solution containing either cephalexin for hydrolysis experiments or phenylglycine amide and 7-ADCA for synthesis experiments was brought to the desired pH and temperature. The enzyme was added in a concentration between 0.8 and 5% w/w, depending on initial substrate concentration and temperature (between 273 and 298 K). A typical synthesis experiment lasted 3–4 hours. Samples were taken at least every 30 minutes, and analyzed by HPLC or capillary electrophoresis.

Calculation of Reaction-Rate Constants Connected to Phenylglycine Amide Hydrolysis at 293 K

For the calculation of the constants connected to phenylglycine amide hydrolysis (k_1 and k_2), the initial reaction rates were used. To give the low initial reaction rates more weight the relative difference was minimized ($\sum(([\text{measured}]-[\text{model}])/[\text{measured}])^2$) by the statistical analysis program SAS (version 6.12, SAS Institute Inc. Cary, NC).

Calculation of the Other Reaction-Rate Constants at 293 K

The results of phenylglycine amide hydrolysis were used as input for the calculation of the other constants at 293 K. For the latter calculation both cephalexin hydrolysis and synthesis experiments were used. The synthesis experiments were carried out both at low (100 m*M* phenylglycine amide and 7-ADCA) and at saturated initial substrate concentration (490 m*M* phenylglycine amide and 300 m*M* 7-ADCA;

the saturation concentration of phenylglycine amide is slightly higher in the presence of 7-ADCA). In the synthesis experiments, 7-ADCA with various concentrations of phenylacetic acid was used. The cephalexin hydrolysis experiments are mainly initial reaction-rate measurements carried out at initial cephalexin concentrations between 20 and 190 m*M*. Also, two experiments in which the reaction was allowed to continue until more than 80% conversion, were used in the calculations.

With the SAS program, reaction-rate constants were calculated based on minimization of the residual sum of squares of phenylglycine and cephalexin. This is the sum of the quadratic difference between the measured concentrations and their respective predicted counterparts (Σ([measured]-[model])^2).

All calculated constants in this article are derived for concentrations in solution. It was checked whether crystallization of phenylglycine took place, and if this was the case, these data points were not used in the calculations.

Calculation of Reaction Enthalpy

The constants from Tables I and II were used as reference constants. The reaction enthalpies were calculated using eq. (9) and experiments carried out at initial substrate concentrations of 100 m*M*, for both phenylglycine amide and 7-ADCA, and at temperatures ranging from 273–298 K. In total, eight experiments were used of which three were carried out with 7-ADCA without phenylacetic acid.

With the SAS program the residual sum of squares of phenylglycine and cephalexin was minimized (Σ([measured]-[model])^2) as was done in the previous paragraph on calculation of constants at 293 K.

Analysis

Sampling and Sample Preparation

Samples of 0.25 mL were taken from the reaction mixture and put into a measuring flask. Distilled water was added until a known volume was reached and the contents were mixed. A 1.0-mL sample was taken from this mixture and centrifuged at 278 K for 15 min at 15,300 rpm (Beckman GS-15R, rotor: Beckman F2402H). After centrifugation, a 0.75-mL sample was taken and either analyzed directly by HPLC or CE, or stored at 277 K and pH 6.0.

Table I. Calculated constants at pH 8.0 and 293 K for phenylglycine amide hydrolysis.

		95% confidence interval	
Constant	Estimated value	Lower	Upper
k_1 (1/min)	6.1	4.8	7.4
k_2' (mmol/l/min)	127	122	132

Table II. Calculated constants for cephalexin experiments at pH 8.0 and 293 K, k_1 and k_2' from Table I are used as input-parameters.

		95% confidence interval	
Constant	Estimated value	Lower	Upper
k_3 (1/min)	4.0	3.3	4.7
k_{-3} (1/min)	5.2	4.0	6.3
k_4/k_{-4} (L/mmol)	239	215	263

HPLC

The HPLC system was purchased from Thermo Separation Products (TSP, Breda, The Netherlands) and consisted of a SCM 1000 vacuum membrane de-gasser, a SpectraSystem P4000 gradient pump, a SpectraSystem AS autosampler, and a SpectraSystem UV3000 detector.

The columns were of type reversed-phase C18 and were purchased from Bester (Amstelveen, The Netherlands). The precolumn was of type SGE W5C18RS, (10 mm · 4 mm). The main column was of type Prodigy ODS(3) (250 mm · 4.6 mm). The particle size was 5 μm and the pore-size 10 nm.

The temperature of the column was 313 K and the tray temperature 278 K. The injected volume was between 1 and 20 μL. For elution, acetonitrile and 18.2 m*M* H_3PO_4 (pH 4.9) were used. The flow rate was 1.25 mL/min. Analysis took place both at wave lengths 191 and 265 nm. The retention times of the components were 3.3 min for phenylglycine (191 nm), 4.1 min for ADCA (265nm), 5.0 min for phenylglycine amide (191nm), and 9.9 min for cephalexin (265 nm). The concentrations were calculated using calibration curves. More details on the analysis method are given in (Schroën et al., 2000).

Capillary Electrophoresis (CE)

Some of the synthesis experiments were analyzed by CE (Hewlett Packard^{3D} Capillary Electrophoresis System). A bubble-cell fused-silica capillary with a length of 48.5 cm and an inner diameter of 50 μm was used. As a buffer, 40 m*M* NaH_2PO_4/Na_2HPO_4 of pH 8.0 was used. The initial voltage was 10 kV; it was increased to 30 kV after 8.0 min, the samples were injected by pressure injection. The capillary temperature was 293 K. The peak area was measured at 191 and 265 nm and the concentrations were calculated using calibration curves. The retention times were 6.7 min for phenylglycine amide (191 nm), 7.6 min for phenylglycine (191 nm), 8.4 min for cephalexin (265 nm) and 9.1 min for ADCA (265 nm). A more detailed description is given elsewhere (Nierstrasz et al., 1997).

RESULTS AND DISCUSSION

Constants at 293 K

Phenylglycine Amide Hydrolysis

The initial hydrolysis rate of phenylglycine amide was measured for initial substrate concentrations between 0 and 430

m*M* (saturation concentration). These initial reaction rates were used to calculate the reaction-rate constants, k_1 and k_2' [Eq. (8)]. The results are given in Table I and Figure 1; the line corresponds to the constants given in Table I.

The initial hydrolysis rates are described well when the constants from Table I are used. The average difference between model and measurement is 3%. The 95%-confidence intervals are very small, especially for k_2', indicating that the obtained constants have a high degree of reliability. Later in this section, the effect of different sets of constants on the description of phenylglycine amide hydrolysis is discussed. Further, Figure 1 shows that the enzyme is indeed not inhibited by phenylglycine amide. This is in agreement with the kinetic model (Theory section).

Remaining Constants at 293 K

The remaining constants were calculated using, in total, 15 cephalexin experiments, including both synthesis and hydrolysis. The results are summarized in Table II and Figure 2a–d for the full concentration range and in Figure 2e–h for the low-concentration range.

The confidence intervals in Table II were again very narrow indicating a high degree of reliability. This also becomes clear from Figure 2 in which the measured concentrations were plotted as a function of the calculated concentration. If the values are close to the line than there is a good agreement between calculated and measured concentration. This is the case both for the high- and for the low-concentration range. The average difference between model and measurements was 3.6 m*M* for the high-concentration experiments (490 m*M* phenylglycine amide, 300 m*M* 7-ADCA, or 200 m*M* cephalexin). The average difference for the low-concentration experiments (100 m*M* phenylglycine amide, 100 m*M* 7-ADCA, or < 100 m*M* cephalexin) was 1.9 m*M*.

It was checked whether the model could be used to describe the course of the reaction at pH 7.5 and 8.5 (temperature 293 K), and it was found that for these pH's as well, the course of the reaction was predicted correctly by the model.

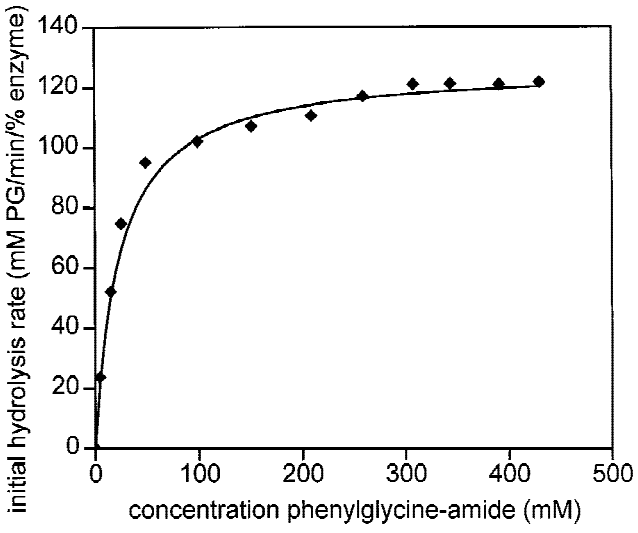


Figure 1. Initial phenylglycine amide hydrolysis rate as a function of the initial phenylglycine amide concentration. The line is based on the k_1 and k_2' given in Table I).

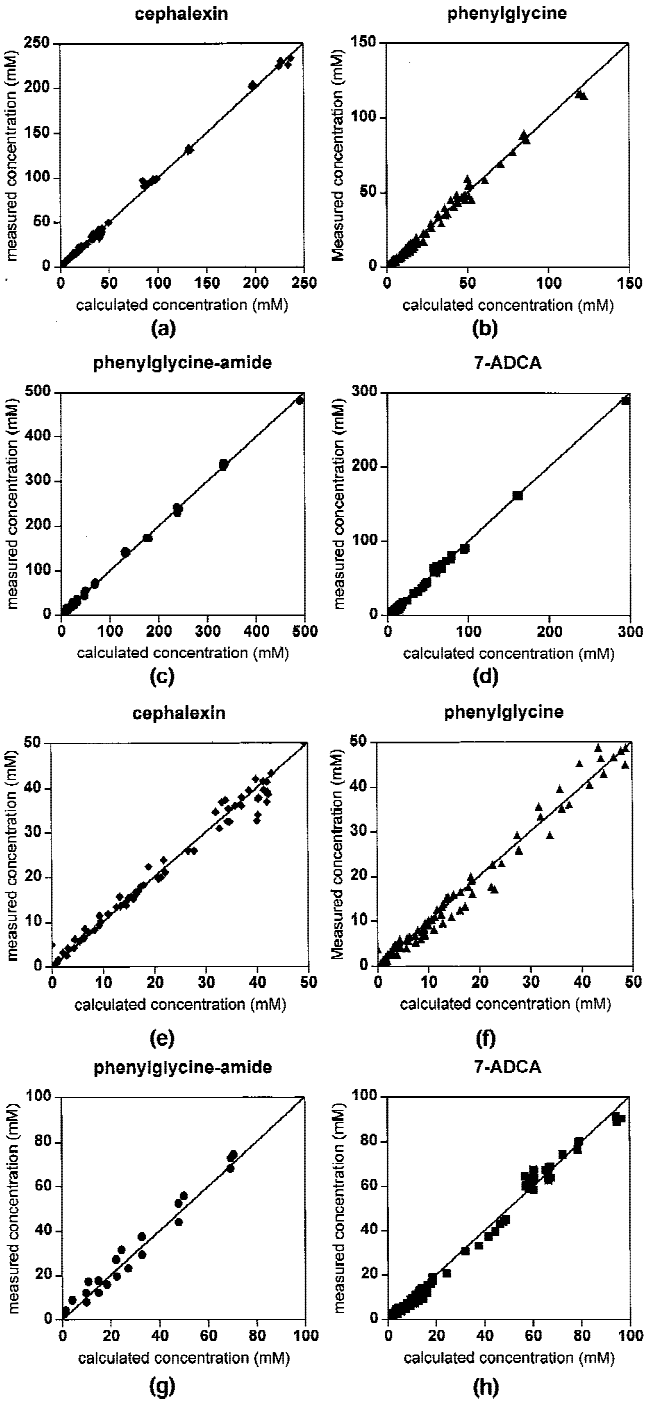


Figure 2. (a)–(d). Parity plots for cephalexin, phenylglycine, phenylglycine amide, and 7-ADCA for the entire concentration range that was investigated. (e)–(h). Parity plots for cephalexin, phenylglycine, phenylglycine amide, and 7-ADCA for the low-concentration range.

Discussion Calculation Method

It is also possible to calculate sets of constants using a different approach. The following two are compared here:

the calculation of all constants simultaneously using only cephalexin experiments as input, and usage of k_1 and k_2' from phenylglycine amide hydrolysis as constants and calculation of all other constants as was described in the previous paragraphs.

For both methods, comparable residual sums of squares were calculated, indicating that both methods should be equally good. However, if all constants are fitted simultaneously, a completely wrong description of the phenylglycine amide hydrolysis experiments was obtained. The contribution of the phenylglycine amide-experiments to the total sum of squares was in that case so low that they are practically neglected in the calculation. Therefore, the method based on k_1 and k_2' obtained from phenylglycine amide hydrolysis, should be the method of choice. The constants that were obtained in this way (Tables I and II) are used in the rest of this article.

In comparison to the models found in the literature (Blinkovsky and Markaryan, 1993; Nam et al., 1985), the model presented here features significantly less parameters and is valid for a wider concentration interval.

Reaction Enthalpies

For the calculation of the reaction enthalpies, the same approach was used as for the calculation of the reaction-rate constants at 293 K. First, the constants for phenylglycine amide hydrolysis at 277 K were calculated and converted to reaction enthalpies [Eq. (9)]. Subsequently, the other reaction enthalpies were calculated. The constants from Tables I and II were used as reference constants.

For the phenylglycine amide hydrolysis-experiments at 277 K, k_1, and k_2' were calculated. It was found that k_2' could be calculated with acceptable accuracy (comparable to that given in Table I for 293 K). However, k_1 showed a wide error margin; therefore, it was decided to use only the enthalpy related to k_2' as a reference point and calculate all other reaction enthalpies from synthesis experiments at different temperatures. The result is given in Table III and Figure 3.

The average difference between measured and calculated concentration equalled 2.2 m*M* and the 95% confidence intervals were very narrow considering the large temperature interval investigated here.

Table III. Calculated reaction enthalpies and their statistical reliability, ΔH_2 is based on phenylglycine amide-hydrolysis at 277 K.

Reaction enthalpy (kJ/mol)	Estimated value	95% confidence interval	
		Lower	Upper
ΔH_1	54.8	42.2	67.3
ΔH_2	45.3	41.3*	49.3*
ΔH_3	13.1	−18.8	44.9
ΔH_{-3}	48.2	15.0	81.4
$\Delta H_4 - \Delta H_{-4}$	6.6	−3.5	16.6

*These values are based on the calculated reaction rate constants at 277 and 293 K plus or minus twice the error. For both temperatures, the error equals 2 kJ/mol.

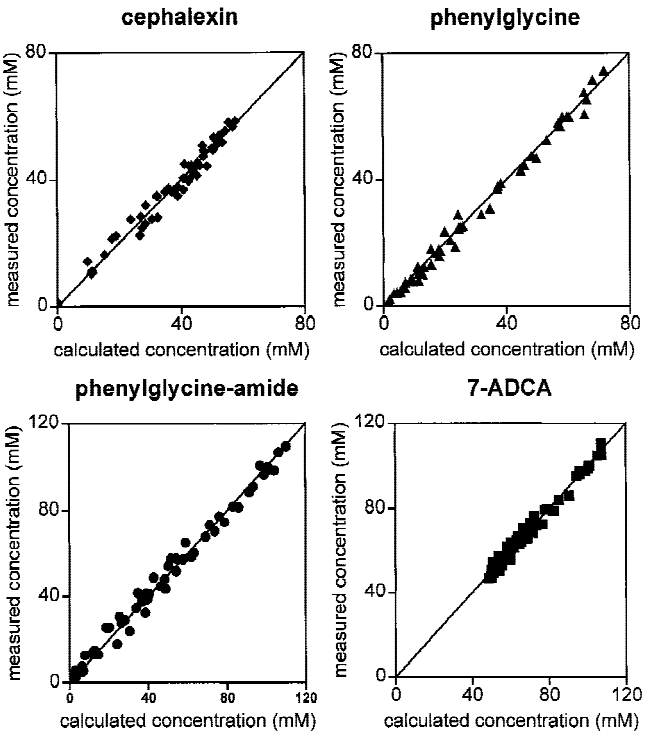


Figure 3. Parity plots for all temperature experiments.

The experiments carried out with 7-ADCA without phenylacetic acid were essential for the calculation of a statistically reliable set of parameters because they reduce the correlation between the reaction enthalpies and thus result in narrower 95%-confidence intervals. The ΔH's of the free enzyme, and especially ΔH_3, are considerably higher; this is an indication that diffusion-limitation occurs (Van'tRiet and J. Tramper, 1991). This effect will be further discussed in a future publication.

It was tested whether the model could also be used to predict experiments slightly outside the range of conditions for which the model was validated. As examples, a synthesis experiment at 273 K with 7-ADCA without phenylacetic acid (impurity) and a synthesis experiment at 303 K with the other 7-ADCA are shown in Figure 4. The model predicts these experiments (reasonably) well. Also, the course of reactions carried out at pH 7.5 and 8.5 (293 K) could be predicted (results not shown). This further indicates that the enzyme activity is constant for this pH-interval.

It is clear that a low temperature is beneficial for the cephalexin synthesis process. The reaction enthalpy for cephalexin synthesis (ΔH_3) is low, indicating that this reaction is hardly influenced by temperature. Both reaction enthalpies for hydrolysis reactions (ΔH_2 and ΔH_{-3}) are notably higher, and therefore, the respective constants are more influenced by temperature. This results in a higher cephalexin and lower phenylglycine concentration at low temperature, which is beneficial for the process (Boesten et

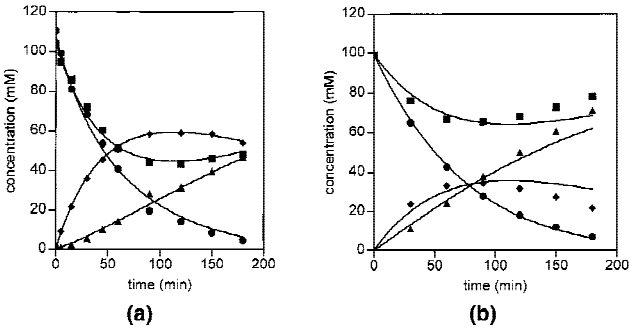


Figure 4. Predictions for synthesis experiments carried out at 273 K (a) with 7-ADCA without phenylacetic acid and carried out at 303 K (b) with 7-ADCA with phenylacetic acid. The symbols are: (♦) cephalexin, (▲) phenylglycine, (●) phenylglycine amide, and (■) 7-ADCA.

al., 1996; Boesten et al., 1997; Kaasgaard and Veitland, 1992, and other related DSM patents).

Evaluation of Effect of Initial Substrate Concentrations

The effect of the initial substrate concentration on the cephalexin synthesis process was evaluated. For initial 7-ADCA concentrations between 50 and 300 m*M* (steps of 50 m*M*) and initial phenylglycine amide concentrations between 100 and 500 m*M* (steps of 100 m*M*) the maximum cephalexin concentration was calculated. This value was used to calculate the maximum conversion yield on 7-ADCA and on phenylglycine amide. The term yield will be used in this article to indicate the maximum conversion yield (without downstream processing). In Figure 5 the results at 293 K are shown.

As expected the yield on 7-ADCA increased with increasing initial phenylglycine amide concentrations. Further, the yield decreased with increasing initial 7-ADCA concentration, although this effect was not as pronounced as for phenylglycine amide, especially if the initial phenylglycine amide concentration was high. For high yields on 7-ADCA, surplus phenylglycine amide is necessary. The opposite is true if the yield on phenylglycine amide is used as a criterion. High initial 7-ADCA and low initial phenylglycine amide concentrations lead to high yields. For a high yield on phenylglycine amide, a surplus of 7-ADCA is required. For all temperatures, the same trends were found, although obviously the actual values of the yields were different as can be deduced from the previous paragraphs. Yield on 7-ADCA and yield on phenylglycine amide are clearly opposing criteria and economics will decide on which weight either one of the yields will carry in the evaluation of the eventual process.

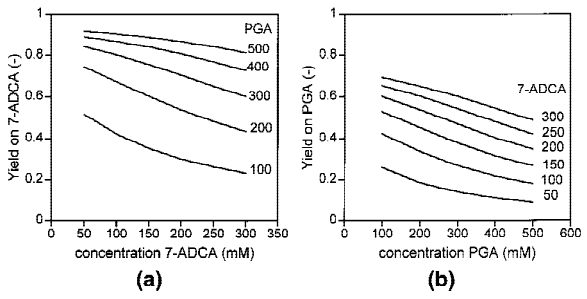


Figure 5. The calculated maximum conversion yield on 7-ADCA (a) and phenylglycine amide (b) as a function of the initial concentration of both the substrates at 293 K. The numbers indicate the initial concentration of the substrate that is *not* on the x-axis.

CONCLUSIONS

In this article, a model is presented for kinetic cephalexin synthesis. The model is validated at pH 8.0, for initial phenylglycine amide concentrations between 0 and 490 m*M* (saturation concentration), initial 7-ADCA concentrations between 0 and 300 m*M* (saturation concentration), and temperatures between 273 and 298 K. In contrast to models found in the literature, the model presented here is valid for a wide range of reaction conditions. Further, it uses only a limited number of constants (five in total) for reactions carried out at one temperature. The influence of temperature is modelled using the Årrhenius equation.

The obtained model is statistically reliable and describes not only cephalexin synthesis but also phenylglycine amide- and cephalexin hydrolysis. The statistical reliability of the model greatly depends on the approach that is used. If all constants are calculated simultaneously then, not only the reliability decreases strongly, but also the individual reactions are no longer described correctly.

The model is a useful tool for the evaluation of reaction conditions. A low temperature is beneficial for the process because more cephalexin and less phenylglycine are formed. For a high yield on 7-ADCA, high initial phenylglycine amide concentrations are required. The opposite is true if a high yield on phenylglycine amide is the aim. In that case, high initial 7-ADCA concentrations should be used. Economics will decide eventually which criterion should carry more weight in the development of an actual process.

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